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# Changes occurring in stabilized ultra-high-temperature-treated whole milk during storage

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This Research Communication describes the relationship between casein, free fatty acids (FFAs) and the storage period of ultra-high temperature-treated (UHT) whole milk observed for a period of 120 d of labelled shelf-life. Moreover, we aim to estimate the daily rate of casein degradation in UHT whole milk, and the total length of time estimated for its full degradation. With this aim, ten sets of samples were evaluated from batches of UHT milk manufactured by a dairy processing plant in Parana State, Brazil on 10 different days. Each set was comprised of one liter of raw milk and 12 units of 1 litre cartons of UHT milk, and represented one batch of production. Total mesophilic (TMC), psychrotrophic (TPC), and somatic cell counts (SCC) of raw milk were assessed. UHT milk was assessed for fat (%), sialic acid (mg/l), casein (%), and FFA contents. TMC ranged from $3{\cdot}5 \times 10^6$ to $3{\cdot}1 \times 10^7$ CFU/ml; TPC, from $10^6$ UFC/ml and higher; and SCC, from $18 \times 10^4$ SC/ml to $4{\cdot}83 \times 10^5$ CS/ml. Casein ($r = -0{\cdot}991$; $R^2 = 0{\cdot}9822$) and FFA ($r = 0{\cdot}962$; $R^2 = 0{\cdot}9245$) contents, and storage time of UHT milk were correlated ($P < 0{\cdot}05$). The rate of casein hydrolysis was estimated as 0·021 g/100 g UHT whole milk/day. A complete breakdown of casein was estimated to occur by the 560th day post-manufacture. Although age gelation was not observed in our study, the report herein corroborates the understanding that the microbiological quality and SCC of raw milk are important components involving the integrity of casein and lipids of UHT milk during shelf-life.

**Keywords:** Age gelation, raw milk quality, heat treated milk.

Ultra-high temperature-treated (UHT) milk in Brazil represents 86·3% of the country's fluid milk market (ABLV – Brazilian Association of the Long Life Milk Industry, unpublished figure retrieved by email, 4 May 2018), and reports indicate that consumers' preferences towards the purchase of UHT milk over pasteurized milk rely predominantly upon the possibility of storing UHT goods for extended periods of time, as long as no modifications in taste and appearance are perceived until the end of the labelled 'use by' dates of the product (Fernandes et al. 2008). These modifications – of concern to UHT fluid milk processors – are credited mainly to changes in proteins and lipids occurring over the period of time that the product is stored. These changes derive mainly from proteolytic and lipolytic activities of heat-stable enzymes in milk, primarily of microbial origin and/or due to inflammatory response cells of the mammary gland (Murphy et al. 2016), which are irreversibly activated during heat treatment (Datta & Deeth, 2001). Hence, it seems evident that the quality of raw milk is pivotal for preventing the onset of such hydrolytic activities in the final product (Murphy et al. 2016). However, as hygienically sourced milk is still a limitation in the dairy industry in some developing economies, we hypothesize that data locally sourced from a dairy cooperative in southern Brazil may contribute to improving the understanding of UHT milk processors that sourcing of higher quality raw milk may represent the key factor towards preservation of fats and proteins in UHT dairy products, particularly fluid milk, over the expected shelf-life period. Thus, the primary objective of this study was to determine the relationship between concentrations of casein and free fatty acids and the storage time of UHT whole milk. Secondly, we calculated the daily rate at which hydrolysis of casein occurred, and

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estimated the length of time for a complete breakdown of the protein in the product. Finally, we described the physicochemical parameters of UHT milk observed at days 0 ($T_0$), 30 ($T_{30}$), 60 ($T_{60}$), 90 ($T_{90}$), and 120 ($T_{120}$) of storage.

## Materials and methods

### Sampling

Raw milk used in this study was kept refrigerated (3–7 °C, 48 h) in farms until haulage to a dairy processing plant in northern Paraná State, Brazil. Processing output of UHT milk at the time of study was ~200 000 l/d. Sampling days were chosen at random so that 10 sets of samples representing 10 different batches produced on 10 different days, from April until May 2013, were collected. Briefly, each batch was comprised of the raw milk content of one isothermal silo of milk (200 000 l) kept under continuous homogenization (1–2 °C, 6 h) until pasteurization (HTST APV® Standard Pasteurizer, Crawley, UK) on the given day. The batch was transferred to a second silo, mixed with a commercial stabilizer ($NaH_2PO_4$, $Na_2HPO_4$ and $Na_3PO_4$, blended, 0·1 g/100 ml), and kept at 1–2 °C, 3 h until sterilization. Then, the milk was subject to UHT treatment (pre-heating: 85 °C; sterilization through direct steam injection: 142–145 °C, 2 s; removal of steamed, injected water: 70 °C, −0·6 MPa; and homogenization: 22–25 MPa) and aseptic packaging (1 litre Tetra Brik® cartons, TETRA PAK® Simply 8, Lund, Sweden). Conditions were strictly repeated for each of the 10 batches assessed. The set of samples representing the batch produced on a given day comprised 1 litre of raw milk and twelve units of 1 litre cartons of UHT milk. The 1 litre sample of raw milk was composed of five 200 ml aliquots taken hourly during milk transfer between silos (from raw milk to pasteurized milk silo prior to UHT processing at a flow rate of milk between silos ~35 000 l/h). The twelve 1 litre carton samples of UHT milk were collected directly from the filling machine (each carton taken hourly, until the end of filling and packaging of the whole batch, i.e. 12 cartons by the end of the 24 h cycle of the batch), boxed in a cardboard box representing the batch of that day, and stored (25 ± 1 °C and RH 70 ± 1%) for 120 d. Finally, the 10 sets of samples collected during the study period was composed of ten 1 litre samples of raw milk and ten cardboard boxes comprising 12 × 1 litre cartons of UHT milk.

### Laboratory analyses

*Microbiological*. Raw milk samples: Total mesophilic bacterial count (TMC) was determined by standard plate count agar (Himedia®, Mumbai, India) (32 ± 1 °C, 48 h) and total psychrotrophic bacterial count (TPC) in PCA (Plate Count Agar) (Himedia®, Mumbai, India) (7 ± 1 °C, 10 d) (Houghtby et al. 1992). Results were expressed in colony forming units per millilitre of milk (CFU/ml). Somatic cell counts (SCC) were determined by flow cytometry (Somacount 300®, Bentley Instruments Inc., Minnesota, USA), and results expressed as somatic cells per millilitre of milk (SC/ml).

### Physicochemical

Raw milk samples were analysed after the completion of silo sampling. Analyses of UHT milk were carried out at $T_0$, $T_{30}$, $T_{60}$, $T_{90}$ and $T_{120}$. UHT milk samples were analysed for fat (%) and nitrogen fractions (%) according to the Association of Official Analytical Chemists (AOAC, 2005). Briefly, total nitrogen (TN), non-casein nitrogen (NCN), and non-protein nitrogen (NPN) contents were assessed for determination of the concentration of casein (TN – NCN, %). Proteolysis and lipolysis in UHT milk samples were estimated through the concentrations of casein (AOAC, 2005) and free fatty acids (FFA, mEq/l) (Mahieu, 1984) at $T_0$, $T_{30}$, $T_{60}$, $T_{90}$, and $T_{120}$. Sialic acid (mg/l) was also determined from UHT milk samples (Neelima et al. 2012). Cartons in which aseptic barriers were found damaged at the time of analysis were rejected and replaced with another carton from the same batch. Gelation was qualitatively evaluated (Datta & Deeth, 2001).

### Statistical analysis

Relationships between the response variables (casein and FFA levels) and the independent factor (storage time) were investigated (Pearson correlation coefficient, MINITAB® Statistical Software, release 13). The hypothetical period of one-quarter life ($t_{1/4}$), one-third life ($t_{1/3}$), half-life ($t_{1/2}$), three-quarters life ($t_{3/4}$) and the complete breakdown of casein were determined, and the kinetics of casein hydrolysis was assessed (Oliveira & Faria, 2010). The reaction constant rate ($k$) was deduced to estimate the daily hydrolysis of casein. Descriptive statistics of CFU and SCC of raw milk summarized the baseline framework. Data from the physicochemical analyses of UHT milk samples obtained during the storage period were submitted to ANOVA and differences were identified by Tukey test ($\alpha = 0{\cdot}05$) over the period considered (MINITAB® Statistical Software, release 13).

## Results and discussion

TPCs of raw milk used to produce the UHT milk evaluated in the present study were as high as $10^6$ CFU/ml, ranging from $4 \times 10^6$ to $4{\cdot}5 \times 10^7$ CFU/ml. TMCs were between $3{\cdot}5 \times 10^6$ and $3{\cdot}1 \times 10^7$ CFU/ml. SCCs ranged from $1{\cdot}8 \times 10^4$ to $4{\cdot}8 \times 10^5$ SC/ml. Casein concentrations and the storage time of UHT milk were correlated ($r = -0{\cdot}991$, $P < 0{\cdot}05$; $R^2 = 0{\cdot}9822$).

As casein undergoes hydrolysis during UHT processing, sialic acid-containing glycomacropeptide is released, casein micelles disassemble and the onset of the phenomenon of gelation is likely to occur (Datta & Deeth, 2001). The majority of indexed research credits high psychrotrophic bacteria and SCC in raw milk to the occurrence of protein-related defects over the shelf-life of UHT milk (Murphy et al. 2016). However, sialic acid concentrations did not vary ($P > 0{\cdot}05$), and gelation of UHT milk was not observed during its storage period. Although increased sialic acid levels in UHT milk suggests that age gelation of proteinase-origin may occur (Datta & Deeth, 2001), the highest value observed (~4·70 mg/l) was still within its warning level in the product (10 mg/l). Elsewhere (Pinto et al. 2016), casein hydrolysis and gel formation occurred by the 120th day of storage of UHT milk produced from raw milk, in which proteolytic psychrotrophic bacterial counts were up to $10^6$ CFU/ml. In our study, per cent casein also decreased significantly ($P < 0{\cdot}05$), between $T_0$ (2·37% ± SEM 0·28) and $T_{120}$ (1·86% ± SEM 0·07). However, it is noteworthy that the addition of stabilizers to UHT fluid milk (regulated for use in Brazil) may have prevented peptides from becoming insoluble (Datta & Deeth, 2001), and this possibly explains why such a phenomenon was not evidenced, even though casein hydrolysis occurred.

The half-life of casein was estimated to be approximately 276 d from processing; $t_{1/4}$ at 134 d; $t_{1/3}$ at 181 d, and $t_{3/4}$ at 418 d. In the present study, the total length of time for the complete hydrolysis of casein was estimated to occur by the 560th day after processing of raw milk into UHT whole milk, and $k$ was determined (Equation 1):

$$k = \frac{(1,33)^{n-1} - 1}{(n-1)t_{1/4}C_{A0}^{n-1}} \quad \frac{(1,50)^{n-1} - 1}{(n-1)t_{1/3}C_{A0}^{n-1}} \quad \frac{2^{n-1} - 1}{(n-1)t_{1/2}C_{A0}^{n-1}} \quad \frac{4^{n-1} - 1}{(n-1)t_{3/4}C_{A0}^{n-1}} \qquad (1)$$

whereas the net weight of a one-litre carton of UHT milk unit manufactured in the dairy plant studied was 1·031 g milk (~1·031 g/cm$^3$), the rate of casein hydrolysis ($k$) was estimated at 0·021 g of the protein fraction/100 g UHT whole milk/day, and the kinetics order of the reaction was 0·36. Such a non-integer figure may indicate that more complex biological mechanisms than those involved in classical models explaining reaction kinetics might be occurring (Oliveira & Faria, 2010). This has been made evident elsewhere (Vorob'ev & Kochetkov, 2016), where the kinetic order of reaction equalled unity when the proteolysis of casein – solely mediated by chymotrypsin – was modelled. Likewise, due to the range of lipolytic enzyme-embedding cells comprising the raw milk employed in our study, *i.e.* bacterial and somatic cells, the hydrolysis of milk lipids during storage time described here ($r = 0{\cdot}962$, $P < 0{\cdot}05$; $R^2 = 0{\cdot}9245$; Fig. 1) followed a similar trend as that depicted elsewhere (Panfil-Kuncewicz et al. 2005), although changes in total fat were not evident ($P > 0{\cdot}05$; $T_0 = 3{\cdot}04\%$ ± SEM 0·02 and $T_{120} = 3{\cdot}03\%$ ± SEM 0·03).

In our study, although gelation could not be detected, casein hydrolysis and FFAs in UHT-treated milk were correlated with the storage time of the product. The daily rate of casein hydrolysis observed decreased protein levels by 21% by the end of the expected 'use by' date of the product. Although a cause-and-effect relationship could not be identified here, UHT whole milk employed in our investigation was produced from high SSC, low hygienically sourced raw milk. This suggests that the hydrolysis of casein and lipids occurring over the storage period of UHT milk may proceed differently from that reported in our study, should a higher-quality raw milk be employed in further investigations.

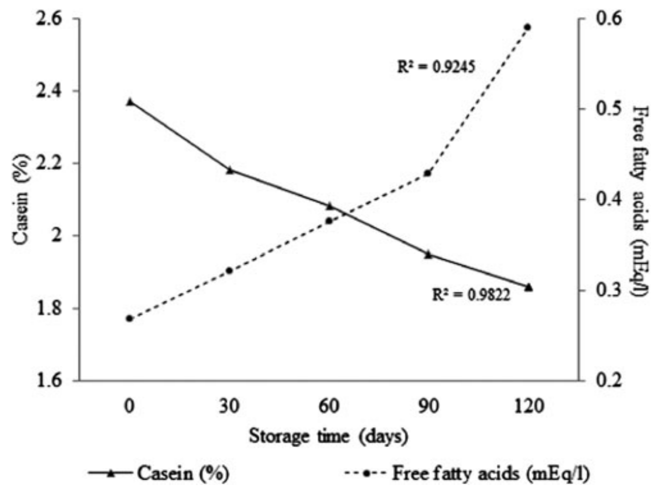


**Fig. 1.** Relationship between concentrations of casein (%) ($r = -0{\cdot}991$; $R^2 = 0{\cdot}9822$), free fatty acids (mEq/l) ($r = 0{\cdot}962$; $R^2 = 0{\cdot}9245$), and the storage time of 10 batches of UHT whole milk (evaluated at days 0, 30, 60, 90 and 120) produced by a processing plant in northern Parana, Brazil.

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