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Evaluation of the Quality and Acceptability of Milk Drinks Added of Conjugated Linoleic Acid and Canola Oil and Produced in Pilot Scale

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ABSTRACT

Considering that the interest of consumers for healthy foods is increasing more and more, the objective of this study was to scale-up the production of two chocolate milk drinks, CLABE (added of conjugated linoleic acid) and CANBE (added of canola oil), by adjusting the parameters used in the laboratory scale. The evaluation of the chemical composition included the determination of moisture, ashes, proteins, lipids and the calculation of the carbohydrate content and caloric value. The drink stability was assessed by measuring the pH and oxidative stability using the quantification of 2-thiobarbituric Acid Reactive Substances (TBARS). The overall drink acceptability and the intention to purchase were evaluated, using a hedonic scale of 9 and 5 points, respectively. The results indicated that the caloric value of CANBE was higher than CLABE, followed by a commercial drink that was used for comparison in this work. Also, CANBE showed higher levels of carbohydrates and proteins than the other two drinks. The amount of ashes of CANBE was similar to CLABE and superior to the commercial product. Among the three drinks, the latter showed the smallest lipid and the highest moisture contents. The addition of conjugated linoleic acid or canola oil had no influence on the stability of the drinks during the period of study, since no significant difference in pH and TBARS between the two drinks was found. The CLABE and CANBE showed better acceptance than the commercial drink, however no significant difference in the intention to purchase among the three drinks was achieved.

Key words: Conjugated linoleic acid, chemical composition, milk drink, scaling-up, sensory evaluation

INTRODUCTION

The development of new products represents one of the major sources of income in a Company, because this consists of a complex and multidisciplinary process. Aiming to obtain the desired success, a large effort involving a close relationship between the company management, the team of R and D department and the sectors of marketing, production, purchasing, quality control, sales, consumers and furnishers is required (Wille *et al.*, 2004).

After the initial steps, which involve the study of market and product definition, the development of prototypes and industrial tests take place, in order to define the process parameters,

the formulation and the final specification (Kotler, 2000). The chemical composition and the study of shelf life are important to characterize the product, i.e., to meet its nutritional and caloric values, as well as to follow its behavior during the storage period (Wille *et al.*, 2004). The prototypes are then subjected to a market research to evaluate the validity of the concept test of the product as well as the consumer preference (Kotler, 2000). Sensory evaluation is an important tool in the food industry for the decision of the production process in order to minimize the risks associated with the introduction of new products in the market (Bech *et al.*, 1994).

Considering that the consumption of milk drinks has been raised in recent years (ABIQ, 2004) and that the interest of consumers for healthy foods is increasing more and more, the dairy industries, attentive to this fact, search for adding value to their products. This occurs mainly by the formulation of new food with functional properties (Antunes *et al.*, 2007), which promotes beneficial physiological health effects (Padilha and Pinheiro, 2004).

Dairy products have been identified as good sources of Conjugated Linoleic Acid (CLA) and this has increased the positive nutritional image of these foods (Meraz-Torres and Hernandez-Sanchez, 2012). The CLA is a set of positional and geometric isomers of linoleic acid with conjugated double bonds. Studies with animals and humans suggest some health benefits of CLA, such as a protective effect against some type of cancer (Ip *et al.*, 1997; Kritchevsky, 2000) and modulation of body composition by reducing fat deposition and increasing lean body mass (Park *et al.*, 1997; Ostrowska *et al.*, 1999; Blankson *et al.*, 2000; Riserius *et al.*, 2001; Gaullier *et al.*, 2004). Ramaswamy *et al.* (2001) demonstrated consumer interest in food enriched with CLA associated to these effects.

Along with a number of beneficial effects of CLA, there are safety considerations for CLA supplementation in humans, which include effects on liver functions, milk fat depression, glucose metabolism and oxidative stresses (Dilzer and Park, 2012). Although some studies concerning the effects of CLA on body had already been reported in the literature, data with humans are scarce, which demonstrates the necessity of further studies with this group (Blankson *et al.*, 2000; Zambell *et al.*, 2000; Gaullier *et al.*, 2004; Malpuech-Brugere *et al.*, 2004; Larsen *et al.*, 2006; Nazare *et al.*, 2007; Laso *et al.*, 2007; Raff *et al.*, 2009). The continuation of the present study consists of carrying out a clinical trial with humans in order to evaluate the effect of dietary supplementation of CLA incorporated into a milk drink. Therefore, the present work was developed with the aim of scaling-up the production of two chocolate milk drinks, one added with CLA and another with canola oil, because this latter will be used as placebo in the clinical study. The present work involved also the analysis of these two drinks concerning the determination their chemical composition, the evaluation of their oxidative stability and of the acceptance of the final product.

MATERIALS AND METHODS

Chocolate milk drink: The fluid milk standardized to 1.5% of fat, fluid whey and mix of ingredients for chocolate milk drinks were kindly provided by CEMIL (Patos de Minas, MG, Brazil). The canola oil was purchased in the market of Patos de Minas (Minas Gerais, Brazil) and the CLA (Clarinol®G 80, contains 80% of CLA in the triacylglyceride form and consists of a 50:50 mixture of cis-9, trans-11-CLA and trans-10, cis-12-CLA) was kindly provided by Lipid Nutrition (Wormerveer, Netherlands). In the production of milk drinks, the equipment tri-blender Almix 210 L and an Ultra-high Temperature (UHT) plant, both from Tetra Pack (São Paulo, Brazil) were used.

A pH meter Tecnal (model TEC-2, Piracicaba, Brazil) was used for the determination of the pH of the drinks and a spectrophotometer CECIL (CE204 model, Buck Scientific, East Norwalk, USA) was used for the evaluation of lipid oxidation.

Scaling-up production of chocolate milk drinks: The chocolate milk drinks added of CLA (CLABE) or canola oil (CANBE) were produced at the dairy industry Cemil (Patos de Minas, Minas Gerais, Brazil), using the data previously obtained in the laboratory development of the dairy drink (Lopes *et al.*, 2009), especially regarding the incorporation of CLA in oil form. All industrial processes were followed in order to adapt the laboratory techniques to the industry.

Initially, whey was added to milk in the 210 L tri-blender equipment under constant agitation (770 rpm). This mixture was heated at 30°C and then a mix of ingredients for chocolate milk drink was added. After 10 min of stirring, CLA or canola oil was incorporated (1 g/100 mL), also under stirring for 40 min.

The drink was then submitted to the UHT process in a Tetra Pack plant (São Paulo, Brazil), where it was homogenized (200 bar), sterilized (145°C for 4 min), cooled (30°C) and packaged in 200 mL boxes. The diagram of the production of the drinks is shown in Fig. 1.

Determination of chemical composition of milk drinks: All analysis of the chemical composition were performed in triplicate, according to the methods described by the Association of Official Analytical Chemists (AOAC, 1995). Moisture was determined by drying at 105°C to constant weight. Ash or minerals by burning in the oven at 550°C; the proteins were determined by micro-Kjeldahl method (AOAC, 1995) using a nitrogen conversion factor of 6.25. The lipids were determined by the method of Bligh and Dyer (1959). Carbohydrate was calculated by difference and the calorific value from the data of chemical composition, according to Resolution N° 360 of Ministry of Health. The conversion factors of 4 kcal g⁻¹ for carbohydrates and proteins and 9 kcal g⁻¹ for lipids were used.

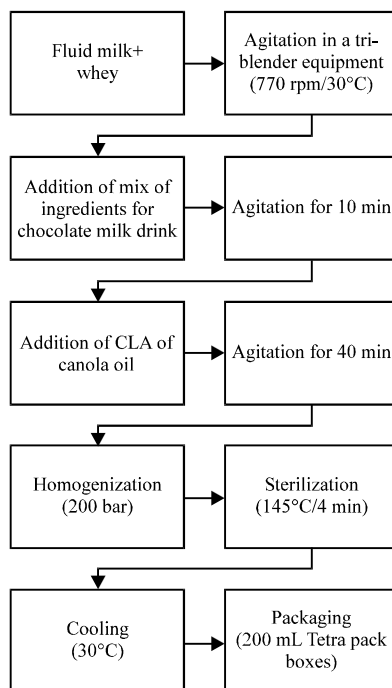


Fig. 1: Main steps of the scaling-up process for producing chocolate milk drinks added of CLA (CLABE) or canola oil (CANBE)

Evaluation of the milk drink stability:

Determination of pH: The pH value of the samples of chocolate milk drinks was determined by the method of AOAC (1995).

Evaluation of lipid oxidation: The lipid oxidation of milk drinks was followed by measuring the 2-thiobarbituric acid reactive substances (TBARS) according to the method described by Rosmini *et al.* (1996), with some modifications like the sample amount (0.04 g) and the reagent volumes (distilled water = 5 mL; trichloroacetic acid = 5 mL and 2-thiobarbituric acid = 2.5 mL). The samples were analyzed immediately after the end of their preparation (month zero) and after 1, 2, 3 and 4 months of storage at room temperature.

Sensory analysis of milk drinks: The affective test was carried out in a specific laboratory equipped with individual booths with white light. The panelists signed a term of agreement and answered a questionnaire about their consumption of milk drinks.

The overall acceptability of CLABE was evaluated in comparison with CANBE and with a commercial milk drink from CEMIL, using a group of 72 potential consumers of such drinks, untrained, college students, mostly women (92.33%) aging between 18 and 25 years old (80%), selected in function of their availability, interest and habit of taking milk drinks. A hedonic scale from 1 to 9 points was used, where point 1 corresponded to "dislike extremely" and point 9 to "like extremely".

In the same record, a scale for intention to purchase was included, containing 5 points, where 1 corresponded to "certainly would buy" and 5 "certainly would not buy". The samples were presented in a monadic sequential form, using a complete balanced block statistical design and were served at 10°C in a volume of 20 mL.

Statistical analysis: All results were evaluated by Analysis of Variance (ANOVA). The Duncan test at 5% probability was used to determine the difference between means (Pimentel-Gomes, 2000).

RESULTS AND DISCUSSION

Chemical composition of milk drinks: The CLA concentration in triacylglyceride form in the CLABE was of 0.8% (w/v). The quantities of the other components of milk drinks are shown in Table 1.

Table 1: Chemical composition of milk drinks

	Quantity (100 mL)		
	CLABE	CANBE	Commercial drink
Energy value (Kcal)	73.63±0.11 ^b	78.70±0.53 ^a	66.72±0.40 ^c
Carbohydrates (g)	11.34±0.02 ^b	12.32±0.34 ^a	11.37±0.20 ^b
Proteins (g)	1.78±0.02 ^b	2.24±0.25 ^a	1.26±0.11 ^c
Lipids (g)	2.35±0.03 ^a	2.27±0.12 ^a	1.80±0.09 ^c
Ash (%)	0.68±0.03 ^a	0.69±0.02 ^a	0.58±0.04 ^b
Moisture (%)	83.85±0.03 ^b	82.48±0.03 ^b	85.00±0.02 ^a

CLABE: Chocolate milk drink with added of conjugated linoleic acid, CANBE: Chocolate milk drink added of canola oil, Commercial drink: Chocolate milk drink manufactured by Cemil, Values (Mean±SD) are mean of three replicates, Values followed by the same letter do not differ at 5% probability in the same row

Initially, it is important to point out that all the three drinks may be classified as milk drinks, because they contain 30% of whey (maximum 49%) and meet the minimum protein content of dairy origin (1.2 g/100 g) established by the Brazilian legislation (Brasil, 1999).

Moreover, it can be observed that, in general, the results for the chemical composition of the three drinks are close. The significant differences found by comparing the values obtained for CLABE with those of CANBE, refer to higher levels of lipids and moisture, to smaller carbohydrate and protein contents as well as to lower energy value, respectively.

The difference in lipid content may probably be explained by the losses that happen during the production of drinks, especially when the ingredients are mixed in the tri-bender. At this moment, some sticking of oil to the wall of the equipment may occur, hindering the mixture of the ingredients and their incorporation into the final product. In the present work, this problem must have occurred to a greater extent for CANBE compared with CLABE. Considering this problem, the National Agency for Sanitary Surveillance of Brazil (ANVISA) allows for a 20% variation in the caloric value and nutrients informed on the label of industrialized foods. It is noteworthy that, for purposes of calculating the diets, it is assumed a 10% variation in the calorie and nutrient content (NRC, 2001).

The comparison of the chemical composition between the drinks produced here, CLABE and CANBE, with the commercial one shows higher levels of proteins, lipids, ashes and energy value, respectively. However, the values obtained for moisture of the first two drinks were lower than the commercial one. Also, the carbohydrate content of CANBE was higher than that of the commercial drink, whereas there was no significant difference between the results for the second CLABE and this latter drink.

The differences found in lipid content and energy value can be explained by the addition of lipids (CLA or canola oil) to the drinks produced here. Moreover, a milk containing 3% of lipids was used for preparing the commercial drink, while the preparation of CLABE and CANBE involved the use of 1.5% fat milk.

Despite the current trend for replacing fat ingredients of food by less energy input ones (Zambrano *et al.*, 2004), the addition of CLA in this milk drink could have some health benefits, including effects such as anticarcinogenic (Ip *et al.*, 2002; Ma *et al.*, 2002), antiatherogenic (Sher *et al.*, 2003), changes in body composition (Park *et al.*, 1997; Blankson *et al.*, 2000; Riserius *et al.*, 2001; Gaullier *et al.*, 2004), modulation of immune function (Cook *et al.*, 1993), among others.

No report was found in the literature concerning the determination of the chemical composition of unfermented milk drinks. Therefore, the results of the present work were compared with those of fermented dairy drinks. Thus, Almeida *et al.* (2001) evaluated the chemical characteristics of fermented dairy drinks prepared with milk added of 30, 40 and 50% of whey. The authors found that the higher the whey addition, the lower the amount of lipids in the drinks. No statistical difference was observed for the other nutrients. The levels of lipids and proteins were close to those of the drinks found in this work, ranging from 1.59 to 2.01% and 1.94 to 2.14%, respectively.

In another study, the chemical characteristics of fermented dairy drinks by probiotics and added of prebiotics were analyzed. The protein (2.18 to 1.96%) and ash (0.61 to 0.53%) contents were similar to those of the present work. However, the amount of lipids ranged from 0 to 0.1%, which differs from that one found here, due to the fact that skim milk was used in the formulation of the fermented drinks. Likewise, the carbohydrate content showed a higher value compared with the drinks developed here, reaching 16.27%, because their calculation was made by difference (Thamer and Penna, 2006).

Stability of milk drinks: It can be observed in Fig. 2 and 3 no significant difference between the values obtained for pH and lipid oxidation, respectively, during the 4 months of study, either for CLABE or for CANBE, demonstrating the chemical stability of the beverages.

In the case of pH, the values remained around 6.5 for both drinks, whereas with regard to the measurement of lipid oxidation, the levels of malondialdehyde (MDA) were in the range of 0.66 for CLABE and 0.71 for CANBE during the 4 months of study.

No report was found in the literature concerning the evaluation of the stability of unfermented milk drinks carried out by lipid oxidation, measurement of pH or other method of stability study. However, this assessment has been used in fermented dairy beverages due to their shorter shelf-life associated with the presence of alive microorganisms. Thus, Sivieri and Oliveira (2002) estimated the shelf-life of fermented dairy drinks formulated with fat replacers, using the measurement of the acidity and of the tyrosine content. The optimal storage time, estimated by these authors was of 28 days.

In another study, the stability of buttermilk, a type of fermented milk drink, was evaluated by microbiological analysis. The authors investigated the time needed for the probiotic bacteria to remain viable, according to Brazilian legislation, aiming a beneficial effect to consumer health.

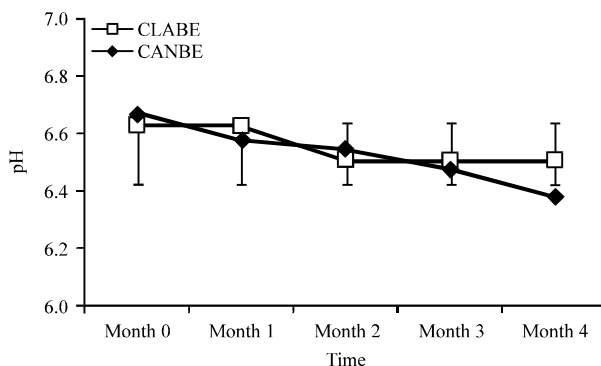


Fig. 2: Effect of storage time on pH values of milk drinks added of CLA (CLABE) or canola oil (CANBE), Values (Mean \pm SD) are mean of triplicates

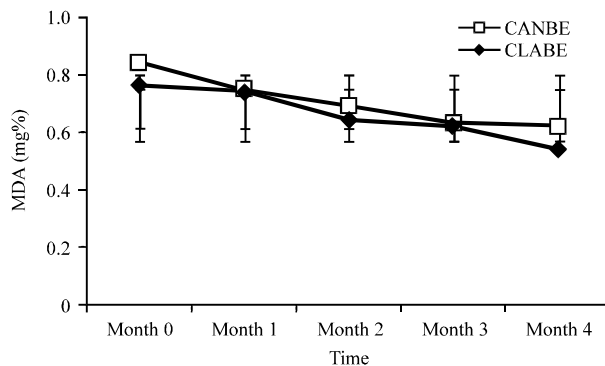


Fig. 3: Effect of storage time on TBARS values (thiobarbituric acid reactive substances) of milk drinks added of CLA (CLABE) or canola oil (CANBE), MDA: Malondialdehyde concentration (mg/100 g drink), Values (Mean \pm SD) are mean of triplicates

Moreover, with regard to parameters of hygiene, all samples met the requirements of the Agriculture Brazilian Ministry of Agriculture, Livestock and Supply (Antunes *et al.*, 2007) during the storage time, which was of 30 days.

The results found here were also compared with those obtained for milk, since it is an oil in water emulsion, similar to CLABE and CANBE. Thus, the oxidative stability of milk from cows fed with extruded soybean and organic selenium was evaluated by TBARS and the peroxide index (Paschoal *et al.*, 2007). According to these authors, the consumption of diets based on extruded soybeans increased the milk contents of polyunsaturated fatty acids and CLA and therefore its lipid oxidation, which differs from the result found in the present study. This difference could probably be explained by the fact that the milk used by dairy industries and consequently in the production of beverages developed here is probably originated from cattle that was fed with unmodified diet, which produces a milk fat with smaller content of polyunsaturated fatty acids, which is therefore more stable to lipid oxidation.

Sensory parameters of dairy drinks: The results of sensory evaluation of acceptance and intention to purchase for CLABE, CANBE and the commercial drink are shown in Table 2.

First, it can be observed that the milk drink added of CLA showed the best acceptance followed by CANBE and, finally, the commercial drink. Furthermore, with regard to intention to purchase, the data in Table 2 indicate no significant differences among the 3 drinks.

In this way, the set of these results reveals the superiority of milk drink added of CLA over the others, at the sensory point of view.

No study was found in the literature concerning the sensory evaluation of either fermented or unfermented milk drinks. Thus, the results of the present work were compared with those of milk, which is an oil in water emulsion like CLABE and CANBE. Thus, Ramaswamy *et al.* (2001) carried out a sensory evaluation of cow's milk with increased content of CLA by modifying the diet of cattle. The authors found a good acceptance, similar to the result found in the present study.

Despite CLABE and CANBE showed a better acceptance in relation to commercial milk drink, there was no significant difference among these 3 drinks for the intention to purchase. This result can be explained by the fact that these three drinks have shown similar sensory characteristics.

Figure 4 illustrates the frequency of acceptance levels expressed by the panelists, based on a hedonic scale ranging from dislike extremely and like extremely.

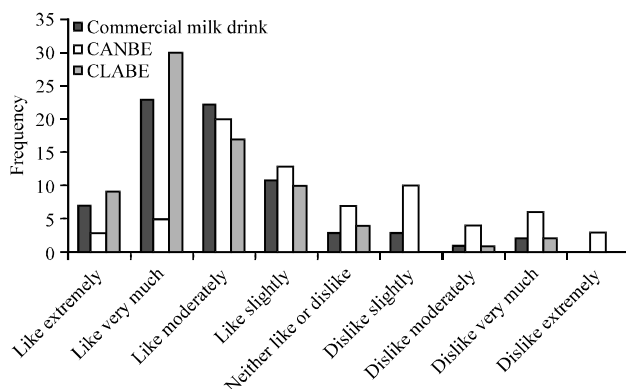


Fig. 4: Frequency of responses regarding the acceptance of chocolate milk drinks added of CLA (CLABE), canola oil (CANBE) and the commercial one

Table 2: Acceptance and intention to purchase of milk drinks added of CLA or canola oil

Samples	Acceptance	Intention to purchase
CLABE	7.22±1.46 ^a	2.12±0.97 ^a
CANBE	6.96±2.04 ^a	3.26±1.22 ^a
Commercial milk drink	5.48±1.52 ^b	2.36±1.05 ^a

CLABE: Chocolate milk drink added of CLA, CANBE: Chocolate milk drink added of canola oil, Values are Mean±SD, Concerning the Acceptance, the value of 1 corresponds to "dislike extremely" and 9 to "like extremely". In case of intention to purchase, the value of 1 is "certainly would buy" and 5 correspond to "certainly would not buy", Values with the same superscripts in the same column do not differ statistically ($p < 0.05$)

It can be noted in this Fig 4 that the drink added of CLA was the one that received the highest frequency of expression as like very much and like extremely, indicating that the addition of CLA may have favored the acceptance of the final product, in the case of drinks prepared in the present study.

CONCLUSION

The two chocolate milk drinks, added of CLA and of canola oil, produced in large scale in this work, showed differences in chemical composition, since CANBE contained higher caloric value and contents of carbohydrates and proteins. However, the lipid content was higher for CLABE and the quantities of moisture and ashes were close for both drinks. The stability of CLABE and CANBE was similar during the period of study and these two drinks showed better acceptance than the commercial one. However, the intention to purchase was similar for these three drinks.

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