



UNIVERSIDADE ESTADUAL DE MARINGÁ

CENTRO DE CIÊNCIAS AGRÁRIAS

Programa de Pós-Graduação em Ciência de Alimentos

**AVALIAÇÃO SENSORIAL, COMPOSIÇÃO PROXIMAL E DE ÁCIDOS
GRAXOS DO LEITE, SORVETE E QUEIJO MUSSARELA DE VACAS
MISTIÇAS SUPLEMENTADAS COM ÓLEO DE PALMA OU DE COCO**

SILVANA APARECIDA DA SILVA CORRADINI

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Tese apresentada ao programa de Pós-Graduação de
Ciência de Alimentos da Universidade Estadual de
Maringá, como parte dos requisitos para obtenção de
título de Doutor em Ciência de Alimentos

Maringá

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Orientador

Ivanor Nunes do Prado

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BIOGRAFIA

SILVANA APARECIDA DA SILVA CORRADINI nasceu em CURITIBA no estado do PARANÁ. Possui graduação em ENGENHARIA DE ALIMENTOS pela PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ e MESTRADO EM ENGENHARIA QUÍMICA pela UNIVERSIDADE ESTADUAL DE MARINGÁ. Tem experiência nas áreas de desenvolvimento de produtos novos, desenvolvimento de processo, controle de qualidade, supervisão de laboratório de análises físico-químicas e professora colaboradora atuando principalmente nos seguintes temas: processos industriais, operações unitárias e laboratório básico.

Dedico, especialmente, as minhas filhas Geovana, Sofia e meu esposo Rodolfo, que estiveram sempre ao meu lado, entendendo-me nos momentos de ausência, dando-me apoio e carinho.

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A todos, muito obrigada.

EPÍGRAFE

"Até hoje os cientistas discutem como a vida começou. Até hoje não se tem certeza de onde viemos. Os filósofos ainda querem entender quem somos. E existem umas duzentas teorias para onde vamos. Se a orientação sexual é definida pela genética. Por que você boceja quando alguém boceja. Os biólogos querem entender como os pássaros migram. E os nutricionistas se o ovo faz mal à saúde. Os economistas querem explicar a crise. E os cientistas como o cérebro funciona. Como você pode ver, não são as respostas que movem o mundo, são as perguntas.."

(Criação da F/NAZCA para o Canal Futura)

APRESENTAÇÃO

Esta Tese está apresentada na forma de dois artigos científicos:

- 1 Silvana Aparecida da Silva Corradini, Grasielle Scaramal Madrona, Nilson Evelázio de Souza, Elton Guntendorfer Bonafe, Camila Barbosa Carvalho, Ivanor Nunes do Prado. FATTY ACIDS MOZZARELLA CHEESE FROM CROSSBRED COWS WITH PALM OIL AND COCONUT FAT SUPPLEMENTATION, *Acta Scientiarum*.
- 2 Silvana A. S. Corradini, Grasielle S. Madrona, Jesuí V. Visentainer, Elton G. Bonafe, Camila, B. Carvalho, Péricles M. Roche, Rodolpho M. Prado, Ivanor N. Prado. PALM OIL AND COCONUT FAT SUPPLEMENTATION ON MILK PRODUCTION AND COMPOSITION FROM CROSSBRED COWS AND ICE-CREAM FATTY ACIDS COMPOSITION, *International Journal of Dairy Technology*.

GENERAL ABSTRACT

Milk is considered the most nutritionally complete beverage for human consumption. Children of all ages, the elderly, and the convalescent are groups which need considerable amount of milk in their diet. Milk exceptional nutrition value is a result of its constituents such as proteins, carbohydrates, fats, minerals, and water.

Nowadays, there is not only natural milk but also a diverse range of milk sub products which are manufactured and consumed in large scale worldwide. Among milk sub products, cheese and ice cream are the most widely consumed.

By definition, cheese can be either a fresh or an aged product resultant of milk coagulation with subsequent processing necessary to give each kind of cheese its characteristics and flavors. The coagulation process during cheese production may occur by means of physical action of rennet or other kinds of coagulants, with partial separation of whey.

Mozzarella cheese accounts for the highest amount of production in Brazil. Estimates indicate that mozzarella cheese production will exceed 200 million tons (FILHO, 2010). The overall augmentation of population income contributes to increasing cheese consumption. Cheese is no longer considered a product exclusively for elite consumption and can be included on a daily basis in the diet of everyone. In addition, cheese is widely utilized in fast food processing, given its properties such as slicing possibility and melting, which are essential to food attractiveness and flavor.

Ice cream belongs to the category of edible frozen food. Ice cream is obtained from an emulsion of fats and proteins, with or without addition of other ingredients and substances submitted to freezing, under conditions that guarantee the product conservation in its frozen or partially frozen state during storage, transportation, and delivery (ANVISA, 2005).

Breakthroughs in the dairy sector during the last few years are responsible for the diversity and quality of dairy food products available nowadays. That was a result of the kind of nourishment given to cattle, rich in fatty acids. These fats are essential for the functioning of the human body. Are essential nutrients, ie there is no endogenous production, as well must be present in the diet.

In this context, this work contributes to efficiency improvement in the milk production chain by means of finding nutritional strategies to increase the quality of the milk, the primary product, emphasizing mainly factors related to fat content, and later applying the primary product to make dairy foods (cheese, mozzarella, and ice cream).

The first phase of the project happened in Marques Farm, located in the city of Mirador-PR. Three treatments were applied with 23 healthy animals, in the same stage of lactation in December, 2010 i.e., a rainy season. The feeding treatments were: control group, palm oil, and coconut fat.

The first gathering of milk occurred 21 days after the diet change and the second after 36 days. Milk physicochemical analysis (humidity, protein, fat, ashes, lactose, total dry extract, and acidity) was performed utilizing the Ekomilk ultrasonic analyzer. Milk samples were taken to a laboratory called Clinica do Leite, located in Piracicaba-SP where bacteria counting (CBT) and somatic cells counting (SCC) were determined.

From the obtained milk, many samples of mozzarella cheese and ice cream were prepared and analyzed in the university laboratory. Several evaluations were performed in this laboratory, including determination of centesimal composition such as humidity and ashes (IAL, 2005), fat content (Bligh & Dyer, 1959), protein (AOAC, 1995), carbohydrates by difference, and pH.A physicochemical analysis was conducted after manufacturing of the dairy products and were performed in triplicate.

Samples of both cheese and ice cream utilized in the sensorial analysis were microbiologically tested to verify if thermo tolerant *Coliforms*, *Staphylococcus* positive coagulase, or *Salmonella* were present. Microbiological analyses were performed according to the methodology recommended by MAPA (2003).

Samples of ice cream and cheese were given in an alternated manner as a strategy to prevent taster fatigue, considering the high number of samples given to each taster. Fifty untrained tasters proved the two products separately. Sensorial analysis utilized hedonic scale of nine points for aroma, flavor, color, and texture. This process was approved by the UEM's ethics committee with number 703/2011 and CAAE: 0415.0.093.000-11. Tasters received the samples in containers randomly numbered and were instructed to clean their palate by drinking water in the period between tasting different samples.

Color was evaluated by means of the portable colorimeter Minolta® CR10, with integration sphere and vision angle of 3°, i.e., d/3 illumination and D65 illuminant. The system utilized was the CIEL a*b*.

Texture analysis was performed in a Stable Micro Systems Texture Analyzer TAXT2 (Texture Technologies Corp, England) utilizing a HDP/WBV probe when testing cheese and a P-36mm probe for ice cream testing.

Fatty acids chromatographic analysis for milk, ice cream, and cheese were performed in the Chemistry laboratory at UEM. The trace ultra 3300 chromatograph works with Thermo gas and is equipped with a flame ionization detector and a fused capillary column CP – 7420 (Select FAME, 100 m long, 0.25 mm inner diameter, and 0.25 µm of cuanopropyl).

Melting and yield capabilities were studied in determining cheese characteristics, while the ice cream was evaluated for overrun.

Data statistical analysis included variance analysis (ANOVA) and average calculation by Bonferroni with 5% significance level, evaluating the influence of feeding (three treatments: control, coconut, and palm) in the separated period.

Milk's physicochemical analysis showed no significant changes in humidity, protein, fat, ashes, lactose, total dry extract, and acidity ($P < 0.05$). Besides, they were found to be within the acceptable range according to values established by the code called Instruction Normative N°62 (2011). All samples that presented differences in results of SCC and CBT's analysis were immediately disposed. Therefore, they were not employed in the subsequent phases of this project.

Milk's fatty acid tests show a reduction of saturated fatty acids content during the two periods of milk gathering for both treatments (coconut and palm). In both gathering periods the presence of rumenic acid, also called linoleico conjugated acid (CLA, 18:2c9t11) was detected.

Cheese and ice cream physicochemical analyses revealed no significant difference ($P < 0.05$) including all tested parameters. Microbiological analyses proved that the three parameters examined were within limits established by legislation (RDC n12, ANVISA).

Regarding sensorial analyses, all samples evaluation were rated in a range from liked moderately to liked a lot, showing a great acceptance from the tasters (acceptance index $\geq 70\%$). Attributes such as aroma, color, and texture did not change significantly ($P < 0.05$). Products prepared with milk obtained from cows fed with coconut oil received better scores regarding flavor (7.56) in comparison with 6.78, obtained for products prepared with milk obtained from cows fed with palm oil.

Cheese sensorial analysis revealed no significant difference ($P < 0.05$) regarding aroma, flavor, and color. Texture was better ranked for the witness and coconut treatment when compared to palm oil, similarly the same findings obtained for flavor in ice cream.

Color for both cheese and ice cream were within acceptable range of values found in literature. Ice cream instrumental texture analysis and overrun showed no substantial difference for

the three samples in any of the periods evaluated. Cheese yield analysis revealed no significant change as well. However, cheese texture values were higher when derived from cows fed with coconut oil, with a 75.10 Kg in time 1 and 64.55 Kg in the time 2.

In gas chromatographic analysis of ice cream for all treatments fatty acids has been highlighted that the palmitic acid (16:0), vaccenic (18:1 n-7), stearic (18:0), myristic (14:0) and lauric (12 : 0) respectively. Trans fatty acids identified were elaidic acid (18:1-9t) and CLA (C18:2c9t11). Among saturated fatty acids found in cheese, a greater amount of palmitic acid (16:0), stearic acid (18:0), and myristic acid (14:0) were detected.

Results show that the kind of cow feeding has influence on the quality of milk and its sub products. Therefore, increasing the source and variety of fat in cow feeding is a feasible strategy towards obtaining milk and milk sub products with a better quality.

RESUMO GERAL

O leite é considerado o alimento mais completo que existe para o ser humano e o mais próximo da perfeição. Crianças de todas as idades, idosos e convalescentes compõem os grupos nos quais o leite deve fazer parte integrante da dieta. Seu excepcional valor nutritivo deve-se a seus componentes principais como proteínas, carboidratos, gorduras, sais minerais e água.

Atualmente o seguimento além do leite *in natura* conta ainda com uma ampla gama de derivados, produzidos e consumidos em grande escala mundial. Entre os derivados do leite, o queijo e o sorvete podem ser classificados como um dos principais produtos, tendo alta demanda para consumo.

Por definição, queijo é o produto fresco ou maturado que se obtém pela coagulação de leite, por ação do coalho ou outros coagulantes apropriados, com separação parcial do soro e submetidos aos processamentos necessários à formação das características próprias de cada tipo.

No Brasil, o queijo de maior produção nacional é o queijo mussarela, e segundo estimativas sua produção deve superar 200 mil toneladas (FILHO, 2010). Isso se deve principalmente ao aumento da renda da população, que deixa de considerar o queijo como produto nobre e passa a adotá-lo em seu consumo cotidiano e ao fato do mesmo ser amplamente utilizado no preparo de alimentos “fast food”, já que este queijo apresenta propriedades funcionais de fatiamento e derretimento, por exemplo, essenciais para o bom resultado dos pratos.

Sorvetes são alimentos enquadrados na categoria de gelados comestíveis. São produtos alimentícios obtidos a partir de uma emulsão de gorduras e proteínas, com ou sem adição de outros ingredientes e substâncias que tenham sido submetidos ao congelamento, em condições que garantam a conservação do produto no estado congelado ou parcialmente congelado durante a armazenagem, o transporte e a entrega ao consumo (ANVISA, 2005).

Os avanços tecnológicos verificados, no segmento de laticínios nos últimos anos, são responsáveis pela diversidade e qualidade dos produtos colocados à disposição dos consumidores. Isto pode ser obtido através da alimentação oferecida aos bovinos, rica em ácidos graxos, que podem modificar a composição do leite. Estes ácidos ajudam no desenvolvimento humano. Eles são gorduras essenciais para o funcionamento do organismo humano. São nutrientes essenciais, ou seja, não há produção endógena, assim devem estar presentes na dieta.

Neste sentido, este trabalho visa contribuir para melhoria na eficiência na cadeia produtiva do leite através da identificação de estratégias nutricionais que aumentem a qualidade do seu produto primário, o leite, principalmente aquelas ligadas ao teor e a composição da gordura e aplicando este produto primário em seus derivados (queijo mussarela e sorvete).

A primeira etapa do trabalho foi conduzida na Fazenda Marques, localizada em Mirador-PR. Foram aplicados três tratamentos com 23 animais saudáveis, no mesmo estágio de lactação no mês de dezembro/2010, ou seja, em período chuvoso, variando a alimentação: controle, óleo de palma e gordura de coco.

Realizou-se uma coleta com 21 dias após o início da alimentação e a segunda coleta com 36 dias. As análises físico-químicas do leite (umidade, proteína, gordura, cinzas, lactose, extrato seco total e acidez) foram realizadas pelo Analisador Ultrasônico Ekomilk. Amostras de leite foram encaminhadas para o laboratório da Clínica do Leite em Piracicaba/SP, onde foram determinadas contagem bacteriana (CBT) e contagem de células somáticas (CCS).

A partir do leite obtido foram produzidas amostras de queijo mussarela e sorvete que foram analisados no laboratório de Engenharia de Alimentos da UEM. Foram realizadas análises de composição centesimal como umidade e cinzas (IAL, 2005), Gordura (Bligh & Dyer, 1959), Proteína

(AOAC, 1995), Carboidratos por diferença e pH. As análises físico-químicas foram realizadas logo após a fabricação. Todas as determinações foram feitas em triplicata.

As amostras utilizadas na análise sensorial foram avaliadas microbiologicamente. Para as análises microbiológicas do queijo e do sorvete foi verificada a presença de *Coliformes termotolerantes*, *Staphylococcus* coagulase positiva e *Salmonella* segundo metodologia preconizada pelo MAPA (2003).

Considerando o grande número de amostras e para não causar fadiga ao provador, para a análise sensorial, escolheu-se aleatoriamente três amostras de queijo e três de sorvete dos dois períodos. Realizou-se análise sensorial com 50 provadores não treinados, dos dois produtos separadamente, uma seção para o queijo e outra para o sorvete. Na análise sensorial aplicou-se um teste de escala hedônica de nove pontos para os atributos aroma, sabor, cor e textura, com o parecer aprovado pelo comitê de ética da UEM com número: 703/2011 e CAAE: 0415.0.093.000-11. Os provadores receberam as amostras em copos identificados com números aleatórios e foram orientados a limpar o palato com água entre as amostras.

A cor foi avaliada com um colorímetro portátil Minolta® CR10, com esfera de integração e ângulo de visão de 3°, ou seja, iluminação d/3 e iluminante D65. O sistema utilizado foi o CIEL*a*b*.

A análise de textura instrumental foi realizada em um Texturômetro modelo Stable Micro Systems Texture Analyser TAXT2 (Texture Technologies Corp, Inglaterra) para o queijo utilizou-se a probe HDP/WBV e para o sorvete a Probe P-36 mm.

As análises cromatográficas dos ácidos graxos presentes no leite, sorvete e queijo mussarela, foram realizadas no laboratório de Química da UEM, onde utilizou-se um cromatógrafo a gás Thermo, modelo trace ultra 3300, equipado com um detector de ionização de chama e coluna capilar de sílica fundida CP - 7420 (Select FAME, 100 m de comprimento, 0,25 mm de diâmetro interno e 0,25 µm de cianopropil).

Para o queijo mussarela foi avaliada a capacidade de derretimento e avaliação do rendimento. Para o sorvete foi avaliado overrun.

A análise estatística dos dados foi realizada utilizando-se análise de variância (ANOVA) e cálculo de médias por Bonferroni ao nível de 5% de significância, avaliou-se a influência da alimentação (três tratamentos: testemunha, coco e palma) nos dois tempos separadamente, ou seja, com 21 dias e 36 dias.

Na análise físico-química do leite, quando da avaliação de umidade, proteína, gordura, cinzas, extrato seco total e acidez, observou-se que para estes componentes não houve diferença significativa ($P < 0,05$) e que se encontram de acordo com o estabelecido pela Instrução Normativa N° 62 (2011). Após resultado das análises de CCS e CBT, as amostras que apresentaram alterações foram descartadas, não sendo utilizadas nas próximas etapas do trabalho.

Na análise de ácidos graxos do leite, com relação aos ácidos graxos saturados houve uma redução nos dois períodos de coleta de leite para os dois tratamentos (coco e palma). Nos dois períodos de coleta foi observado a presença do ácido rumênico, como também é chamado o ácido linoléico conjugado (CLA, 18:2c9t11).

Para a análise físico-química do sorvete e do queijo, verificou-se que não houve diferença significativa ($P < 0,05$) para todos os parâmetros analisados. Na análise microbiológica dos dois produtos foi possível observar que para os três tratamentos todas as amostras encontram-se dentro do limite estabelecido pela legislação (RDC n° 12, ANVISA).

Avaliando os resultados da análise sensorial das amostras de sorvete, observou-se que as notas atribuídas pelos provadores se encontram na faixa de gostei moderadamente a gostei muito, indicando boa aceitação dos provadores (Índice de aceitação $\geq 70\%$). Não foi encontrada diferença significativa ($P < 0,05$) para os atributos aroma, cor e textura. Para o atributo sabor, observou-se que a

amostra preferida foi o sorvete com tratamento com óleo de coco (7,56) e o tratamento com palma obteve menor nota (6,78).

Na análise sensorial do queijo, para os atributos aroma, sabor e cor não encontrou-se diferença significativa ($P < 0,05$). Para o atributo textura as maiores notas foram para o tratamento testemunha e coco e a menor para o tratamento com óleo de palma, resultado este semelhante ao encontrado no atributo sabor para o sorvete.

A análise de cor, tanto do sorvete quanto do queijo apresentou valores condizentes aos encontrados por outros autores. As análises de textura instrumental e *overrum* do sorvete não apresentaram diferença significativa para as três amostras em nenhum dos tempos avaliados. Para o queijo a na análise de rendimento também não se encontrou diferença significativa. Porém, na textura observou-se que o tratamento com óleo de coco, obteve maiores valores nos dois tempos, 75,10 (Kgf) no tempo um e 64,55 (Kgf) no tempo dois.

Na análise cromatográfica de sorvete para todos os tratamentos os ácidos graxos que se destacaram foi o palmítico (16:0), vacênico (18:1n-7), esteárico (18:0), mirístico (14:0) e láurico (12:0) respectivamente. Os ácidos graxos *trans* identificados foram o ácido elaídico (18:1-9t) e o CLA, (C18:2c9t11). Para o queijo entre os ácidos graxos saturados, os majoritários foram o ácido palmítico (16:0), esteárico (18:0) e mirístico (14:0).

Os resultados indicaram que as diferentes alimentações podem afetar a qualidade do leite e seus derivados. Conclui-se, portanto, que é viável a adição de diferentes fontes de gordura na alimentação animal para obter leite e conseqüentemente derivados lácteos de boa qualidade.

ARTICLE 1

ÁCIDOS GRAXOS DE QUEIJO MUSSARELA DE VACAS MISTIÇAS COM A SUPLEMENTAÇÃO DE ÓLEO DE PALMA E GORDURA DE COCO**Ácidos graxos de queijo mussarela****FATTY ACIDS MOZZARELLA CHEESE FROM CROSSBRED COWS WITH PALM OIL AND COCONUT FAT SUPPLEMENTATION**

Silvana Aparecida da Silva Corradini¹, Grasielle Scaramal Madrona², Nilson Evelázio de Souza³, Elton Guntendorfer Bonafe³, Camila Barbosa Carvalho⁴, Ivanor Nunes do Prado⁵

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Resumo. O objetivo foi melhorar a qualidade nutricional do leite de vaca e usá-lo como matéria-prima para o queijo mussarela. Foram testados três tratamentos com 23 animais saudáveis: o controle, óleo de palma e de coco. A coleta foi realizada em 21 dias e 36 dias. Foram feitas análises de composição centesimal (umidade, cinzas, gordura, proteína, carboidratos), sensorial, cor (CIE L *, a *, b *) e textura para o queijo mussarela. Os ácidos graxos (AG) presentes no queijo mussarela foram determinados por cromatografia. Os ácidos graxos saturados (AGS) foram as mais abundantes em queijo. Os resultados indicam que é possível adicionar várias fontes de gordura na alimentação animal para obter leite e produtos lácteos de boa qualidade.

Palavras-chave: leite de vaca, ácidos graxos, análise sensorial.

1 **Summary.** The aim was to improve nutritional quality of cows' milk and use it as raw material for
2 mozzarella cheese. Three treatments were tested with 23 healthy animals ranging power: control,
3 palm oil and coconut oil. Collection was performed in 21 days and another after 36 days. Proximate
4 composition (moisture, ash, fat, protein, carbohydrates), sensory, color (CIE L*, a*, b*) and texture
5 were made for mozzarella cheese. The fatty acids (FA) present in mozzarella cheese were
6 determined by chromatography. Saturated fatty acids (SFA) were the most abundant in cheese. The
7 results point that it is feasible to add various fat sources in animal feed for milk and milk products
8 therefore of good quality.

9
10 **Keywords:** dairy milk, fatty acids, sensory analysis

11 12 **INTRODUCTION**

13
14 Milk production systems in countries such as Argentina, Australia, New Zealand and many other
15 countries in Europe are the basis to use pasture for lactating cows ([SCHROEDER ET AL., 2004](#)). The
16 authors in this review identified eighteen experiments with supplemental fat to the diet of grazing
17 dairy cows involving 25 comparisons and more than 480 multiparous cows. The type of fat usually
18 used in animal feed is oilseeds (sunflower, cottonseed, soybeans and canola) ([SCHROEDER ET AL.,](#)
19 [2004](#)). Coconut fat and palm oil were used in this study; such products have fatty acid (FA), a kind of
20 fat that is essential for human body to function. They are not produced by the organism and that is
21 why they must be present in food. Milk production is generally increased by adding fat into confined
22 feeding systems ([WU &HUBER, 1994](#)). Food also changes milk composition, thereby this study
23 intends to use this milk with different composition while preparing cheese.

24 Cheese is defined as a fresh or matured product obtained by draining the whey (the moisture or
25 serum of the original milk) after casein coagulation. Casein is coagulated by acid produced by
26 selected microorganisms, by coagulating enzymes, or by adding food-grade acidulates ([PLANZER JR](#)
27 [ET AL., 2008](#)). The great diversity in technological processes used in such manufacture results in
28 different physical, chemical, and microbiological cheese qualities, Mozzarella is a type of cheese.
29 Mozzarella is a semisoft/semi hard, plastic-curd cheese. Mozzarella was originally produced from
30 buffalo milk, but it is now made from cow's milk as well. It is a pasta filata cheese, which means that
31 it goes through cooker-stretcher steps while being processed (Ismail et al, 2011). Mozzarella cheese
32 is an essential ingredient of pizza ([TANWEER &GOYAL, 2011](#)).

1 The increase in consumption and production of cheese around the world is continually and
 2 directly proportional to food safety's aspects related to this product ([PLANZER JR ET AL., 2008](#)).
 3 Therefore, this study aims to conduct a diet supplementation with palm oil and coconut fat in 23
 4 animals and obtain milk in two different periods (21 and 36 days); to produce Mozzarella cheese
 5 from the collected milk as well as carrying out physical-chemical and sensory analyses for the
 6 products.

8 MATERIAL AND METHODS

10 Cow feeding and milk selection

12 The experiment was carried-out at the Marques Farm, located in Mirador city, Paraná State
 13 (Geographical location: Latitude: -23.2561, Longitude: -52.7745, 23° 15' 22" South, 52° 46' 28"
 14 West), in December (summer season). Three different diets were conducted according to the
 15 formulation application described in Table 1.

17 **Table 1.** Centesimal composition of experimental diets

Ingredients	Diets		
	CON ^a	PAL ^b	COC ^c
Soybean meal	30.0	30.0	30.0
Corn	57.0	57.0	57.0
Salt mineral	3.00	3.00	3.00
Caulin	10.0	-	-
Palm oil	-	10.0	-
Coconut fat	-	-	10.0

18 ^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat.

20 The treatments were applied to 23 healthy crossbred animals (half Holstein x zebu) by the 60th
 21 day of their third lactation. They were milked once a day and randomly distributed in three groups in
 22 which three isoenergetic treatments were evaluated, CON (Control – 8 animals), PAL (Palm – 8
 23 animals) and COC (coconut – 7 animals). In addition to the treatments offered to animals once a day
 24 they were also fed with pasture. The first collection was carried-out 21 days after the diet started and
 25 the second one 36 days after.

The collected milk was transported in brasses under refrigeration to the State University of Maringá, where the cheese samples were immediately processed.

Preparation of mozzarella cheese

Mozzarella cheese was manufactured separately for the three treatments. Such procedure was performed in the Milk Laboratory of Food Engineering Department, State University of Maringá all on the same day. The flowchart of cheese manufacturing can be seen in Figure 1 and the formulation in Table 2.

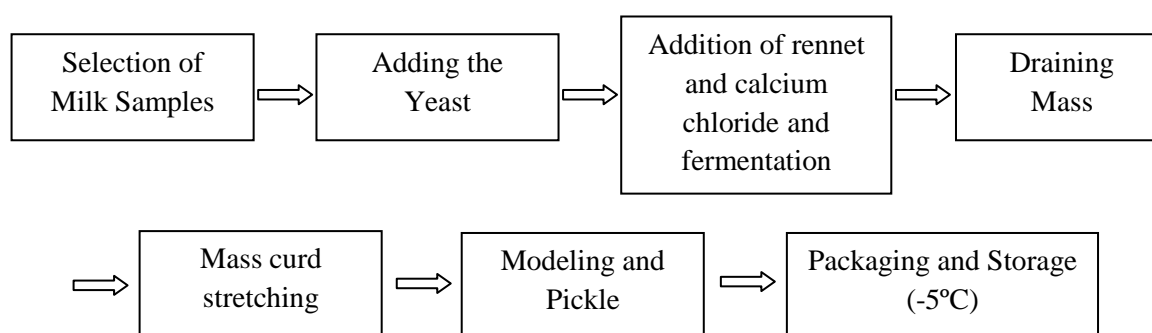


Figure 1. Flowchart of the production of mozzarella cheese.

Table 2. Formulation of mozzarella cheese

Ingredients	%
Milk	99.9
Ferment	0.06
Calcium chloride	0.05
Rennet	0.003

Mozzarella physical chemical analysis

The physical chemical analyses were carried out right after the production in the Food Engineering laboratory at State University of Maringa (UEM). The samples were homogenised and analysed in triplicate. Milk moisture and ash contents were determined according to [AOAC \(1998\)](#). The crude protein content was obtained through the Kjeldahl method ([AOAC, 1998](#)). The total lipids were extracted using the [Bligh and Dyer \(1959\)](#) method with a chloroform/methanol mixture.

1 **Mozzarella microbial analyses**

2

3 The samples were microbiologically evaluated in duplicate and randomly separated (between two
4 periods), at this stage were used for sensory analysis, that is, two samples from each treatment. The
5 presence of thermo tolerant *coliforms*, coagulate-positive *Staphylococcus* and *Salmonella* was
6 investigated according to [Vanderzant and Splittstoesser \(1992\)](#).

7

8 **Mozzarella colour evaluation**

9

10 Colour was evaluated through a portable Minolta ® CR10 colorimeter, with integrating sphere
11 and viewing angle of 3°, that is, D3 lighting and illuminant D65. The system used was CIEL*a*b*,
12 in which the measured coordinates were: L* = black (0) to white (100); a* = green (-) to red (+); b*
13 = blue (-) to yellow. Other parameters obtained were color saturation rate (C*) and tone angle (H*).
14 All determinations were made in duplicate.

15

16 **Melting capacity**

17

18 The melting capacity for Mozzarella cheese was determined by adapting the method of
19 Schreiber's to processed cheese, described by [Pizaia et al. \(2003\)](#). With the help of a 36 mm diameter
20 cylinder the sample was cut into slices of 7 mm thickness, the first one and last one were discarded.
21 Each slice was placed in the center of a petri dish, covered and left at room temperature for 30
22 minutes. Plates were previously labeled with four lines arranged at angles of 45°. The diameter of
23 each sample was calculated as the diameters average in four directions, measured before and after
24 melting for 7 minutes in an oven at 107° C. All analyzes were performed in triplicate. The average
25 diameter was calculated from the percentage melting of cheese slices according to the equation:

26

$$27 \quad \% \text{ Melt} = \frac{A_f - A_i}{A_i} \times 100$$

28

29 Where:

30 A_f : slice area after melting (calculated using the average diameter).

31 A_i : slice area before melting (calculated using the average diameter).

32

1 **Yield evaluation**

2

3 The crude yield of cheese obtained in the treatments was determined by the formula:

$$4 R (\%) = (P_q / P_f) \times 100$$

5 where:

6 R = crude yield,

7 P_q = finished cheese weight,

8 P_f = formulation weight (milk plus ingredients) according [Yunes and Benedet \(2000\)](#).

9

10 **Texture Analysis**

11

12 This analysis was carried out in a Stable Micro Systems Texture Analyser TAXT Plus
13 Texturometer (Texture Technologies Corp, England). The testing characteristics were: Accessory:
14 Probe HDP/WBV; Mode: strength measured in compression; Pre-test speed: 2,0 mm/s; Test speed:
15 3.0 mm/s; post-test speed: 7.0 mm/s; Distance: 10 mm.

16

17 **Sensorial analysis**

18

19 For sensorial analysis two cheeses from each treatment with the best physical chemical and
20 microbial results from milk were randomly selected at both production stages. The analysis was
21 carried out at the Sensorial Analysis Laboratory in the State University of Maringá (UEM), with an
22 approving position by the UEM Ethics Committee numbered 703/2011 e CAAE: 0415.0.093.000-11.

23 The acceptance test was applied using a nine-point hedonic scale, from 1-Highly disliked it to 9-
24 Highly liked it ([Moreira et al., 2010](#)). The formulations were evaluated considering the attributes of
25 color, smell, texture and taste using a team of nearly 50 randomly selected non-trained tasters both
26 male and female. Samples of nearly 20g were presented to the tasters in a balanced manner, in
27 disposable white plastic containers codified with three-digit random numbers. The tasters received a
28 glass of water to be consumed between samples.

29 Equation was adopted to calculate the acceptability rate for the product.

$$30 IA (\%) = A \times 100/B$$

31 Where:

32 A = average score for the product

1 B = highest score for the product

2

3 **Chromatographic analyses**

4

5 According to the method ([ISO-R-5509, 1978](#)) a triacylglycerols transesterification was carried
6 out in order to obtain methyl esters from fatty acids. The fatty acids esters were separated in a
7 Thermogas chromatograph, model trace ultra 3300, equipped with a flame-ionization detector and
8 fused-silica capillary column CP – 7420 Select FAME, 100 m will be of 1.2 mL/min, with 30
9 mL/min of N₂ (make up); and 35 and 300 mL/min, for H₂ and synthetic air and for flame detection.
10 The volume injected was nearly 2.0 µL, using a sample division of 1:80, with injector and detector
11 temperatures of 220 and 230° C respectively, and the column of 65° C during 4 minutes then increased
12 to 185°C at a rate of 4° C/min, kept for 0.75 minutes. The percentage was set by integrating peak
13 areas through Chronquest Software version 5.0.

14

15 **Statistical analysis**

16

17 The data statistical analysis was carried out by using variance analysis (ANOVA) and average
18 calculus by Bonferroni at 5% significance level through Statistics 7.0 software ([SAS, 2004](#)). The
19 treatments for each parameter were compared in two different periods.

20

21 **RESULTS AND DISCUSSION**

22

23 **Mozzarella Composition and Physical Chemical Parameters**

24

25 The Mozzarella cheese samples centesimal composition from different treatments is presented in
26 table 3. There was no meaningful difference ($P > 0.05$) for all parameters analyzed. The average
27 values of physico-chemical characteristics are within the average composition for mozzarella cheese
28 according to [Ferreira et al. \(2006\)](#). Therefore, the sources of fat can be added into animal feed
29 without changing mozzarella cheese standard quality.

30

31

32

1 **Table 3.** Results from mozzarella cheese physical chemical analyses of (%)

Item	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
Moisture	46.0 ± 2.97	45.9 ± 2.95	43.8 ± 1.78	46.2 ± 2.96	45.1 ± 3.61	43.8 ± 2.21
Protein	21.4 ± 0.87	21.0 ± 0.80	21.0 ± 0.95	21.3 ± 1.12	21.0 ± 1.25	21.2 ± 0.99
Fat	24.1 ± 0.85	24.0 ± 1.53	23.8 ± 1.89	25.0 ± 1.41	24.4 ± 1.34	24.2 ± 1.80
Carbohydrate	3.87 ± 0.51	4.55 ± 0.65	7.01 ± 0.71	2.14 ± 0.95	4.64 ± 0.86	5.93 ± 0.89
Ash	4.66 ± 0.28	4.52 ± 0.33	4.47 ± 0.30	5.36 ± 1.24	4.93 ± 1.24	4.84 ± 1.07
pH	5.24 ± 0.49	5.30 ± 0.32	5.37 ± 0.23	5.40 ± 0.34	5.42 ± 0.31	5.24 ± 0.36

2 ^aCON - Control diet; ^bPAL, - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from
3 analyses in triplicate. Means followed by different letters in the same line are different into each period by Bonferroni
4 test at a 5% probability.

6 **Mozzarella microbial analysis**

7
8 The results from mozzarella cheese microbial analysis are shown in Table 4. This results point
9 that cheeses are produced in good microbiological conditions, i.e., around < 1.0 x 10¹ CFU/g for
10 thermo tolerant coliforms, 1.0 x 10² CFU/g for coagulate-positive *Staphylococcus* and no
11 *Salmonella* 25g. Sanitation processes and milk pasteurization are fundamental to control pathogenic
12 bacteria and also result in a significant reduction of natural bacterial populations involved in cheese
13 production.

15 **Table 4.** Mozzarella cheese Microbial

Mozzarella Cheese	<i>Thermotolerant</i> coliforms	Cogulase-positive <i>Staphylococcus</i>	<i>Salmonella</i> 25g
	CFU/g	CFU/g	
CON ^a	< 1.0 x 10 ¹	< 1.0 x 10 ²	Out
PAL ^b	< 1.0 x 10 ¹	1.0 x 10 ²	Out
COC ^c	< 1.0 x 10 ¹	1.0 x 10 ²	Out
RDC n° 12 Anvisa	1 x 10 ³	1 x 10 ³	Out

16 ^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat.

18 **Mozzarella color analysis**

19
20 Table 5 relates the averages from values found for Luminosity (L*), for chrome a*, chrome b*,
21 saturation rate (C*) and tone angle (H*) for cheese samples in both periods. For statistical analysis, it

1 is noticeable that there was no significant difference between the three samples of cheese treatments
 2 for L *, chroma a * and H * in both manufacture periods for such parameters. We realize that for all
 3 cheeses that to reflect more light is to present a more positive value for the white tone (L * \pm 61),
 4 which may rank mozzarella cheese as a translucent food due to the ability to reflect most of the light.

6 **Table 5.** Results from mozzarella cheese colorimetric analysis

Item	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
L*	63.1 \pm 5.14	61.9 \pm 3.80	60.6 \pm 2.86	61.9 \pm 2.51	61.3 \pm 4.04	62.4 \pm 4.32
a*	1.93 \pm 0.52	2.58 \pm 0.58	1.98 \pm 0.80	2.20 \pm 0.59	1.77 \pm 0.44	1.69 \pm 0.24
b*	19.2 ^a \pm 1.49	21.4 ^{ab} \pm 2.60	22.8 ^b \pm 1.68	23.5 ^A \pm 1.45	24.7 ^{AB} \pm 1.26	22.6 ^{AC} \pm 1.72
C*	20.0 ^a \pm 1.36	21.6 ^{ab} \pm 2.59	22.9 ^b \pm 1.78	23.7 \pm 1.38	24.4 \pm 1.70	22.7 \pm 1.38
H*	94.0 \pm 1.65	95.1 \pm 8.87	94.5 \pm 8.39	94.2 \pm 3.12	91.5 \pm 2.93	93.0 \pm 2.61

7 ^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from
 8 analyses in triplicate. Means followed by different letters in the same line are different into each period by Bonferroni test
 9 at a 5% probability.

10

11 [MacDougall \(2002\)](#) attributes the processing time and exposure to light a tendency to browning or
 12 at least to the maturation degree of this sample, when relative to the other. The L * values tend to
 13 increase as higher temperatures are used.

14 For Chroma a * parameter all cheeses tended to negative green color. Regarding Chroma b *, with
 15 meaningful difference between cheese samples for the three treatments in both manufacturing
 16 periods, and for samples produced in 21 days there was difference between coconut and control
 17 treatment samples (P < 0.05) and samples produced in 36 days there was a significant difference
 18 between treatments and coconut palm (P < 0.05), tending to a positive staining yellow color. While
 19 mozzarella cheese at curd stretching step cheese is subjected to a temperature around 85 °C, which is
 20 the breakdown of sugars (lactose) with heating and swelling by the action of water, which may have
 21 triggered the Maillard reaction where there is a tendency to brown and caramelize the product.

22 [Matuska et al. \(2006\)](#) reported that a process using high temperatures (> 50 °C) results in color
 23 degradation in accordance with time. There is thus a positive and meaningful relationship between
 24 colors, types of heating, sugar content and darkening measurement, in the samples. For C *
 25 parameter there was a meaningful difference between cheese samples for three treatments in 21 days

occurring in coconut samples and control treatment ($P < 0.05$). For cheeses made in 36 days no meaningful difference between the three treatments samples was found. The rate of saturation (C^*) points that in curd stretching process where there is impregnation of water greater saturation pigments tend to brown and to have the highest average. The variability values in the cheese hue (H^*) increase because of thermal treatment (curd stretching).

Melting capacity for mozzarella cheese

Assessing the percentage for melting capacity for mozzarella cheese (Table 6) it was found to be irrelevant ($P > 0.05$). The longer the mozzarella settling time, the better it will melt as there will be greater proteolysis. However, the higher the pH, the higher the mass calcium content, to be submitted firmer and less prone to melting.

Table 6. Analysis of the melting capacity of the mozzarella cheese.

	Diets		
	CON ^a	PAL ^b	COC ^c
Ability to Melt (%)	18.7 ± 2.15	19.6 ± 2.02	18.5 ± 1.58

^aCON - Control diet; ^bPAL, - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in triplicate. Means followed by different letters in the same line are different into each period by Bonferroni test at a 5% probability.

Yield and texture

Table 7 relates the mean values found for mozzarella cheese yield and texture manufactured in both periods. The mozzarella cheeses showed a yield mean of 9.52%. The cheese yield is mainly influenced by milk composition, cheese composition and cutting losses. Other factors such as milk pasteurization, curd and kind of psychrotrophic count, can also affect cheese yield. Simultaneous losses of dry extract components in stretching water with particular emphasis on fat and protein incorporation occurs in this water mass, with more or less retention of these components in cheese, consequently affecting the manufacturing performance (VALLE ET AL., 1996). Mozzarella cheese made within 21 days with milk from cows fed with coconut oil had higher firmness values (75.10 kgf) when compared to other cheeses, showing that there was a meaningful difference compared to the other treatments ($P > 0.05$).

1 **Table 7.** Mozzarella cheese samples yield and texture.

Item	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
Yield, %	8.13 ± 1.86	8.05 ± 3.68	8.61 ± 1.66	10.29 ± 1.91	10.05 ± 1.66	8.79 ± 1.30
Texture, kgf	0.04 ^a ± 5.67	0.04 ^a ± 12.9	0.07 ^b ± 9.05	0.05 ^a ± 7.14	0.04 ^b ± 6.23	0.06 ^c ± 15.4

2 ^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Results in percentage as average standard
3 deviation of results from analyses in triplicate. Averages followed by different letters in the same line are meaningfully
4 different by the Bonferroni test, at a 5% probability level.

5

6 For the second period (36 days) there was a significant difference between palm and coconut
7 treatments, where coconut also presented the greatest firmness (0.065 kgf). [Valle et al. \(1996\)](#)
8 analyzed the different fat contents in functional analysis and verified that with fat content firmness,
9 elasticity and chewiness increase as fat content rises.

10

11 **Mozzarella cheese sensory analysis**

12

13 Table 8 presents the results for attributes evaluated in mozzarella cheese samples. It was observed
14 that all treatments presented scores from moderately liked it to really liked it, indicating good
15 acceptance from tasters (acceptance index ≥ 70%). Comparing instrumental texture and sensory
16 results it is noted that coconut fat treatment presented the highest instrumental and sensory means.

17

18 **Table 8.** Results from mozzarella cheese sensorial analysis.

Sensorial Characteristics	CON ^a	PAL ^b	COC ^c
Smell	6.46 ± 1.36	6.38 ± 1.35	6.74 ± 1.32
Taste	5.96 ± 2.13	6.44 ± 1.90	6.48 ± 1.73
Color	6.62 ± 1.59	6.44 ± 1.67	6.84 ± 1.67
Texture	6.76 ^a ± 1.89	5.74 ^b ± 2.16	6.08 ^{a,b} ± 1.84
Acceptance rate	70.0%	71.6%	72.0%

19 ^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Results in percentage as average standard
20 deviation of results from analyses in triplicate. Averages followed by different letters in the same line are meaningfully
21 different by the Bonferroni test, at a 5% probability level.

22

1 **Mozzarella Chromatographic Analysis**

2

3 In Table 9 links fatty acids (FA), where the majority was of palmitic acid (16:0), stearic (18:0)
4 and myristic (14:0). Palmitic acid is the predominant saturated fatty acid in all samples analyzed. As
5 in previous reports (SECKIN ET AL., 2005) saturated fatty acids were the most abundant in cheese.

6

7 **Table 9.** Result from mozzarella cheese chromatographic analysis (% relative area).

Fatty Acid	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
12:0	3.16 ^a ± 0.56	2.64 ^a ± 0.46	5.94 ^b ± 2.05	3.41 ^A ± 0.82	2.62 ^A ± 0.39	4.76 ^B ± 0.90
14:0	11.9 ^a ± 0.82	10.6 ^a ± 0.87	17.2 ^b ± 4.84	11.9 ^A ± 1.04	10.9 ^{AB} ± 0.63	13.2 ^{AC} ± 1.27
16:0	36.2 ± 3.93	34.6 ± 2.15	33.7 ± 1.51	35.4 ± 4.21	35.4 ± 4.33	33.5 ± 2.95
18:0	12.2 ± 2.07	12.6 ± 2.28	15.1 ± 2.95	12.5 ± 1.25	12.7 ± 2.79	12.8 ± 2.45
18:1n-9t	0.21 ^a ± 0.17	0.26 ^a ± 0.01	0.44 ^c ± 0.26	0.21 ± 0.16	0.18 ± 0.16	0.18 ± 0.20
18:1n-9c	2.91 ± 0.61	3.15 ± 1.78	2.91 ± 0.52	2.98 ± 0.71	3.28 ± 0.57	2.64 ± 0.32
18:1n-7	19.1 ^a ± 2.76	22.6 ^{ab} ± 2.77	24.1 ^b ± 4.88	19.8 ± 2.76	21.2 ± 4.20	19.9 ± 2.41
18:3n-3	1.00 ± 0.20	1.02 ± 0.13	1.01 ± 0.27	1.02 ± 0.17	1.14 ± 0.18	0.85 ± 0.28
18:2 c9t11	0.20 ± 0.02	0.20 ± 0.03	0.23 ± 0.04	0.19 ± 0.02	0.19 ± 0.03	0.18 ± 0.02
Others	13.2 ± 0.15	12.6 ± 0.12	12.1 ± 0.13	12.9 ± 0.17	12.4 ± 0.14	12.0 ± 0.15
AGS	70.3 ± 3.41	66.9 ± 2.73	63.94 ± 7.56	69.5 ± 3.02	68.0 ± 4.31	70.4 ± 3.13
AGMI	27.6 ± 3.20	30.8 ± 2.36	33.8 ± 7.04	28.4 ± 2.89	29.7 ± 4.46	27.7 ± 2.90
AGPI	2.10 ± 0.23	2.31 ± 0.46	2.29 ± 0.56	2.08 ± 0.26	2.25 ± 0.24	1.89 ± 0.28
ω - 6	0.89 ± 0.08	1.08 ± 0.50	1.05 ± 0.31	0.87 ± 0.13	0.91 ± 0.11	0.86 ± 0.10
ω - 3	1.00 ± 0.20	1.03 ± 0.13	1.01 ± 0.27	1.02 ± 0.17	1.14 ± 0.18	0.85 ± 0.28
ω - 9	5.84 ± 0.36	5.80 ± 0.80	6.34 ± 1.36	5.88 ± 0.45	6.04 ± 0.32	5.22 ± 0.47
ω-6/ω-3	0.92 ± 0.16	1.09 ± 0.56	1.06 ± 0.20	0.87 ± 0.13	0.81 ± 0.14	1.09 ± 0.37
AGS/AGPI	33.9 ± 5.44	29.8 ± 5.24	30.0 ± 10.7	33.9 ± 5.77	30.3 ± 2.41	38.1 ± 6.52

8 ^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in
9 triplicate. ^bResults in percentage as average standard deviation of results from analyses in triplicate. Averages followed by
10 different letters in the same line are meaningfully different by the Bonferroni test, at a 5% probability level.

11 ^d Fatty acid: 4:0; 6:0; 8:0; 10:0; 14:1n-11; 14:1n-9; 14:1n-7; 15:0; 15:1n-9; 16:1n-11; 16:1n-9; 16:1n-7; i17:0; 17:0; 17:1n-9;
12 18:2n-6t; 18:2n-6c.

13

14 Saturated fatty acids remained almost the same for both periods. Recommended fatty acids intake
15 such as omega-3 family essential to the body and also those bringing health benefits such as the

1 CLAs ([SIMIONATO ET AL., 2010](#)). For rumenic acid, as it is called conjugated linoleic acid (CLA,
2 18:2 c9t11), found in both periods, yet there was no meaningful difference ($P < 0.05$) between
3 samples. It is known that several factors affect the CLA amount in milk, such as season and
4 physiological factors ([SIMIONATO ET AL., 2010](#)).

5 A meaningful difference ($P < 0.05$) was found for the following fatty acids: 12:00, 14:00, 14:1 n-
6 11, 14:1 n-9, 14:1 n-7, 15:00, 15: 1n-9 16:1 n-7, 17: 00, 17:00, 18:1 n-9t and 18:1 n-7, the highest
7 levels being in general in coconut fat application into the diet.

8 Lauric acid is present in greater quantity in coconut oil (43% - 51%), and this increase was
9 observed in both treatment periods using coconut fat cheese. The same observation used for oleic
10 acid (18:1 n-9c), present in larger amount in palm oil (36% - 47%), where there was an increase for
11 both treatment periods using palm oil in cheese.

12

13 CONCLUSION

14

15 It was possible to produce mozzarella cheese with excellent sensory characteristics, presenting a
16 great acceptability in attributes.

17 Quantification of fatty acids present in mozzarella cheese showed homogeneity, since the cheese
18 manufacturing involves adding lactic ferments and precipitating agents as well as the use of
19 temperature.

20 We thus conclude that it is possible to add different fat sources while feeding animals for milk and
21 milk products of good quality.

22

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24

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27

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25 GROTTA, D. C. C.; LOPES, A.; FURLANI, C. E. A.; SILVA, R. P.; REIS, G. N.; CORTEZ, J. W.
26 Biodiesel etílico filtrado de óleo residual de soja: desempenho de um trator agrícola na operação de
27 gradagem. **Acta Scientiarum. Technology**, v. 30, n. 2, p. 135-138, 2008.

28 **Livros**

29 COWIE, J. M. G. **Polymers: chemistry and physics of modern materials**. Cheltenham: Nelson
30 Thornes: Chapman & Hall, 2001.

31 EL-REWINI, H.; ABD-EL-BARR, M. **Advanced computer architecture and parallel processing**.
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1 MELO JÚNIOR, P. A. (Ed.). **Fronteiras da Engenharia Química I**. Rio de Janeiro: E-papers,
2 2005. p. 21-50.

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ARTICLE 2

Palm oil and coconut fat supplementation on milk production and composition from crossbred cows and ice-cream fatty acids composition

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ABSTRACT

This work was carried out to study the nutritional milk quality of cows fed palm oil or coconut fat, and the use of that milk in ice-cream production. Three treatments were tested (CON, PAL and COC). Proximate composition and fatty acids composition were evaluated on milk and ice-cream, and sensorial analysis, color, overrun and texture were evaluated on the ice-cream. Sensory analysis of the ice cream acceptance rate was above 70%. Chromatographic analysis showed an increase of some fatty acids in milk and ice cream. The results indicate that it is feasible to add sources of fat on the animal feed for fatty acid composition modulation on milk and ice-cream.

Keywords: dairy products, CLA, fatty acids, iced-cold foods.

31 INTRODUCTION

32

33 During the past few years there have been some technological advances towards dairy products,
34 reflecting diversity and quality of what is nowadays consumed. This was attained through a fatty
35 acids modulation on diets offered to dairy cows. Some of these fatty acids are beneficial in human
36 health, which makes them important as daily dietary supplement.

37 A healthy fat composition of milk and derivatives are required to balance a diet, due to the
38 presence of essential fatty acid and vitamins, usually the fat-soluble ones ([Chen et al., 2004](#)). The
39 high percentage of short and long chain fatty acids is associated to low density lipoprotein synthesis
40 (LDL), responsible for cardiovascular diseases ([Funck et al., 2006](#)). However, there is the possibility
41 for low levels of monounsaturated (AGMI) and polyunsaturated (AGPI) fatty acids to be found in
42 milk. Conjugated linoleic acid, is an important polyunsaturated FA, which even in low
43 concentrations, improves health functions, such as reduction of coronary diseases incidence, increase
44 of high density lipoprotein (HDL), reduction of body fat, protection against cancer and anti-diabetic
45 and antioxidant effect ([Demeyer and Doreau, 1999](#)).

46 Some FA's are essential to human body normal function. They are not produced in the amounts
47 the organism need, so they must be in human diet. An example of such FA is the "monolaurin",
48 which has antiviral property and is found in coconut fat ([Machado et al., 2006](#)). Palm oil has large
49 industrial application and is one of the major natural sources of carotenoids ([Nozière et al., 2006](#)).

50 Ice-cream is a great energy source, of which the content is almost totally assimilated ([Marshall et](#)
51 [al., 2003](#)). Mainly constituted of carbohydrates and fat, ice cream also contains protein, minerals and
52 vitamins. A high standard ice cream requires a mixture of good quality raw material, meaning that
53 the selection of ingredients should take into account the dairy products availability and perish ability,

54 equipment availability, mixture beating effects, processing effects on taste, solid costs and others
55 ([Turnbow et al., 1947](#)).

56 An ideal ice-cream should correspond to the trust limits concerning quality criteria for taste, body,
57 texture, melting characteristics, color, packing, microbial content and composition ([Marshall et al.,
58 2003](#)).

59 The objective this study was to feed a supplemented diet with palm oil or coconut fat for 23 dairy
60 cows and obtain the milk in two periods (at 21 and 36 days); produce ice-cream from the collected
61 milk as well as carrying out physical-chemical and sensorial analyses for the products.

62

63 **MATERIAL AND METHODS**

64

65 *Cow feeding and milk collection*

66

67 The experiment was realized at Marques' Farm, at Mirador city, Paraná State, south Brazil
68 (Geographical location: Latitude: -23.2561, Longitude: -52.7745, 23° 15' 22" South, 52° 46' 28"
69 West), during December (summer season). Three different diets were fed to cows according to the
70 composition described in table 1.

71 Experimental diets were fed to 23 healthy crossbred dairy cows (Holsteins vs. zebu) by the 60th
72 day of their third lactation. Cows were milked daily and were randomly distributed in three groups of
73 three isoenergetic diets, control (CON – 8 cows), palm oil (PAL – 8 cows) and coconut fat (COC – 7
74 cows). Concentrate was offered to the cows once a day they after milking, and then cows remained in
75 pasture (*Brachiaria decumbes*). The first milk sampling was carried out at day 21 and the second, at
76 day 36 after the beginning of the experiment.

77 The physical chemical analyses (moisture, fat, protein, ashes, lactose, acidity and total dry
78 extract) of milk were conducted in an Ekomilk Ultrasonic Milk Analyzer (Ekomilk total). Milk
79 samples were analyzed at the Milk Clinic in Piracicaba-SP, where total bacteria count (TBC) was set
80 through bacteria counter with flow cytometry. Milk samples which were not according the
81 Regulation 62 ([MAPA, 2011](#)) were ruled out.

82 The collected milk was transported in brasses under refrigeration to the State University of
83 Parana (UEM), where the ice-cream was immediately processed.

84

85 *Ice-cream production*

86

87 The ice-cream was produced separately by treatment, by sampling day and by cow, which means,
88 23 ice-creams for the first collection (21 days) and 23 ice-creams for the second collection (36 days).
89 This procedure was carried out in the Food Engineering Milk and Derivatives Laboratory at State
90 University of Maringa (UEM). The ice-cream production is presented in table 2. After each process,
91 the equipment was washed and sanitized. After the processing samples were identified and stored at
92 a temperature of -18°C.

93

94 *Ice-cream physical chemical analysis*

95

96 Centesimal composition analyses of moisture, protein and ashes were made according [AOAC](#)
97 [\(1998\)](#), fat ([Bligh and Dyer, 1959](#)), and carbohydrates by difference. The physical chemical analyses
98 were carried out right after the production at the Food Engineering laboratory at State University of
99 Maringa (UEM). All determinations were made in duplicate.

100

101 *Ice-cream microbial analysis*

102

103 Samples were microbiologically evaluated in duplicate. Samples were randomly separated
104 (between two periods) and at this stage the same samples were used for sensorial analysis. The
105 presence of thermo tolerant *coliforms*, coagulase-positive *Staphylococcus* and *Salmonella* was
106 investigated according to the Regulation 62 ([MAPA, 2011](#)).

107

108 *Ice-cream color evaluation*

109

110 Color was evaluated through a portable colorimeter Minolta ® CR10, with integrating sphere and
111 viewing angle of 3°, D3 lighting and illuminant D65. The system used was CIE L*a*b*, where: L*,
112 representing luminosity in a scale from 0 (black) to 100 (white); a* representing a tone scale varying
113 from red (0+a) to green (0-a) and b* representing a scale from yellow (0+b) to blue (0-b). Color
114 saturation rate (C*) and tone angle (H*) were obtained according to [Sousa et al. \(2003\)](#). All
115 determinations were made in duplicate.

116

117 *Overrun*

118

119 The volumes for mixture samples and final aerated products were measured to set the overrun
120 calculated according to equation 1, as established by [Silva Junior and Lannes \(2011\)](#).

121
$$\% \text{ Overrun} = \frac{(V_{\text{initial}} - V_{\text{final}})}{V_{\text{initial}}} \times 100 \quad (\text{Equation 1})$$

122 Where:

124 V_{final} is the aerated product volume.

125 V_{initial} is the non-aerated mixture initial volume.

126 *Texture analysis*

127

128 Texture analysis was carried out in a Stable Micro Systems Texture Analyser TAXT Plus
129 Texturometer (Texture Technologies Corp, England). According to [Silva and Bolini \(2006\)](#), the
130 testing characteristics were: Accessory: Probe 36 mm; Mode: strength measured in compression;
131 Pre-test speed: 2.0 mm/s; Test speed: 3.0 mm/s; post-test speed: 7.0 mm/s; Distance: 10 mm;

132

133 *Sensorial analysis*

134

135 Two samples of ice-cream from each treatment with the best physical chemical and microbial
136 results from milk were randomly selected at both production stages. The analysis was carried out at
137 the Sensorial Analysis Laboratory at State University of Maringá (UEM), with approving position by
138 the UEM Ethics Committee (number: 703/2011 e CAAE: 0415.0.093.000-11).

139 The acceptance test was applied using a nine-point hedonic scale, from 1-Highly disliked it to 9-
140 Highly liked it. The formulations were evaluated considering the attributes of color, smell, texture
141 and taste using a team of 50 consumers of both genders randomly selected. Ice-cream samples of
142 nearly 20g were offered to the consumers in a balanced manner, on disposable white plastic
143 containers codified with three-digit random numbers. The consumers received a glass of water to be
144 consumed among samples ([Meilgaard et al., 1991](#)).

145 To calculate the acceptability rate for the product the equation 2 was adopted.

146 $AR (\%) = A \times 100/B$ (Equation 2)

147 Where:

148 A = average score for the product

149 B = highest score for the product

150 *Chromatographic analyses*

151

152 Triacylglycerols transesterification was carried out in order to obtain methyl esters of fatty acids,
153 according to the method [ISO \(1978\)](#). The esters of fatty acids were separated in a Thermogas
154 chromatograph, model trace ultra 3300, equipped with a flame-ionization detector and fused-silica
155 capillary column CP – 7420 (Select FAME, 100m will be of 1.2mL/min, with 30mL/min of N₂
156 (make up); and 35 and 300 mL/min, for H₂ and synthetic air and for detector flame. The volume
157 injected was 2.0 µL, using a sample division of 1:80, with injector and detector temperatures of 220
158 and 230° C, respectively, and the column of 65° C during 4 minutes and increased to 185° C at a rate
159 of 4° C/min, kept for 0.75 minutes. The percentage was set through the integration of peak areas by
160 Chronquest Software version 5.0.

161

162 *Statistical Analysis*

163

164 Statistical data analysis was carried out by using variance analysis (ANOVA) and average
165 calculus by Bonferroni at 5% significance level through Statistics 7.0 software. Each parameter was
166 compared for the two different periods ([Bertoldo et al., 2011](#)).

167

168 **RESULTS AND DISCUSSION**

169

170 *Characterization of milk*

171

172 Only lactose presented significant difference, all other milk components there was no significant
173 difference (Table 3) and they are in accordance with what established by Regulation 62 in Brazil

174 (MAPA, 2011), which requires minimums of 3% fat, 0.14 acidity – 0.18 (g lactic acid/100 ml), 8.4%
175 fat-free dry extract and 2.9% protein.

176 Lactose level in milk from cows fed with PAL was lower ($P < 0.05$) in the first collection period,
177 which did not occurred with the results from the second period. According to [Fonseca \(2000\)](#) the
178 composition of milk involves the effects of feeding, reproductive management and genetic heritage
179 with a possible occurrence of difference in the collected milk.

180 Table 4 presents the results from CBT and CCS of milk collected from 23 cows in two collection
181 periods. Most of *in natura* milk samples evaluated were in accordance to the current legislation both
182 for CBT and CCS (6.0×10^5 CS/ml). Milk samples that were found to be out of Regulation 62
183 ([MAPA, 2011](#)) were ruled out.

184 [Machado et al. \(2000\)](#) analyzed 920 milk samples from tanks in herds from São Paulo and Minas
185 Gerais, and found no difference for total solids related to scores of CCS varying from 12 to 3.2×10^5
186 CS/mL. The same study presented an increased fat level (3.58 to 4.15%) in milk from cows with
187 mastitis, as well as in the study carried out by [Shuster et al. \(1991\)](#) varying from 3.7 to 4.8%.

188

189 *Chromatographic analysis of in natura milk*

190

191 The FA composition of *in natura* milk samples are presented on table 5. The major fatty acids
192 were: palmitic (16:0), vaccenic (18:1n-7), stearic (18:0), myristic (14:0) e lauric (12:0) respectively.

193 There was a predominance of alpha-linolenic (LNA) acid (18:3n-3), in both periods for the palm
194 treatment, from n-3 series FA's. Trans fatty acids were found in the three treatments of both periods,
195 in which the predominant form was the elaidic acid (18:1n-9t).

196 Coconut fat has high (43 – 51%) lauric acid (12:0), which increased this FA in milk, in both
197 periods for cows fed COC. Palm oil has high (36 – 47%) oleic acid (18:1n-9c), which increased this

198 FA in milk, in both periods for cows fed PAL. Scientific researchers showed that the lauric acid has
199 the capacity to improve the immunological system, by unleashing a substance called interleukin,
200 which when activated, leads the bone marrow to produce more white cells. Furthermore, the lauric
201 acid acts as an anti-inflammatory through the inhibition of prostaglandins local synthesis (PGE2) and
202 interleukin 6, a pro-inflammatory substances present in rheumatic diseases, arthritis and muscle
203 inflammations ([Machado et al., 2006](#)).

204 There was a lower percentage of saturated fatty acids in both periods of milk collection for cows
205 fed PAL and CON. The saturated fatty acids were lower in the PAL and COC diets, thus, a lower
206 ingestion of vegetal fatty acid is an aspect that has been highlighted.

207 A omega-3 FA' daily ingestion is recommended ([Simionato et al., 2010](#)). The rumenic acid,
208 another name for conjugated linoleic acid (CLA, 18:2c9t11), was found in both collection periods.
209 Rumenic acid is formed in the rumen through the action of an isomerase which converts linoleic acid
210 connections into conjugated connections. However, its main milk source is from endogenous
211 mammary glands synthesis through vaccenic acid. Vaccenic acid (18:1n-7t) is an intermediate in the
212 ruminal biohydrogenation, being converted into CLA through the action of enzyme Δ -9 desaturase,
213 in mammary tissues and glands. It is known that several factors affect the quantity of CLA in milk,
214 such as the season of the year and physiological factors ([Simionato et al., 2010](#)).

215

216 *Ice-cream composition and physical chemical parameters*

217

218 The ice-cream centesimal composition from different treatments is presented in table 6. There was
219 no significant difference for the analyzed parameters, and the results obtained are according to
220 [Chiara et al. \(2003\)](#), in which the approximate composition should generally be 7.46% for fat,

221 63.96% for moisture, 24.80% for carbohydrate and 0.70% for ashes. The results are also according to
222 the ones described in Regulation 379 ([ANVISA, 2010](#)) (min 3% for fat and 2.5% for protein).

223 The data found for pH in ice-cream are according to [Shaviklo et al. \(2011\)](#), who evaluated the
224 chemical properties of ice-cream fortified with fish protein and had a pH of 6.7 for control
225 formulation (without adding protein).

226 [Pereira et al. \(2011\)](#) evaluated the influence of a partial replacement of milk by soymilk and found
227 0.94% for ash and 22.41% for carbohydrate, in ice cream, which is similar to those found in this
228 study. The protein content found by the authors was lower (3.99%) and fat higher (10.15%)
229 compared to the values found in this work.

230 Ice-cream is mainly constituted of carbohydrates and fat, and also contains protein, minerals and
231 vitamins. Ice-cream composition also varies according to location and market, considering not only
232 manufactures' opinions, but also the conditions under which they are made ([Marshall et al., 2003](#)).

233

234 *Ice-cream microbial analysis*

235

236 The results from ice-cream microbial analysis are shown in table 7. For the experimental diets all
237 samples were found to be according to the limits set by the Brazilian legislation ([ANVISA, 2010](#)).
238 Great portion of commercialized ice-cream are consumed by children, including those in frail age,
239 and so it is necessary to set special care when choosing raw material and processing ([Warke et al.,](#)
240 [2000](#)).

241

242 *Ice-cream color analysis*

243

244 In table 8 the averages from values found for luminosity (L^*), for chrome a^* , chrome b^* ,
245 saturation rate (C^*) and tone angle (H^*) for ice-cream samples in both periods are presented. During
246 the first period, L^* was higher for PAL. During the second period, L^* was lower for CON, which is
247 the capacity to reflect great part of the light.

248 The variance analysis for Chrome a^* parameter revealed that CON was higher in the first period.
249 During the second period, there was no significant difference for the ice-cream produced.

250 Regarding Chrome b^* , there was no significant difference in ice-cream samples for the three
251 treatments in both production periods, with a tendency to a positive yellow color.

252 [Sagdic et al. \(2011\)](#) evaluated probiotic ice-cream and found values for color analysis of nearly
253 60.03 for L^* ; 1.58 for a^* and 16.75 for b^* , which are similar to the ones found in this study. For C^*
254 and H^* parameters there was no significant difference in ice-cream samples for the three treatments
255 in both production periods, showing no variation for tonality in ice-cream samples.

256

257 *Ice-cream Overrun and Texture Analysis*

258

259 The overrun and texture values are presented in table 9. There was no significant difference in all
260 treatments regarding overrun. [Karaca et al. \(2009\)](#) evaluated fat reduction in various levels for ice-
261 cream and found overrun values among 10 and 58%. According to the authors, low fat quantity leads
262 to low overrun. According to [Goff \(2002\)](#), the air incorporation in ice-cream must be among 10 and
263 50%. Thus, this study presents overrun values in accordance to the ones in literature.

264 [Silva and Bolini \(2006\)](#) worked with ice-cream with different sorts of whey from bovine milk and
265 noted an overrun of 50% for samples with 100% milk, according to the proposed procedure.

266 No significant difference was found for texture parameter in samples evaluated. Texture was
267 measured as the peak of strength necessary to shear the sample. It indicates the product structural

268 rigidity, thus, the firmer the samples, greater the strength necessary to shear it ([Silva and Bolini,](#)
269 [2006](#)). The ice-cream with similar characteristics was the standard without milk replacement by
270 whey, with rigidity of 0.27 Kgf.

271

272 *Ice-cream sensorial analysis*

273

274 According to data shown in table 10, all treatments obtained scores among “slightly liked it” and
275 “highly liked it”, indicating good acceptance from tasters (acceptance rate $\geq 70\%$).

276 [Shaviklo et al. \(2011\)](#) fortified ice-cream with fish protein and found that concentration of 5 and
277 30 g/kg of protein did not influence sensorial attributes evaluated and also increased protein levels in
278 ice-creams.

279 [Silva and Bolini \(2006\)](#) evaluated the sensorial analysis of ice-cream produced with different
280 concentrations of whey. The products formulated with replacement of skimmed milk powder by 30%
281 dry acid whey product, or 100% de-mineralized whey, or 100% CPS-35 (35% whey protein
282 concentrate), had a sensorial acceptance as the formulated pattern with only skimmed milk powder.

283 Ice-cream should be considered not as a mere delicacy or summer pleasure, but a valuable and
284 nutritive dessert which contributes with highly relevant elements to a balanced diet both for children
285 and adults.

286

287 *Ice Cream chromatographic analysis*

288

289 The major FA composition was: palmitic (16:0), vaccenic (18:1n-7), stearic (18:0), myristic
290 (14:0) and lauric (12:0) respectively. Fatty acids identified were elaidic acid (18:1-9t) and CLA,
291 (C18:2c9t11). [Chiara et al. \(2003\)](#) analyzed fatty acids in ice-cream consumed in Brazilian found a

292 similar FA composition from the present work. The author states that fat from both animal and
293 vegetal origin are constituted by saturated and unsaturated fatty acids (mono and polyunsaturated).
294 Oils and fat are essential components to a human diet once they perform different roles in human
295 organism such as energetic reserve, phospholipids source, essential sterols and FA, and also assist the
296 transport and absorption of fat-soluble vitamins, and still makes food more tasteful.

297 The difference among trans fatty acids from lactic fat and the ones from hydrogenated fat is not
298 related only to quantity but also to the sort of predominant isomer in one or the other. Among trans
299 fatty acids resultant from biohydrogenation process, vaccenic acid is predominant, while elaidic
300 acids prevails in fat which suffers hydrogenation. Elaidic acid is considered to be the main
301 competitor of linoleic acid in human metabolism, especially when its ingestion is reduced ([Chiara et](#)
302 [al., 2003](#)).

303

304 **CONCLUSION**

305

306 It was possible to develop ice-creams with excellent sensorial characteristics presenting great
307 acceptability regarding evaluated attributes. The physical chemical and sensorial analyses of the
308 products proved that is possible to conduct animal feeding supplementing without causing alterations
309 in the final product (ice-cream). When analyzing FA's composition in milk and ice-cream, it is noted
310 that in the ice-cream, lauric acid (12:0), myristic acid (14:0) and elaidic acid (18:1n-9t) were higher
311 for COC treatment. Thus, feeding different fat sources to dairy cows in order to obtain milk and
312 consequently good quality dairy products is concluded to be positive.

313

314

315

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386

387

Table 1 Centesimal composition of experimental diets

Ingredients	Diets		
	CON ^a	PAL ^b	COC ^c
Soybean meal	30.0	30.0	30.0
Corn	57.0	57.0	57.0
Salt mineral	3.0	3.0	3.0
Caulin	10.0	-	-
Palm oil	-	10.0	-
Coconut fat	-	-	10.0

^aCON – Control diet; ^bPAL – Diet with palm oil; ^cCOC – Diet with coconut fat.

Table 2 Ice-cream production

Ingredients	Percentage
Base Power	1.48%
Milk	74.07%
Sugar	18.52%
Cream	4.44%
Liga-Neutra	0.74%
Emustab	0.74%

Table 3 Physical chemical analyses of milk (%)

Item	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
Moisture, %	85.3 ± 2.25	84.7 ± 2.96	86.0 ± 2.62	82.3 ± 2.83	81.8 ± 3.47	79.9 ± 4.37
Protein, %	3.35 ± 0.07	3.31 ± 0.14	3.34 ± 0.11	3.29 ± 0.10	3.31 ± 0.20	3.33 ± 0.10
Fat, %	4.00 ± 0.81	3.95 ± 1.21	3.18 ± 0.68	4.88 ± 1.20	4.96 ± 1.16	3.65 ± 0.71
Ash, %	0.73 ± 0.04	0.74 ± 0.04	0.77 ± 0.02	0.66 ± 0.04	0.66 ± 0.05	0.67 ± 0.05
Lactose, %	4.64 ^a ± 0.10	4.31 ^b ± 0.31	4.67 ^a ± 0.14	4.54 ^a ± 0.08	4.34 ^a ± 0.23	4.68 ^a ± 0.22
Dry extract, %	12.9 ± 0.68	12.7 ± 1.29	12.1 ± 0.64	13.5 ± 0.82	13.7 ± 1.50	12.5 ± 0.68
Dornic acidity	16.3 ± 1.46	15.1 ± 2.30	15.8 ± 1.35	17.1 ± 1.96	16.5 ± 1.85	15.9 ± 0.90

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in triplicate. Means followed by different letters in the same line are different into each period by Bonferroni test at a 5% probability.

Table 4 Microbial analyses of milk

Item	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
CBT (600×10^3 CS/ml)	16	12	48	6	7	8
CCS (600×10^3 CS/ml)	88	84	85	183	338	101

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in triplicate.

Table 5 Fatty acid of milk (% relative area)

Fatty acid	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
12:0	3.52 ^a ± 1.23	2.28 ^b ± 0.40	4.85 ^c ± 0.81	3.36 ^A ± 0.77	2.23 ^B ± 0.46	4.38 ^C ± 0.57
14:0	12.27 ^a ± 1.88	10.27 ^b ± 1.09	13.51 ^a ± 1.30	11.66 ^A ± 0.83	10.14 ^B ± 0.77	12.68 ^A ± 1.37
16:0	38.95 ^a ± 3.33	35.54 ^{ab} ± 2.67	33.73 ^b ± 1.95	35.25 ^A ± 3.87	34.59 ^A ± 2.21	33.63 ^A ± 2.90
18:0	13.54 ^a ± 3.34	13.30 ^a ± 2.99	13.13 ^a ± 3.03	13.28 ^A ± 0.99	13.35 ^A ± 2.94	13.19 ^A ± 2.94
18:1n-9t	0.52 ^a ± 0.24	0.78 ^b ± 0.14	0.51 ^a ± 0.11	0.44 ^A ± 0.07	0.71 ^B ± 0.10	0.49 ^A ± 0.08
18:1n-9c	2.62 ^a ± 0.26	2.88 ^{ab} ± 0.39	2.25 ^{ac} ± 0.39	2.84 ^A ± 0.59	3.04 ^{AB} ± 0.40	2.40 ^{AC} ± 0.25
18:1n-7	18.70 ^a ± 3.31	22.93 ^b ± 1.44	20.04 ^{ab} ± 2.00	20.31 ^A ± 2.74	23.75 ^B ± 1.26	21.17 ^{AB} ± 2.78
18:3n-3	0.85 ^a ± 0.17	1.07 ^{ab} ± 0.18	0.75 ^{ac} ± 0.18	0.99 ^A ± 0.20	1.03 ^A ± 0.43	0.86 ^A ± 0.30
18:2 c9t11	0.21 ± 0.04	0.21 ± 0.04	0.20 ± 0.04	0.20 ± 0.01	0.19 ± 0.05	0.19 ± 0.02
Others	11.3 ± 0.14	10.7 ± 0.16	11.0 ± 0.16	11.6 ± 0.15	11.0 ± 0.16	11.0 ± 0.13
AGS	73.7 ^a ± 7.98	66.4 ^b ± 1.89	70.6 ^{ab} ± 2.62	69.2 ^A ± 2.92	65.5 ^B ± 1.82	68.9 ^{AB} ± 3.33
AGMI	24.5 ^a ± 7.66	31.5 ^b ± 1.80	27.7 ^{ab} ± 2.51	28.9 ^A ± 2.89	32.5 ^B ± 1.49	29.1 ^{AB} ± 3.13
AGPI	1.77 ^a ± 0.37	2.11 ^b ± 0.16	1.74 ^a ± 0.30	1.88 ± 0.37	2.05 ± 0.56	1.98 ± 0.30
ω6	0.71 ± 0.27	0.82 ± 0.11	0.80 ± 0.24	0.69 ± 0.27	0.84 ± 0.11	0.93 ± 0.12
ω3	0.85 ^a ± 0.17	1.07 ^b ± 0.18	0.75 ^a ± 0.18	0.99 ± 0.20	1.03 ± 0.43	0.86 ± 0.30
ω9	5.75 ^a ± 0.42	6.24 ^a ± 0.32	5.06 ^b ± 0.39	5.93 ^A ± 0.59	6.37 ^B ± 0.36	5.34 ^A ± 0.50
ω6/ω3	0.84 ± 0.31	0.79 ± 0.18	1.11 ± 0.35	0.71 ± 0.30	1.24 ± 4.18	1.21 ± 0.53
AGPI/AGS	0.03 ± 0.15	0.03 ± 0.21	0.03 ± 0.20	0.03 ± 0.17	0.03 ± 0.12	0.03 ± 0.18

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in triplicate. Averages followed by different letters in the same line are meaningfully different by the Bonferroni test, at a 5% probability level.

^dFatty acid: 4:0; 6:0; 8:0; 10:0; 14:1n-11; 14:1n-9; 14:1n-7; 15:0; 15:1n-9; 16:1n-11; 16:1n-9; 16:1n-7; 17:0; 17:1n-9; 18:2n-6t; 18:2n-6c.

Table 6 Results from ice-cream physical chemical analyses

Item	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
Moisture (%)	63.7 ± 3.14	64.9 ± 2.44	64.5 ± 2.30	61.8 ± 7.3	64.2 ± 3.8	63.5 ± 4.9
Protein (%)	6.51 ± 0.50	7.37 ± 1.31	6.22 ± 0.76	6.83 ± 0.58	6.69 ± 0.99	6.61 ± 0.49
Fat (%)	6.70 ± 0.48	7.05 ± 0.56	6.82 ± 0.75	6.94 ± 0.63	6.82 ± 0.60	7.05 ± 0.75
Carbohydrate (%)	22.4 ± 0.86	19.9 ± 0.74	21.7 ± 0.78	23.9 ± 0.5	21.8 ± 0.6	22.2 ± 0.9
Ash (%)	0.71 ± 0.02	0.71 ± 0.02	0.71 ± 0.02	0.47 ± 0.03	0.48 ± 0.05	0.51 ± 0.07
pH	6.70 ± 0.33	6.62 ± 0.31	6.49 ± 0.27	6.66 ± 0.23	6.46 ± 0.24	6.51 ± 0.19

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in triplicate.

Table 7 Ice-cream microbial

Diets	Thermo tolerant <i>coliforms</i> UFC/g	Cogulase-positive <i>Staphylococcus</i> UFC/g	<i>Salmonella</i> 25g
CON ^a	3×10^1	$< 1.0 \times 10^2$	Out
PAL ^b	$< 1.0 \times 10^1$	$< 1.0 \times 10^2$	Out
COC ^c	$< 1.0 \times 10^1$	$< 1.0 \times 10^2$	Out
RDC nº 12 Anvisa	1×10^3	1×10^3	Out

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat.

Table 8 Results from ice-cream colorimetric analysis

Parameters	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
L*	57.7 ^b ± 1.27	64.5 ^a ± 2.72	55.1 ^b ± 3.09	58.9 ^B ± 3.28	62.7 ^A ± 2.28	66.2 ^A ± 2.09
a*	2.51 ^a ± 0.51	1.50 ^b ± 0.24	1.61 ^b ± 0.29	1.99 ± 0.54	2.00 ± 0.30	2.45 ± 0.99
b*	25.2 ± 2.29	25.3 ± 1.46	25.2 ± 1.59	23.7 ± 1.36	23.4 ± 1.86	25.45 ± 1.84
C*	25.2 ± 2.37	25.3 ± 1.46	25.4 ± 1.59	23.9 ± 1.36	23.4 ± 7.37	25.7 ± 1.73
H*	86.3 ± 2.68	87.2 ± 1.54	87.1 ± 1.72	86.6 ± 1.80	86.4 ± 1.64	85.0 ± 2.26

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in triplicate. Means followed by different letters in the same line are different into each period by Bonferroni test at a 5% probability.

Table 9 Ice-cream samples overrun and texture

Item	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
Overrun. %	48.9 ± 2.83	48.8 ± 2.53	50.3 ± 3.09	50.0 ± 2.23	49.1 ± 2.62	50.4 ± 2.46
Texture, Kgf	0.53 ± 241	0.47 ± 149	0.35 ± 96.9	0.44 ± 76.6	0.40 ± 109	0.49 ± 275

^aCON – Control diet; ^bPAL – Diet with palm oil; ^cCOC – Diet with coconut fat. Results in percentage as average standard deviation of results from analyses in triplicate. Averages followed by different letters in the same line are meaningfully different by the Bonferroni test, at a 5% probability level.

Table 10 Results from ice-cream sensorial analysis

Sensorial characteristics	COC	PAL	COC
Smell	6.72 ± 1.31	6.50 ± 1.52	6.74 ± 1.38
Taste	7.36 ^a ± 1.37	6.78 ^b ± 1.67	7.56 ^a ± 1.30
Color	7.58 ± 1.20	7.12 ± 1.38	7.56 ± 1.28
Texture	6.91 ± 1.77	6.59 ± 1.67	7.33 ± 1.30
Acceptance rate, %	81.78	75.33	84.00

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Results in percentage as average standard deviation of results from analyses in triplicate. Averages followed by different letters in the same line are meaningfully different by the Bonferroni test, at a 5% probability level.

Table 11 Result from ice-cream chromatographic analysis (% relative area)

Fatty acid	Period I			Period II		
	Diets			Diets		
	CON ^a	PAL ^b	COC ^c	CON ^a	PAL ^b	COC ^c
12:0	2.81 ^a ± 0.11	2.30 ^b ± 0.26	3.31 ^c ± 0.40	2.66 ^A ± 0.16	2.53 ^A ± 0.52	3.29 ^B ± 0.39
14:0	11.3 ^a ± 0.28	10.4 ^b ± 0.24	11.7 ^c ± 0.40	11.0 ± 0.44	10.8 ± 0.76	11.5 ± 0.76
16:0	34.4 ± 2.42	35.3 ± 1.68	34.6 ± 2.31	34.4 ± 2.04	34.6 ± 1.22	33.3 ± 1.53
18:0	13.9 ± 1.54	13.8 ± 1.34	13.9 ± 1.38	14.1 ± 1.18	13.7 ± 1.07	14.0 ± 0.84
18:1n-9t	0.43 ^a ± 0.06	0.19 ^b ± 0.32	0.51 ^a ± 0.08	0.41 ^A ± 0.18	0.18 ^{AB} ± 0.22	0.51 ^{AC} ± 0.07
18:1n-9c	20.8 ^a ± 1.30	22.6 ^b ± 0.79	21.0 ^{ab} ± 1.72	21.7 ± 1.39	22.6 ± 1.25	22.3 ± 1.20
18:1n-7	2.90 ^a ± 0.19	3.47 ^b ± 0.18	2.64 ^c ± 0.17	3.01 ^A ± 0.38	3.37 ^{AB} ± 0.43	2.73 ^{AC} ± 0.16
18:3n-3	1.00 ^a ± 0.08	1.06 ^{ab} ± 0.08	0.92 ^{ac} ± 0.09	1.06 ± 0.12	1.07 ± 0.07	0.96 ± 0.10
18:2 c9t11	0.22 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.21 ^A ± 0.01	0.21 ^A ± 0.02	0.21 ^A ± 0.01
Others	11.8 ± 0.09	10.3 ± 0.12	10.8 ± 0.08	11.4 ± 0.13	10.9 ± 0.10	11.2 ± 0.07
AGS	67.9 ^a ± 1.44	66.3 ^{ab} ± 0.9	68.41 ^{ac} ± 1.93	67.5 ± 1.83	66.6 ± 1.36	67.4 ± 1.35
AGMI	29.4 ± 1.39	31.1 ± 0.94	29.1 ± 1.88	30.2 ± 1.64	31.1 ± 1.28	30.5 ± 1.28
AGPI	2.26 ^a ± 0.08	2.24 ^a ± 0.13	2.08 ^b ± 0.14	2.27 ± 0.20	2.26 ± 0.15	2.19 ± 0.11
ω6	1.04 ^a ± 0.04	0.96 ^a ± 0.13	0.95 ^a ± 0.16	0.99 ^A ± 0.15	0.98 ^A ± 0.13	1.02 ^A ± 0.13
ω3	1.00 ^a ± 0.08	1.06 ^{ab} ± 0.08	0.92 ^{ac} ± 0.09	1.06 ^A ± 0.12	1.07 ^A ± 0.07	0.95 ^A ± 0.10
ω9	5.95 ^a ± 0.13	6.17 ^b ± 0.16	5.61 ^c ± 0.10	6.08 ^A ± 0.25	6.10 ^A ± 0.17	5.72 ^B ± 0.23
ω-6/ω-3	1.04 ^a ± 0.09	0.91 ^a ± 0.17	1.05 ^a ± 0.22	0.95 ^A ± 0.16	0.92 ^A ± 0.13	1.08 ^A ± 0.20
AGPI/AGS	0.03 ^a ± 0.13	0.03 ^a ± 0.19	0.03 ^a ± 0.17	0.03 ^A ± 0.14	0.03 ^A ± 0.17	0.03 ^A ± 0.15

^aCON - Control diet; ^bPAL - Diet with palm oil; ^cCOC - Diet with coconut fat. Means and standard deviation from analyses in triplicate. Results in percentage as average standard deviation of results from analyses in triplicate. Averages followed by different letters in the same line are meaningfully different by the Bonferroni test, at a 5% probability level.

^dFatty acid: 4:0; 6:0; 8:0; 10:0; 14:1n-11; 14:1n-9; 14:1n-7; 15:0; 15:1n-9; 16:1n-11; 16:1n-9; 16:1n-7; i17:0; 17:0; 17:1n-9; 18:2n-6t; 18:2n-6c.

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