



Factors affecting buffalo Mozzarella cheese yield: a study using regression analysis

Danielle Cavalcanti SALES^{1*} , Stela Antas URBANO¹ , Dorgival Morais de LIMA JÚNIOR² ,
José Geraldo Bezerra GALVÃO JÚNIOR³ , André F. BRITO⁴ , Claudio CIPOLAT-GOTET⁵ ,
Luís Henrique Fernandes BORBA¹ , Adriano Henrique do Nascimento RANGEL¹ 

Abstract

Cheese production such as mozzarella is affected by several factors including the processing technology (e.g., cuts, fermentation, curd stretching), as well as quantity and quality of raw materials and other ingredients. The aim of this study was to investigate the relationship between mozzarella cheese yield and buffalo milk composition, processing factors, and losses of whey constituents using polynomial regression and adjusted R². All regression coefficients associated with each explanatory variable were significantly different from zero. The models explained an average of 99% of the variation in the dataset. The regression models showed that the concentration of milk components (e.g., fat, protein, casein, lactose, total solids), somatic cell score, age and acidity of the starter culture, time between curd cuts, and percentage of lost whey constituents highly influenced the mozzarella cheese yield. The buffalo dairy industry should focus on rigorous control of milk quality and processing factors to standardize and optimize mozzarella cheese yield efficiency.

Keywords: cheese manufacture; dairy industry; efficiency; losses of constituents; milk.

Practical Application: Know the factors influencing mozzarella cheese yield to better production efficiency.

1 Introduction

Factors influencing dairy product yield are generally associated with animal and milk quality (e.g., genetics, diet, physiological state, sanitary conditions) or handling of raw materials (e.g., hygiene conditions, manufacturing techniques) (Abd El-Gawad & Ahmed, 2011; Cipolat-Gotet et al., 2015; Cecchinato & Bittante, 2016; Sabia et al., 2020).

Industrial yield and the quality of products made from buffalo milk are better than those from cow milk due to the greater concentration of solids in milk produced by buffaloes (Rangel et al., 2011; Tonhati et al., 2011; Cecchinato & Bittante, 2016; Ahmad & Saleem, 2020). In the Italian industry, about 20 to 22 kg of mozzarella can be obtained from processing 100 L of buffalo milk, yielding almost 50% more than what has been reported for cow milk (Citro, 2010).

Cheese production such as mozzarella is affected by several factors including the processing technology (e.g., cuts, fermentation, curd stretching), as well as quantity and quality of raw materials and other ingredients [e.g., starter culture (SC), rennet] (Citro, 2010; Hooda et al., 2020). Understanding the factors that influence cheese yield allows dairy industry to have better control over their production efficiency during manufacturing. Sales et al. (2017) evaluated through Pearson linear correlation and multivariate analysis the relationship between buffalo mozzarella cheese yield (MCY) and milk composition, processing

factors, and constituent recovery. Results from Sales et al. (2017) indicated that most variables evaluated could influence MCY. Thus, the objective of the current study was to investigate the relationship between mozzarella cheese yield and buffalo milk composition, processing, and recovery of whey constituents using regression analysis.

2 Materials and methods

2.1 Data collection

Data were obtained from 30 batches of mozzarella cheese processed by a single dairy plant located in the semi-arid region in northeastern Brazil. Milk used for cheese making was produced in the herd owned by the milk processing company consisting of 350 lactating buffaloes milked twice a day (morning and afternoon) with an automated milking system. The milking equipment was a double-20, single line type, with a low line in closed circuit.

2.2 Cheese manufacture

Mozzarella cheese was manufactured according to the enzymatic milk coagulation method with the goal of obtaining cheeses with similar characteristics to those produced in Italy, following the recommendations of Citro (2010). The steps used

Received 26 May, 2020

Accepted 29 July, 2020

¹Unidade Acadêmica Especializada em Ciências Agrárias, Universidade Federal do Rio Grande do Norte – UFRN, Macaíba, RN, Brasil

²Universidade Federal de Alagoas – UFAL, Campus Arapiraca, Arapiraca, AL, Brasil

³Instituto Federal do Rio Grande do Norte – IFRN, Campus Ipanguaçu, Ipanguaçu, RN, Brasil

⁴Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, United States

⁵Department of Veterinary Science, University of Parma, Parma, Italy

*Corresponding author: daniellecsales@hotmail.com

for mozzarella cheese manufacturing consisted of the following: pasteurization at 72 °C for 15 seconds; adding starter culture (natural yeast), calcium chloride, and chymosin rennet powder (coagulant power of 1:50,000); cutting the curd - the first curd cut was a cross-cut (with ruler) performed 10 min after adding rennet. The pH and acidity of the whey were monitored every 15 min; the second curd cut was performed into transversal lines (with lire) only when the whey reached acidity between 16 and 20 °D; curd separation - the curd was transferred from the tank onto a stainless steel table when the cheese curd was close to the stretching point (about 2 hours after the second cut or when the pH was close to 5.0); stretching - the curd was triturated into portions, immersed in water (1:1, 90 °C) and manually stirred in a circular motion until it reached the desirable softness and elasticity. Immediately after stretching, the cheese portions were placed into an Italian Cheese Ball Forming Machine, then molded into large or small balls and immediately cooled in cold water. The cheese was maintained with a 1% saline solution.

2.3 Samples collection

Samples of raw milk were collected upon arrival in the dairy plant. Pasteurized milk and mozzarella cheese whey samples were taken directly from the cheese vat respectively at the beginning and at the end of processing, identified, stored in 40-mL plastic bottles, and kept at a temperature between 4 and 7 °C until analysis.

2.4 Milk and whey analysis

Analyses were conducted at the Milk Quality Laboratory of the Universidade Federal do Rio Grande do Norte (LABOLEITE; Macaiba, RN, Brazil). Milk and whey samples were analyzed in duplicate by infrared absorption (Dairy Spec[®]; Bentley Instruments Inc., Chasca MN, USA) to determine the fat, protein, casein, lactose, and TS concentrations (%). Somatic cell count was determined using Somaticell[®] (Idexx Laboratories Inc., Westbrook, MA) according to the manufacturer's protocol. Values obtained for SCC were transformed into SCS using the procedure: $SCS = \log_2 (SCC \div 100,000) + 3$ (Shook, 1982). Starter culture acidity (°Dornic) was analyzed at the beginning of the processing using NaOH titration with phenolphthalein as indicator (Association of Official Analytical Chemists, 1998).

2.5 Variables

Sixteen (16) independent variables expected to affect MCY (dependent variable) were selected as follows - Time between curd cut (min), SC age (h), milk concentrations of fat, total protein (TP), casein, lactose, SNF, and TS, and proportions of whey recovery of fat, casein, lactose, and TS, as well as both milk SCS and SC acidity (°Dornic). Two additional variables of milk casein:fat ratio and milk casein:TP ratio were selected, both of which are closely associated with mozzarella cheese composition.

Mozzarella cheese yield (MCY, %) was calculated by the ratio between milk (kg) to cheese yield (kg), which represents the milk volume used to obtain one kg of cheese ($MCY = VM/VC$, where VM = volume of milk used in kilograms; and VC = volume of cheese produced in kilograms) (Rossi et al., 1998).

The losses of milk constituents in the whey was obtained by equation: $Loss = \% \text{ component in whey} / \% \text{ component in milk}$, where component = fat, total protein, casein, lactose, TS, or SNF (Furtado & Pombo, 1979).

2.6 Statistical analysis

The relationship between MCY (dependent variable) and milk constituents, processing variables, and recovery of whey constituents was assessed by polynomial regression analysis forced through the origin using 2 criteria for model selection: (1) statistical significance at 5% probability level and (2) adjusted R². Statistical analysis was performed using the PROC REG procedure (ver. 9.0, SAS Institute Inc., 2002).

3 Results and discussion

The descriptive statistics are shown in Table 1. All regression coefficient slopes associated with each explanatory variable were significantly different from zero, except for the milk casein:TP ratio (Figure 1).

Adjusted R² showed that the models explained an average of 99% of the variation in the dataset, thereby confirming our hypothesis that MCY is influenced by the studied selected variables (Figure 1).

For instance, formation, fermentation and curd hardness and syneresis properties are highly dependent on milk composition (Cecchinato & Bittante, 2016; Sales et al., 2016). A significant part of the milk fat usually participates in constituting mozzarella mass. In addition, casein accounts for almost 80% of the total

Table 1. Mean values, standard deviation (SD), minimum (Min) and maximum values (Max) of study variables (n = 30).

Variable	Min	Mean ± SD	Max
Fat, %	5.73	6.11 ± 0.17	6.40
Total Protein, %	3.54	3.81 ± 0.15	4.16
Casein, %	2.76	2.98 ± 0.12	3.25
Casein:Fat Ratio	0.45	0.49 ± 0.02	0.52
Casein:Total Protein Ratio	0.78	0.78 ± 0.00	0.78
Lactose, %	4.90	5.12 ± 0.07	5.24
Total Solids, %	15.61	16.27 ± 0.30	16.81
Solids-Not-Fat, %	9.79	10.16 ± 0.19	10.51
Somatic Cell Count, x10 ³ cells.mL ⁻¹	224.00	405.86 ± 90.66	530.00
Somatic Cells Score, Log cells.mL ⁻¹	0.90	0.99 ± 0.06	1.05
Starter Culture Acidity, °D	58.00	79.07 ± 14.03	115.00
Starter Culture Age, h	12.00	34.96 ± 14.84	84.00
Time Curd Cut, min	30.00	48.39 ± 9.81	66.00
Loss of Fat, %	3.17	5.99 ± 1.88	10.61
Loss of Casein, %	21.59	33.25 ± 2.84	37.01
Loss of Lactose, %	83.56	94.88 ± 4.17	99.22
Loss of Total Solids, %	37.90	43.97 ± 1.99	46.81
Mozzarella Cheese Yield, kg.kg ⁻¹	4.27	4.87 ± 0.33	5.52

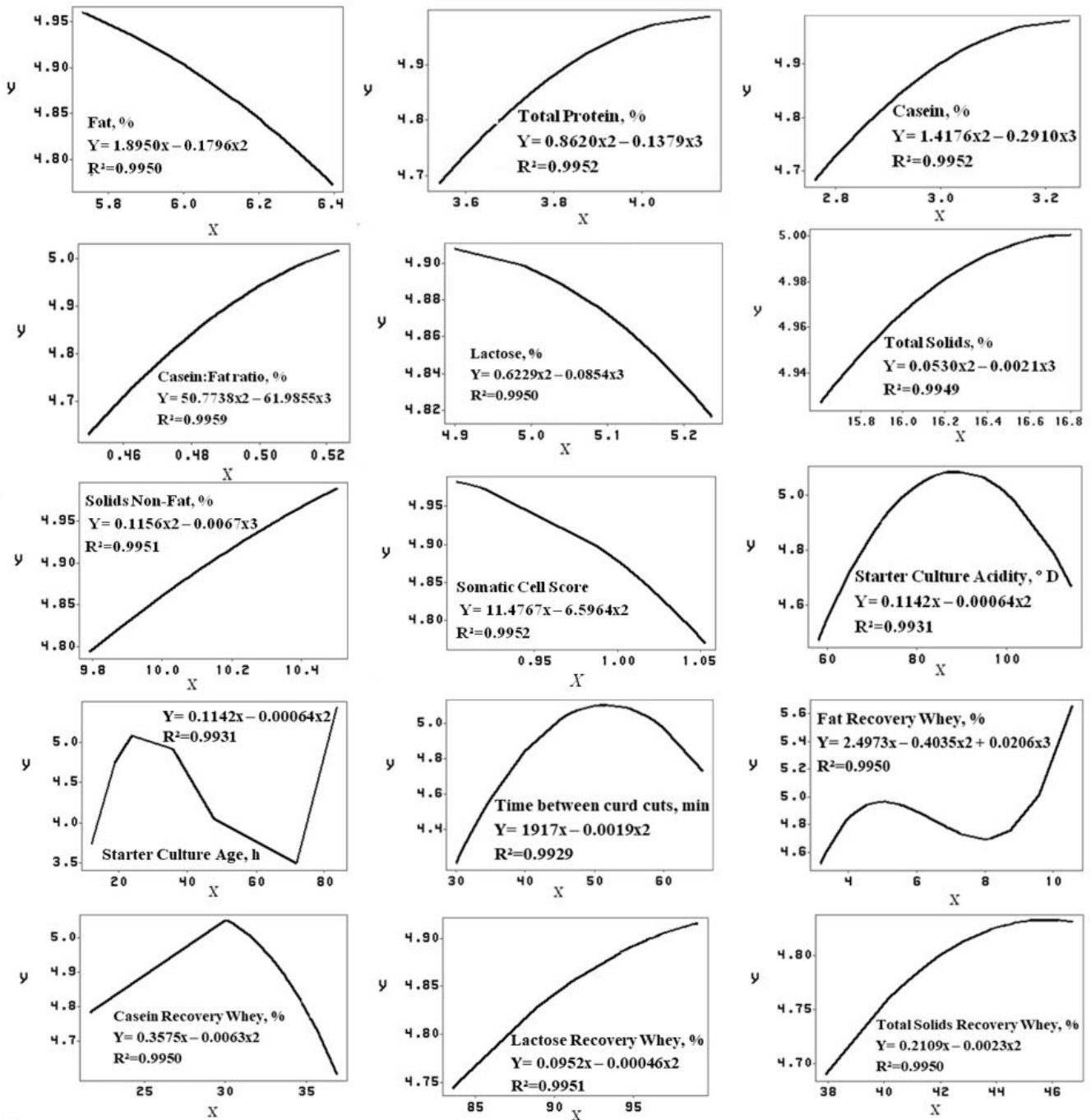


Figure 1. Estimated mozzarella cheese yield (Y) by polynomial regressions equations, without intercept, in functions of the variables X. All the equation coefficient parameters were significant at the 5% probability level ($P < 0.05$).

buffalo milk protein and is responsible for structuring the curd and retaining fat and minerals for cheese formation (Sales et al., 2017). It is important to note that the major goal in cheese making is to improve yield efficiency by using less milk to produce the same amount of cheese (Sales et al., 2016). Thus, MCY efficiency is favored when fat, TP, casein, and TS concentrations, as well as the milk casein:fat ratio increase.

Protein recovery is strongly related to cheese yield (Cecchinato & Bittante, 2016). For this reason, casein recovery is

an important factor influencing cheese manufacturing efficiency. In contrast, lactose is the least utilized milk constituent in manufacturing mozzarella; therefore, it has the greatest losses in whey compared with other milk components (Sales et al., 2017). Despite this, a significant relationship was found between lactose recovery and MCY in the present study.

The SC used in the study was obtained from the processor itself at the end of the last mozzarella manufacturing in the plant. It was stored in a cold room for approximately 36 h (age)

with acidity between 80 and 90 °Dornic to be used in the next cheesemaking batch. The interspersed time between the use of an old SC and a new one (whey “renewal”) ensures that the whey obtained from each cheese batch has an elevated microbial load for producing lactic acid, resulting in increased acidity and improved fermentative efficiency (Sales et al., 2018).

A significant effect of SC age and SC acidity on MCY was observed ($P < 0.05$; Figure 1). A newer SC has a reduced lactic acid concentration and less capacity for increasing the acidity of the medium. Thus, it is necessary to add more SC to the recipe to reach the ideal curd fermentation. However, this can increase the moisture of the mass and artificially reduce the actual MCY efficiency. Therefore, the significant relationship of MCY with SC acidity may be related to the acidification and moisture conditions that the SC confers to the cheese curd. A slightly acidified mozzarella mass does not easily bind to water during the curd stretching, thereby resulting in low moisture concentration, elasticity, and resistance (Altiero et al., 1984), leading to a reduction in the final cheese yield. The fact that SC age and SC acidity influenced the MCY emphasizes the need for using high-quality SC (with approximately 36h of age and acidity between 80 and 90 °Dornic) in mozzarella cheese processing at the plant level.

After adding the necessary ingredients for cheese production, it is important to be careful when handling the curd under formation. This is essential to ensure that the process occurs according to standards without damage to the subsequent stages of fermentation, syneresis, curd stretching, and molding. The interval between curd cuts (variable Time Curd Cut, min) is one of the factors related to handling. Following the recommended time between cuts means assurance of ideal conditions for milk coagulation and curd fermentation, and consequently optimization of MCY.

4 Conclusions

The regression models showed that the concentration of milk components (fat, protein, casein, lactose, total solids), SCS, age and acidity of the starter culture, time between the curd cuts, and percentage of lost whey constituents all impact MCY. Thus, the buffalo dairy industry should focus on rigorous control of milk quality and processing factors to standardize and optimize MCY efficiency. Future research is needed to build a robust dataset to validate equations to predict the MCY of buffalo milk based on actual observations.

References

Abd El-Gawad, M., & Ahmed, N. S. (2011). Cheese yield as affected by some parameters review. *Acta Scientiarum Polonorum. Technologia Alimentaria*, 10, 131-153.

Ahmad, N., & Saleem, M. (2020). Characterisation of cow and buffalo ghee using fluorescence spectroscopy. *International Journal of Dairy Technology*, 73(1), 191-201. <http://dx.doi.org/10.1111/1471-0307.12632>.

Altiero, V. F., Addeo, F., & Masi, P. (1984). Influenza dell'acidificazione della cagliata al momento della filatura sulla qualita e sulla struttura della Mozzarella di bufala. *Il Latte*, 10, 764-774.

Association of Official Analytical Chemists – AOAC. (1998). *Official methods of analysis* (18th ed.). Washington: AOAC International.

Cecchinato, A., & Bittante, G. (2016). Genetic and environmental relationships of different measures of individual cheese yield and curd nutrients recovery with coagulation properties of bovine milk. *Journal of Dairy Science*, 99(3), 1975-1989. <http://dx.doi.org/10.3168/jds.2015-9629>. PMID:26805996.

Cipolat-Gotet, C., Bittante, G., & Cecchinato, A. (2015). Phenotypic analysis of cheese yields and nutrient recoveries in the curd of buffalo buffalo milk, as measured with an individual model cheese-manufacturing process. *Journal of Dairy Science*, 98(1), 633-645. <http://dx.doi.org/10.3168/jds.2014-8308>. PMID:25465562.

Citro, A. (2010). *Classificazione dei formaggi a pasta filata e tecnologia di produzione* (Newsletter, No. 1, pp. 8-12). Cremona: Associazione Italiana Veterinaria Medicina Pubblica. Retrieved from <http://cms.aivemp.it/SiteTailorCommon/showBinary.aspx?id=4731>

Furtado, M. M., & Pombo, A. F. W. (1979). Fabricação de queijo prato e minas: estudo do rendimento/parte I - determinação das cifras de transição. *Revista do Instituto de Laticínios Cândido Tostes*, 34(205), 1-48.

Hooda, A., Mann, B., Sharma, R., & Bajaj, R. (2020). Physicochemical characterisation of native micellar casein concentrates from buffalo and cow skim milk harvested using microfiltration. *International Journal of Dairy Technology*, 1471-0307.12711. <http://dx.doi.org/10.1111/1471-0307.12711>.

Rangel, A. H. N., Oliveira, J. P. F., Araújo, V. M., Bezerra, K. C., Medeiros, H. R., Lima, D. M., Jr. & Araújo, C. G. F. (2011). Influência do estágio de lactação sobre a composição do leite de búfalas. *Acta Veterinaria Brasilica*, 5(3), 306-310. <http://dx.doi.org/10.21708/avb.2011.5.3.2315>.

Rossi, D. A., Abreu, L. R., Furtado, M. M., & Moura, C. J. (1998). Utilização do coalho bovino e coagulantes microbiano e genético na composição e rendimento do queijo Minas Frescal. *Revista do Instituto de Laticínios Cândido Tostes*, 53(305), 8-14.

Sabia, E., Gauly, M., Napolitano, F., Cifuni, G. F., & Claps, S. (2020). The effect of different dietary treatments on volatile organic compounds and aromatic characteristics of buffalo Mozzarella cheese. *International Journal of Dairy Technology*, 73(3), 594-603. <http://dx.doi.org/10.1111/1471-0307.12696>.

Sales, D. C., Rangel, A. H. N., Urbano, S. A., Borges, K. C., Andrade, J. C., No., & Chagas, B. M. E. (2016). Cheese yield in Brazil: state of the art. *Food Science and Technology*, 36(4), 563-569. <http://dx.doi.org/10.1590/1678-457x.17116>.

Sales, D. C., Rangel, A. H. N., Urbano, S. A., Freitas, A. R., Tonhati, H., Novaes, L. P., Pereira, M. I. B., & Borba, L. H. F. (2017). Relationship between mozzarella yield and milk composition, processing factors, and recovery of whey constituents. *Journal of Dairy Science*, 100(6), 4308-4321. <http://dx.doi.org/10.3168/jds.2016-12392>. PMID:28342605.

Sales, D. C., Rangel, A. H. N., Urbano, S. A., Tonhati, H., Galvão, J. G. B., Jr., Guilhermino, M. M., Aguiar, E. M., & Bezerra, M. F. (2018). Buffalo milk composition, processing factors, whey constituents recovery and yield in manufacturing mozzarella cheese. *Food Science and Technology*, 38(2), 328-334. <http://dx.doi.org/10.1590/1678-457x.04317>.

SAS Institute Inc. (2002). *SAS ver. 9.0*. Cary, NC.

Shook, G. G. (1982). Approaches to summarizing somatic cell count which improve interpretability. In *Proceedings of the 21st Nacional Mastitis Council Annual Meeting* (pp. 150-166). Madison: National Mastitis Council.

Tonhati, H., Mendoza-Sánchez, G., & Aspilcueta Borquis, R. R. (2011). Programa de melhoramento genético de búfalos no Brasil: estado da arte. In *Anais do II Simpósio da Cadeia Produtiva da Bubalinocultura* (pp. 1-11). Botucatu: FEPAP.