Ohmic heating does not influence the biochemical properties of Minas Frescal cheese but decreases uric acid levels in healthy Wistar rats

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ABSTRACT

The objective of this study was to evaluate the effects of the consumption of Minas Frescal cheese (2 g for 15 d) produced with pasteurized milk or milk subjected to ohmic heating on the health variables (biochemical, hematological, and uric acid levels) of rats, as well as on the identity variables and proteolysis indices of cheese. Three groups of rats ($n = 6$ per group) were studied, as follows: control group, without cheese consumption (CON), rats fed Minas Frescal cheese produced with milk pasteurized by conventional technology (PC, 72 to 75°C for 15 s), and rats fed Minas Frescal cheese produced with milk subjected to ohmic heating (OHC, 8 V/cm, 72 to 75°C for 15 s). Ohmic heating did not alter the macronutrient levels (moisture, fat, protein) of the cheeses, but an increase in the proteolysis index was observed. For both cheeses, no significant differences were observed for feed intake (139 to 143 g/d), water consumption (196 to 206 mL/d), or final animal weight (236 to 252 g), and no changes were detected in hematological variables (counts of red blood cells, hemoglobin, leukocytes, and platelets) or levels of potassium, calcium, magnesium, urea, creatinine, total cholesterol, high-density lipoprotein cholesterol, blood glucose, and bilirubin (total, direct, and indirect) of rats. In addition, both cheeses led to a reduction of serum triglycerides and an increase in monocyte, eosinophil, and basophil levels. However, consumption of OHC resulted in decreased sodium, uric acid, and lymphocyte levels. The present results suggest that ohmic heating in the

processing of Minas Frescal cheese has positive effects on the health of healthy rats.

Key words: Minas Frescal cheese, ohmic heating, uric acid, rats

INTRODUCTION

Minas Frescal cheese is one of the most popular cheeses in Brazil, characterized by its high yield, high water activity, slightly acidic pH (pH = 5.0), low salt content, absence of synthetic preservatives, and easy technological processing (Sperry et al., 2018). It is a fresh cheese obtained by enzymatic coagulation of milk using rennet or other coagulant enzymes, with or without the addition of starter cultures (Pimentel et al., 2017). For Minas Frescal cheese manufacturing, raw milk is conventionally subjected to a thermal processing called pasteurization, ensuring the microbiological safety of the product (Cappato et al., 2017). However, pasteurization can lead to negative changes in the sensory, nutritional, and physicochemical properties of processed products (Coutinho et al., 2018), which can be prevented by the use of emerging technologies such as ohmic heating (OH; Cappato et al., 2017).

Ohmic treatment promotes rapid and homogeneous heating of milk by converting electrical energy to thermal energy, resulting in greater retention of thermosensitive compounds and sensory attributes due to exposure to a lower thermal load (Cappato et al., 2018). In addition, OH is characterized by uniform heat generation, lower capital cost (not requiring a boiler and a heat transfer surface), lower maintenance cost (no moving parts), and ease of process control through instant switch-on and shutdown (Makroo et al., 2020).

Consumption of Minas Frescal cheese has been associated with several health benefits, such as improved

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lipid indexes (Sperry et al., 2018), lowered blood pressure (Lollo et al., 2015), constipation relief (Favretto et al., 2013), and increased immunity (Lollo et al., 2012), although studies have focused on cheeses containing probiotics and produced with pasteurized milk. To the best of our knowledge, no studies have yet been performed on Minas Frescal cheese made with milk subjected to OH. Thus, the objective of this study was to evaluate the effects of continuous consumption of Minas Frescal cheeses made with pasteurized milk or milk subjected to OH on the health variables (biochemical and hematological properties and uric acid level) in healthy rats.

MATERIALS AND METHODS

Milk Processing

Cooled raw milk (10 L, 3.4% fat) was purchased from the Barra Mansa Milk Producers Cooperative (Barra Mansa, Brazil) and subjected to conventional pasteurization or OH treatment. For conventional pasteurization, milk was heated to 72 to 75°C for 15 s, using a water bath. The OH equipment consisted of 2 iron-containing electrodes (9 cm long, made of stainless steel type 316), a power supply, a heating bowl, a thermocouple, a stirrer, and 3 multimeters for measuring the variables (temperature, voltage, and alternating current generation). Milk was submitted to ohmic treatment of 8 V/cm until reaching 72 to 75°C for 15 s. Then the samples were immediately immersed in an ice bath to promote rapid cooling (7°C).

Manufacture of Minas Frescal Cheese

Minas Frescal cheese was produced according to the methodology described by Dantas et al. (2016). Calcium chloride (0.2 g/100 g; Labsynth, Sao Paulo, Brazil) and coagulant (3 g/L; Halamix, Chr. Hansen, Hørsholm, Denmark) were added to the pasteurized or OH-treated milk, which was stirred and then kept at rest for 35 min at 37°C for coagulation. The curd was then cut, the whey was removed, and the remaining curd was placed in 250-g plastic molds. The cheeses were dry-salted (0.8 g of salt per 100 g of cheese; NaCl from Labsynth), packaged, and stored in a refrigerated chamber at 5°C.

Physicochemical Composition and Proteolytic Activity

The physicochemical composition of the cheese (moisture, protein, and fat levels) was determined using the traditional methods proposed by AOAC International (2002). The moisture content was determined by drying

5 g of sample at 100 to 105°C for 24 h. The protein content was determined via the Kjeldahl method, multiplying the nitrogen content by the conversion factor 6.38. Fat content was determined through the Gerber method. All results were expressed in grams per 100 g of cheese. Proteolytic activity was determined after reaction with a reactive solution (*o*-phthaldialdehyde) containing sodium dodecyl sulfate, dithiothreitol, sodium tetraborate decetrate, ophthalaldehyde, and ethanol. Amino acids and peptides were quantified by absorbance readings of *o*-phthaldialdehyde derivatives at 340 nm (Sperry et al., 2018).

Clinical Study

The in vivo experiment used 8- to 10-wk-old male rats (*Rattus norvegicus*, Wistar variety) weighing 230 to 260 g. This study was approved by the Animal Experimentation Committee of the Federal University of Grande Dourados (Dourados, Brazil), under protocol 21/2018. The animals were supplied by the Federal University of Grande Dourados Central Biottery, kept under controlled conditions (22°C; 40 to 60% humidity, and 12-h light-dark cycle), individually identified, and housed in polypropylene cages with feed and water ad libitum.

The experimental design is shown in Figure 1. Three groups ($n = 6$ per group) were studied, as follows: the control group (**CON**) was fed no cheese; the **PC** group was fed cheese made with pasteurized milk (2 g/d); and the **OHC** group was fed cheese made with milk subjected to ohmic heating (2 g/d). Cheese was administered at the end of the dark cycle, offered along with the commercial ration. The experiment lasted 14 d.

Animals were anesthetized with xylazine (8 to 15 mg/kg) associated with ketamine (60 to 80 mg/kg) via intraperitoneal injection, and blood was collected via cardiac puncture (1 to 2 mL). Blood was used to measure dosage of urea, creatinine, sodium, potassium, and magnesium, total cholesterol and fractions, total bilirubin and fractions, uric acid, blood glucose, and hematological variables (red blood cells, hemoglobin, leukocytes, platelets, monocytes, eosinophils, basophils, neutrophils, and lymphocytes). Dosing was performed via standardized methods, specific to each analysis, performed at the Clinical Analysis Laboratory of the School Hospital at the Federal University of Grande Dourados.

Statistical Analysis

We used ANOVA to compare the responses between groups, and means were compared using Tukey's test, with $P < 0.05$ as the significance level. Statistical anal-

Figure 1. Experimental design to evaluate the effects in rats of consuming Minas Frescal cheese produced with pasteurized milk or milk subjected to ohmic heating on the health variables of rats, as well as on identity variables and proteolysis indices of cheese.

yses were performed using GraphPad Prism software, version 5.0, for Windows (GraphPad Software Inc., La Jolla, CA).

RESULTS AND DISCUSSION

Physicochemical Composition and Proteolytic Activity

Table 1 shows the physicochemical composition and proteolytic activity of the cheeses. Both types of Minas Frescal cheeses presented (per 100 g of cheese) 57 to 58 g of moisture, 16.9 to 17.2 g of protein, and 16 to 16.4 g of lipids, corroborating previous studies (Dantas et al., 2016; Sperry et al., 2018). We found no effect of the type of milk processing (pasteurization or OH) on the physicochemical composition of the cheeses ($P > 0.05$). The OHC cheese had higher proteolytic activity values compared with PC (0.69 vs. 0.43, $P < 0.05$). Ohmic treatment can promote changes in the conformation of enzymes, leading to either increase or reduction of enzyme activity (Cappato et al., 2018) as well as an increase in protein hydrolysis (Ferreira et al., 2019). These changes can lead to the formation of small peptides. Higher proteolysis may be a positive result, as it represents greater digestibility and higher

concentration of bioactive peptides in the OHC cheese (Sperry et al., 2018).

Clinical Study

Table 2 presents the results of feed and water consumption and the final weights of the studied rats. The rats consumed 138.8 to 142.8 g/d of feed and 195.5 to 206.4 mL/d of water and presented a final weight from 236.4 to 251.7 g. We found no effect of cheese consumption (PC or OHC) on the amount of feed or water ingested or on the final animal weight ($P > 0.05$). The results indicate that although food intake was higher

Table 1. Chemical composition and proteolytic activity of Minas Frescal cheeses made with conventional pasteurized milk (PC) or milk treated with ohmic heating (OH); mean $\pm$ SD, $n = 6$ per group

Item ¹	PC	OH
Moisture	57.0 $\pm$ 0.62 ^a	58.0 $\pm$ 0.57 ^a
Protein	17.2 $\pm$ 0.63 ^a	16.9 $\pm$ 0.89 ^a
Lipid	16.4 $\pm$ 0.81 ^a	16.0 $\pm$ 0.40 ^a
Proteolytic activity	0.43 $\pm$ 0.12 ^b	0.69 $\pm$ 0.04 ^a

^{a,b}Different superscript letters in the same row indicate significant differences among treatments ($P < 0.05$).

¹Moisture, protein, and lipid contents are expressed in grams per 100 g of cheese. Proteolytic activity is absorbance at 340 nm.

Table 2. Feed and water consumption and final weight of experimental rats: control (CON) rats were fed no cheese in addition to standard diet, PC group was fed Minas Frescal cheese made with conventional pasteurized milk, and OHC group was fed Minas Frescal cheese made with milk treated with ohmic heating (n = 6 per group; values are mean $\pm$ SD)

Item	CON	PC	OHC
Final weight (g)	238.5 $\pm$ 9.25 ^a	251.7 $\pm$ 4.196 ^a	236.4 $\pm$ 5.61 ^a
Feed consumption (g/d)	141.8 $\pm$ 2.74 ^a	142.8 $\pm$ 3.85 ^a	138.8 $\pm$ 3.77 ^a
Water consumption (mL/d)	206.4 $\pm$ 4.72 ^a	203.6 $\pm$ 2.87 ^a	195.5 $\pm$ 8.13 ^a

^aMeans with identical superscript letters within a row indicate no significant differences among treatments ($P > 0.05$).

in the diets containing cheese, there was no significant effect on body weight gain (Bouton et al., 2019).

Table 3 presents the results of the biochemical variables of uric acid levels in rats. We found no effect of cheese consumption on levels of potassium, calcium, magnesium, urea, creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, direct or indirect bilirubin, and glucose ($P > 0.05$). Cheese consumption reduced serum triglycerides ($P < 0.05$), regardless of the type of cheese. Cheese contains compounds that can inhibit fatty acid desaturation, thereby reducing the availability of monounsaturated fatty acids, which are the preferred substrates for the synthesis of triglycerides, cholesterol esters, and phospholipids (Høstmark and Lunde, 2012). In addition, the calcium in cheese may interact with fatty acids, resulting in the formation of insoluble soaps and excretion in feces, contributing to the decrease in triglycerides (Higurashi et al., 2016). The reduction of triglyceride levels is interesting from a functional point of view.

Continuous consumption of OHC led to a reduction of sodium and uric acid levels ($P < 0.05$) in the studied rats. Lower levels of uric acid and sodium in the blood

of the rats fed OHC are probably due to their higher proteolytic capacity (Table 1) and, consequently, to a higher number of bioactive peptides. Milk proteins, especially short-chain peptides, are able to reduce serum uric acid concentrations, increasing the amount of uric acid excretion (Kaneko et al., 2014). The consumption of these peptides can also be related to increased vasodilation and reduction of blood sodium levels (Schiering et al., 2016). Indeed, a recent study showed that Minas Frescal cheese processed using OH presented greater ACE inhibitory activity compared with cheese manufactured using conventional processing (25.4 to 26.7% vs. 12.5%, respectively; $P < 0.05$; Rocha et al., 2020). In addition, peptide profiling in cheeses manufactured using ohmic heating revealed higher numbers of bioactive peptides, identified using the mass-to-charge ratio (m/z). Ohmic-heated cheese presented 21 to 34 peptides, as opposed to 18 in CP cheese; 7 of these peptides are responsible for the discrimination of the treatments, notably 3 with ACE-inhibitory activity: κ -CN f(157–163, m/z 780), α_{S1} -CN f(18–23, m/z 791), and β -CN f(193–202, m/z 1,158), all found exclusively in OH cheeses (Rocha et al., 2019). Thus, cheeses made

Table 3. Biochemical variables of experimental rats: control (CON) rats were fed no cheese in addition to standard diet, PC group was fed Minas Frescal cheese made with conventional pasteurized milk, and OHC group was fed Minas Frescal cheese made with milk treated with ohmic heating (n = 6 per group; values are mean $\pm$ SD)

Item	CON	PC	OHC
Sodium (mmol/dL)	142.6 $\pm$ 0.54 ^a	141.1 $\pm$ 0.46 ^a	140.6 $\pm$ 0.57 ^b
Potassium (mmol/L)	4.72 $\pm$ 0.10 ^a	4.65 $\pm$ 0.15 ^a	5.08 $\pm$ 0.34 ^a
Calcium (mg/dL)	10.62 $\pm$ 0.12 ^a	10.25 $\pm$ 0.17 ^a	10.32 $\pm$ 0.289 ^a
Magnesium (mg/dL)	2.85 $\pm$ 0.145 ^a	2.76 $\pm$ 0.10 ^a	2.54 $\pm$ 0.04 ^a
Urea (mg/dL)	54.12 $\pm$ 2.25 ^a	51.85 $\pm$ 2.69 ^a	53.13 $\pm$ 1.93 ^a
Creatinin (mg/dL)	0.34 $\pm$ 0.01 ^a	0.40 $\pm$ 0.02 ^a	0.39 $\pm$ 0.02 ^a
Cholesterol (mg/dL)	54.00 $\pm$ 5.81 ^a	53.00 $\pm$ 4.34 ^a	55.00 $\pm$ 4.42 ^a
Triglyceride (mg/dL)	70.33 $\pm$ 10.19 ^a	32.98 $\pm$ 3.36 ^b	47.20 $\pm$ 3.99 ^b
HDL cholesterol ¹ (mg/dL)	60.83 $\pm$ 5.9 ^a	61.17 $\pm$ 3.48 ^a	58.6 $\pm$ 3.61 ^a
Glycemia (no fasting; mg/dL)	295.1 $\pm$ 14.20 ^a	273.1 $\pm$ 20.20 ^a	320.8 $\pm$ 17.56 ^a
Bilirubin (total; mg/dL)	0.06 $\pm$ 0.08 ^a	0.06 $\pm$ 0.09 ^a	0.08 $\pm$ 0.06 ^a
Bilirubin (indirect; mg/dL)	0.04 $\pm$ 0.01 ^a	0.05 $\pm$ 0.01 ^a	0.06 $\pm$ 0.04 ^a
Bilirubin (direct; mg/dL)	0.01 $\pm$ 0.003 ^a	0.01 $\pm$ 0.004 ^a	0.01 $\pm$ 0.003 ^a
Uric acid (mg/dL)	0.8 $\pm$ 0.11 ^a	1.2 $\pm$ 0.11 ^a	0.1 $\pm$ 0.15 ^b

^{a,b}Different superscript letters in the same row indicate significant differences among treatments ($P < 0.05$).

¹HDL = high-density lipoprotein.

with milk subjected to ohmic treatment have antihyperuricemic properties and may help in controlling hypertension. Hyperuricemia is a metabolic disease resulting from overproduction of uric acid or from a disorder of uric acid excretion in the body. Excessive levels of uric acid in the blood can cause nephrolithiasis and gout, resulting in hyperlipidemia, hypertension, type II diabetes, and cardiovascular disease (Li et al., 2018). High blood pressure increases the risk of stroke, heart attack, heart failure, kidney damage, and vision loss (Wright et al., 2018).

We found no effect of cheese consumption on the counts of red blood cells, hemoglobin, leukocytes, and platelets of rats ($P > 0.05$). Cheese consumption increased the number of monocytes, eosinophils, and basophils ($P < 0.05$), and a more pronounced effect was observed for the conventional cheeses (PC). In addition, the number of lymphocytes decreased ($P < 0.05$). Basophils release mediators capable of increasing vascular permeability and inducing migration of inflammatory cells (neutrophils and macrophages). Inflammatory responses are also associated with increased eosinophils (Zakharenko et al., 2017). An increase in neutrophil levels was observed only for the consumption of the control cheese made with pasteurized milk (PC, $P < 0.05$). Increased neutrophil levels in the blood are an indicator of inflammatory processes (Zakharenko et al., 2017). The results indicate that cheese intake, regardless of the type of milk used in cheese manufacture, may have the ability to increase inflammatory processes in rats. However, this increase was smaller in cheeses made with milk subjected to ohmic heating, which represents an advantage of this technology.

CONCLUSIONS

The consumption of Minas Frescal cheese made with milk subjected to ohmic heating may be beneficial for hyperuricemic individuals, maintaining uric acid levels and preventing progression of gout. In addition, it can contribute to controlling hypertension. The present study is the first in vivo investigation involving the consumption of Minas Frescal cheese made with milk subjected to ohmic heating, and the results are important for the dairy industry, as they indicate that this alternative heat treatment may result in health benefits.

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