Physicochemical characterization, fatty acids profile and lipid quality of Gorgonzola cheeses

Caracterização físico-química, perfil de ácidos graxos e qualidade lipídica de queijos Gorgonzola

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ABSTRACT

Gorgonzola cheeses are produced from cow’s milk added to the Penicillium Roqueforti fungus and matured for a minimum of 90 days. They have particular characteristics and flavor that are appreciated by consumers. The objective of this study was to evaluate the physicochemical characteristics, determine the profile of fatty acids and the lipid quality indexes this type cheeses. Eight cheese samples were purchased in municipalities of the Southwest of Paraná and West of Santa Catarina to evaluation. The results physicochemical showed that this cheese contains high levels of proteins and lipids. In the fatty acid profile, twenty-five fatty acids were identified, with palmitic acid being majority with low ratio PUFA/SFA e values of the ratio of n-6/n-3 in accordance with the recommended by the World Health Organization while the lipid quality indexes have characteristic values for dairy products.

keywords: Penicillium roqueforti; Gorgonzola cheese; Fatty acids; Lipid quality.

RESUMO

Os queijos gorgonzola são produzidos a partir de leite de vaca adicionado do fungo Penicillium Roqueforti e maturados por um período mínimo de 90 dias. Possuem características e sabor particulares que são apreciados pelos consumidores. O objetivo deste estudo foi avaliar as características físico-químicas, determinar o perfil de ácidos graxos e os índices de qualidade lipídica deste tipo de queijos. Oito amostras de queijos foram adquiridas em municípios do Sudoeste do Paraná e Oeste de Santa Catarina para avaliação. Os resultados físico-químicos mostraram que esses queijos contêm altos teores de proteínas e lipídios. No perfil de ácidos graxos foram identificados vinte e cinco ácidos graxos, sendo o ácido palmitico majoritário, com baixa razão AGPI/AGS e valores da razão de n-6/n-3 de acordo com o recomendado pela Organização Mundial da Saúde enquanto os índices de qualidade lipídica têm valores característicos para produtos lácteos.

Palavras-chave: Penicillium roqueforti; queijo Gorgonzola; Ácidos graxos; Qualidade lipídica

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INTRODUCTION

In Brazil, Gorgonzola cheeses are produced from cow's milk, with the addition of *Penicillium roqueforti* fungus and matured for a minimum of 90 days. This cheese has a pungent aroma and flavor, an open texture, and a soft, smooth and crumbly consistency (Ribeiro *et al*., 2020).

This type of cheese has gained popularity and has seen significant growth in the Brazilian market. The characteristics and composition of the Gorgonzola cheese produced in Brazil is similar to the Danish Danablu and the American Blue Cheese (Lourenço Neto, 2013).

Lipids are among the essential components of the Gorgonzola cheese, since they give it its distinct aroma and flavor. These lipids are mostly made up of short-chain fatty acids, which are quite volatile, with some being capable of intense fungal lipolysis, characteristics which strongly contribute to the development of flavor (Sbampato *et al*., 2000).

Conjugated linoleic acid, found in the fatty acid profile of milk and dairy products, presents a variety of beneficial properties (Koba; Yanagita, 2014), such as anti-inflammatory action (Reynolds; Roche, 2010) protection of cells against tumor necrosis factor alpha (TNF-α) and induced toxicity (Mohammadi *et al*., 2020), benefits for diseases like asthma (Macredmond; Dorscheid, 2011) and Alzheimer (Lee *et al*., 2013), in addition to an improved immune system (Basaganya-Riera *et al*., 2012). For these reasons, it has been widely studied.

Thus, it is a well-known fact that the composition of fatty acids influences the lipid quality of food and is related to the health of the consumer. The quality of the lipids can be predicted by calculating the atherogenicity and thrombogenicity indexes (AI and TI). Despite these indexes having no reference values, there is a consensus that the lower they are, the higher the healthfulness of the food (Turan *et al*., 2007).

The objective of this study was to characterize Gorgonzola cheeses according to physicochemical standards, assess the fatty acid profile and determine the lipid quality indexes of these dairy products.
MATERIALS AND METHODS

Sampling

Eight samples of Gorgonzola cheese (6 national and 2 imported from Argentina and Italy), were collected in the cities that make up the Southeast of Parana and the West of Santa Catarina. The samples collected were identified with letters (A, B, C, D, E, F, G and H), in order to preserve the identity of each brand. Three samples were collected from the same lot of each brand, totaling 24 samples. Cheeses of different brands had different maturation periods, which varied between 100 and 120 days. The samples were packed and transported in thermal boxes. The analyses were conducted in triplicate.

Physicochemical characterization and Fatty Acid Profile

Gorgonzola cheeses were characterized as to the percentage of moisture, ashes, total proteins, sodium (AOAC, 1997), total lipids, pH, carbohydrates and fat in dry matter (FDM) (IAL, 2008), caloric value (Tagle, 1981); and the water activity using a water activity meter (NovasinaLab Master).

The lipids were extracted using Bligh-Dyer method (1959) and transesterified to according with the ISO 5509 (1978) method. To separation the fatty acids was using a Shimadzu GC 2010 chromatograph, equipped with flame ionization detector and a Supelco fused-silica capillary column (F.S.CA.SP-2560), of 100 m x 0.25 mm x 20 µm of stationary phase.

Splitless injection was used, with the injector at 260 °C and the initial temperature of the column at 140 °C in the first five minutes, then increasing 2.4 °C per minute until reaching 240 °C for 17 minutes, with carrier gas flow (helium 5.0) at 1.21 mL/min, and a flame ionization detector operating at 260 °C utilizing synthetic air and hydrogen, with the total running time being 60 minutes.

The fatty acids were identified by comparing the retention time of the Sigma Aldrich analytical standard mix, identified as 18919-1 AMP, which presents 37 standards of Methyl Esters of fatty acids, and their values were expressed in adjusted peak area percentages.

The lipid quality indexes, known as atherogenicity and thrombogenicity indexes (AI and TI) were determined according to specifications from Ulbricht and Southgate (1991), following Equations 1 and 2.
(IA): Index of atherogenicity

\[
\begin{align*}
\text{[(C12:0+(4xC14:0)+C16:0)]/(\Sigma\text{MUFA}+\Sigma\text{n-6}+\Sigma\text{n-3})} \quad \text{Eq (1)}
\end{align*}
\]

(IT): Index of thrombogenicity

\[
\begin{align*}
\text{(C14:0+C16:0+C18:0)/[\left(0.5x\Sigma\text{MUFA}\right)+\left(0.5x\Sigma\text{n-6}\right)+\left(3x\Sigma\text{n-3}\right)+\left(\Sigma\text{n-3}/\Sigma\text{n-6}\right)}} \quad \text{Eq (2)}
\end{align*}
\]

Data Treatment

The analysis of variance (ANOVA), Tukey test or Kruskal Wallis test were applied to compare the results between Gorgonzola cheese brands. For all of the tests, a confidence interval of 95% was set. The ACTION® software, version 2014, was used for the analyses.

RESULTS AND DISCUSSION

Physicochemical characterization of Gorgonzola cheeses

The content of moisture did not present difference \((P>0.05)\) between the eight samples of Gorgonzola evaluated, classifying them as medium-hard cheeses, according to the Technical Regulation of Identity and Quality of Cheeses (Brasil, 1996).

Manzi et al. (2007) reported higher values (49.00 to 51.50\%) of moisture when they evaluated Gorgonzola cheese in Italy. The low moisture content found can indicate a problem with dryness in this cheese due to a lack of humidity control in the ripening chamber, low fat content, incorrect curd cutting, waiting too long to begin the pressing process, excess of acidity, among others (Furtado, 2013).

According to Sbampato et al. (2000), Gorgonzola cheeses made in Brazil have in mean 45.44\% to 47.65\% of moisture, after five days from production. However, these values tend to decrease during the maturation.

Table 1: Physicochemical characterization of Gorgonzola cheeses sold in Southeast Parana and West Santa Catarina.

| Samples | M (%) | Ash (%) | PT (%) | LP (%) | CH (%) | FDE (%) | EV   |
|---------|-------|---------|--------|--------|--------|---------|------|
| A       | 39.01±0.81a | 4.29±0.16b | 20.14±0.55a | 34.00±0.25b | 2.56±0.16a | 55.75±0.34bc | 396.80±4.06b |
| B       | 40.15±1.29a | 3.86±0.25bc | 20.66±0.47a | 34.92±1.15ab | 0.41±0.92a | 58.34±1.39bc | 399.07±9.42b |
| C       | 38.44±2.13a | 3.59±0.22b | 1.22±0.59a | 36.75±0.75a | 0.01±2.40a | 59.77±3.25bc | 419.20±1.13a |
| D       | 42.48±3.27a | 3.76±0.20bc | 16.45±0.74a | 37.08±0.52a | 0.23±3.02a | 64.64±4.48a | 404.16±6.60a |
| E       | 41.16±2.11a | 4.23±0.23b | 17.05±0.47b | 36.83±0.14a | 0.74±2.05a | 62.65±2.16b | 404.73±4.50ab |
| F       | 36.62±2.30a | 5.18±0.18b | 20.08±1.37a | 36.17±0.63ab | 1.96±1.22a | 57.09±1.08abc | 413.63±12.02ab |
| G       | 41.64±2.43a | 5.14±0.21b | 21.47±0.61a | 31.33±1.91c | 0.42±3.81a | 53.82±5.23c | 374.32±5.72c |
| H       | 41.4±1.15a  | 3.63±0.20b | 21.25±0.34a | 30.33±0.38c | 3.34±1.19a | 51.82±1.52c | 371.37±4.20b |
The content of ashes in the samples Gorgonzola cheeses varied from 3.59% to 5.18% ($P<0.05$). This variation can be due the amount of calcium chloride and sodium added to the cheese, as does the milk used as raw material. Manzi et al. (2007) also reported a large variation in the ashes for different Gorgonzola cheese Italian.

The protein value of cheeses varied between 16.45% and 21.47%, with difference ($P<0.05$) of samples D and E to the other samples. According to Lourenço Neto (2013), the average protein level for these cheeses varies between 18.00 and 22.00%, these similar to the results found in this study. Similar protein percentage (19.20%) was reported by Manzi et al. (2007) for Italian Gorgonzola.

The lipid content of the cheese samples varied ($P<0.05$) from 30.33% to 37.08%. Sbampato et al. (2000) found lipid values between 31.88% and 33.00% in Gorgonzola cheeses after 5 days of maturation. However, according to Furtado (2013), the expected average lipid level is between 28.00 and 30.00%, indicating that the content in these cheeses is altered. Lower lipid content (27.90%) was found in Italian Gorgonzola cheeses by Manzi et al. (2007), and similar values (57.29%) were reported by Diezhandino et al. (2015) for Valdeón blue cheese.

The fat in dry extract (FDE) varied between 51.82% and 64.64%, classifying the cheeses as fat and extra-fat, according to the Technical Regulation of Identity and Quality of Cheeses (Brasil, 1996). For the FDE, higher values (58.43 and 63.03%) were observed by Sbampato et al. (2000) in Gorgonzola cheeses after five days of maturation.

The carbohydrate values found in the cheeses were lower than 3.34%, and this fact, according to Lourenço Neto (2013), is due to the maturation process, which reduces the sugar levels in the cheese. According to this author, in this process, lactose is hydrolyzed into galactose and glucose by the lactic bacteria originating, later on, lactic acid. This can be proven by the Diezhandino et al. (2015) study, where it was observed that in the beginning of the maturation process, the Valdeón blue cheese had 0.78% of lactose and, after 120 days of maturation, the lactose value was 0.07%.

Caron et al. (2021) analyzed the physicochemical measurements in raw milk and cheeses at 9, 20, 90 and 180 days of maturation and the maturation stage effect changed
11 of the 16 physicochemical parameters studied - dry matter content, fat, pH, water activity, non-protein nitrogen, soluble nitrogen, lactose, lactate, acetate and butyrate.

The energetic value (EV) of Gorgonzola cheeses differed ($P<0.05$) between the samples, making it similar to other cheeses regarding energetic value (Prato cheese, – 360.00 Kcal/100g; Parmesan cheese – 453.00 kcal/100g). Thus, considering a diet of 2000 calories/day, a serving of this type of cheese (average slice – 30 g) would provide, on average, 6% of the total energy value for a day.

The water activity (Aw) varied ($P<0.05$) between the evaluated samples. Diezhandino et al. (2015) reported similar values (0.917) in Valdéon blue cheese at 120 days of maturation. According to Franco and Landgraf (2008), the water activity can be affected by several factors, among them, the relative humidity of the environment (UH).

Table 2: Water activity (WA), pH and sodium (Na) concentration of different brands of Gorgonzola cheese sold in Southeast Parana and West Santa Catarina

| Samples | Aw      | pH       | Na (g/100g) |
|---------|---------|----------|-------------|
| A       | 0.925±0.007$^{abc}$ | 7.12±0.19$^{bc}$ | 0.65±0.01$^{bc}$ |
| B       | 0.939±0.002$^{ab}$  | 7.15±0.09$^{bc}$ | 0.61±0.06$^{bc}$ |
| C       | 0.931±0.004$^{abc}$ | 7.32±0.13$^{b}$  | 0.57±0.03$^{b}$  |
| D       | 0.940±0.006$^{a}$  | 7.43±0.14$^{b}$  | 0.71±0.03$^{bc}$ |
| E       | 0.938±0.006$^{abc}$ | 7.23±0.20$^{b}$  | 0.74±0.05$^{b}$  |
| F       | 0.917±0.008$^{a}$  | 7.43±0.20$^{b}$  | 1.10±0.02$^{a}$  |
| G       | 0.933±0.005$^{ab}$  | 8.12±0.10$^{b}$  | 0.96±0.08$^{b}$  |
| H       | 0.924±0.003$^{bc}$  | 6.65±0.30$^{b}$  | 0.63±0.05$^{bc}$ |

Results refer to the mean ± standard deviation of the replicates analyzed in triplicate. Same letters in the same column represent same means according to Tukey test, ($P>0.05$).

The pH of Gorgonzola cheeses varied between 6.65 and 8.12, with sample G having the highest pH, significantly different ($P<0.05$) from the remaining samples. Similar pH values (6.85) were reported by Diezhandino et al. (2015) for Valdeón blue cheese on the 120th day of maturation, and by Furtado et al. (1984) for Gorgonzola cheese on the 45th day of maturation, with a pH of 6.89.

In Gorgonzola cheeses, the pH evolves proportionally as the fungus develops, utilizing lactic acid for its growth and releasing compounds resulting from proteolysis, which aid in the pH increased. The salt and the temperature during the maturation of the cheese also influence the pH value, developing the flavor and aroma specific for this cheese (Furtado, 2013). Moreover, according to Cantor et al. (2004), the internal pH of the cheese increases more rapidly than the surface pH, because lower concentrations of salt favor the early growth of Penicillium roqueforti cultures on the inner part of the cheese.
Cheeses with pH level over 7.0, on the other hand, as is the case with Gorgonzola samples A, B, C, D, E, F and G, can develop ammoniacal taste, due to high proteolysis or excessive growth of mold, which forms and accumulates ammonia by the action of deaminases and results in a defect for the product (Furtado, 2013).

The sodium (Na) values in Gorgonzola cheeses presented different \((P<0.05)\) among samples. The samples with the highest sodium level (F and G) also presented the highest level of ashes – inorganic matter. Sbampato et al. (2000) found similar values, from 1.83 g to 2.13 g of salt (NaCl) per 100 g of Gorgonzola cheese, that is, approximately 0.8 g of sodium/100g after five days of maturation. The *Penicillium roqueforti* have capacity of tolerating high concentrations of sodium chloride and, for this reason, blue cheeses are usually saltier than other types of cheese (Furtado, 2013).

The World Health Organization (WHO, 2004) advocates that the adult population should consume no more than 5,0 g of NaCl or 2,0 g of Na a day. And the daily sodium need is met with 500 mg of sodium/day (Nakasato, 2004). Thus, considering the values found in the study, a (1) slice of Gorgonzola cheese (30 g) contributes to 11% of the total daily sodium need, and two (2) slices would fulfill the total physiological need.

**Fatty Acid Profile and Lipid Quality Indexes of Gorgonzola type Cheeses**

Twenty-five fatty acids were identified in the Gorgonzola samples, with the majority being the palmitic (16:0), stearic (18:0) and oleic (18:1n-9) acids (Table 3).

The linoleic fatty acids 18:2n-6c and 18:2n-6t are two geometric and positional isomers (Andrade et al., 2012) which make up the conjugated linoleic acid (CLA). From these isomers, rumenic acid (cis-9, trans-11 C18:2) is the most predominant in milk and dairy products, and it is formed by bacteria present in the rumen of animals (Buccione et al., 2012; Santos et al., 2013).

In the fatty acid profile, all fatty acids present a significant difference \((P<0.05)\), except the butyric acid (4:0). In addition, the saturated fatty acids (SFA) make up most of the fatty acids, followed by the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), totaling from 27.19 to 35.77% of unsaturated fatty acids (UFA). Fuke et al. (2012) found a similar amount of SFA (67.71%) and UFA (32.51%) in milk produced in various regions in the state of Rio Grande do Sul. The predominance of saturated fatty acids in cheeses was also reported by other authors (Oliveira et al., 2009; Pellegrini et al., 2013), and can be considered harmful if consumed in high quantities in
diets (Olivo et al., 2021). Oleic (18:1n-9); linoleic (18:2n-6) and alpha-linolenic (18:3n-3) acids are among the polyunsaturated fatty acids (PUFA) present in the Gorgonzola cheese profiles.

Table 3: Fatty acid profile of various Gorgonzola cheese brands sold in Southeast Parana and West Santa Catarina.

| Fatty Acids | Samples of cheese Gorgonzola |
|-------------|------------------------------|
| A (%)       | B (%)                        |
| 4:0*        | 0.76±0.72*                   |
| 6:0*        | 0.45±0.18*                   |
| 8:0*        | 0.34±0.02*                   |
| 10:0        | 1.18±0.01*                   |
| 12:0        | 2.00±0.02*                   |
| 14:0        | 10.30±0.10*                  |
| X1          | 0.56±0.01*                   |
| 16:0*       | 0.66±0.01*                   |
| 17:0*       | 1.11±0.01*                   |
| 17:1        | 0.95±0.01*                   |
| 18:0        | 17.33±0.16*                  |
| 18:1:n9     | 3.77±0.03*                   |
| 18:1:n9c*   | 19.5±0.19*                   |
| 18:1:n1 *   | 0.60±0.01*                   |
| 18:2:n6*    | 0.16±0.01*                   |
| 18:2:n6c*   | 1.04±0.01*                   |
| 18:3:n6*    | 0.16±0.01*                   |
| 18:3:n3*    | 0.28±0.01*                   |
| 20:0        | 0.29±0.01*                   |
| 21:0        | 0.90±0.01*                   |

Results refer to the mean of the peak area in percentage ± standard deviation of the replicates analyzed in duplicate. * Fatty acids tagged with an asterisk were analyzed using the Kruskal Wallis test. Same letters in the same column represent same means according to Tukey test or Kruskal Wallis test (P<0.05).

Table 4: Summations, fatty acid ratios and Lipid Quality indexes of various Gorgonzola cheese brands sold in Southeast Parana and West Santa Catarina.

| Sum        | Samples of cheese Gorgonzola |
|------------|------------------------------|
| A (%)      | B (%)                        |
| n-6/n-3*   | 4.82±0.04*                   |
| UFA/SFA*   | 0.39±0.05*                   |
| PUFA/SFA*  | 0.02±0.01*                   |
| IA         | 2.87±0.01*                   |

Lipid Quality Indexes

| IA         | 2.87±0.01*                   | 2.64±0.01* | 2.47±0.05* | 2.40±0.04* | 1.96±0.01* | 2.29±0.01* | 2.53±0.01* |
Results refer to the mean ± standard deviation of the replicates analyzed in duplicate. * Fatty acids tagged with an asterisk were analyzed using the Kruskal Wallis test. Same letters in the same column represent same means according to Tukey test or Kruskal Wallis test (P>0.05). The summations are those of the fatty acids: PUFA (polyunsaturated); MUFA (monounsaturated); SFA (saturated); X (not identified by the standard); n-6 (omega-6) and n-3 (omega-3). The ratios are those between the summations of the groups: unsaturated/saturated fatty acids (UFA/SFA) polyunsaturated/saturated fatty acids (PUFA/SFA) and omega-6/omega-3 (n-6/n-3); AI – Atherogenicity Index; IT – Thrombogenicity Index.

The UFA/SFA ratios were different (P<0.05) among Gorgonzola cheese brands (Table 4). For cow's milk from Southern Brazil this ratio is approximately 0.48 (Fuke et al., 2012). In sheep-milk cheeses, these results varied between 0.25 and 0.60 (Pellegrini et al., 2013). According to the National Cholesterol Education Program (1988), in order to maintain one's health and control coronary diseases and hypercholesterolemia, the UFA/SFA ratios should be higher than 1, which was not achieved by any of the assessed brands. Therefore, they should be consumed in moderation.

The PUFA/SFA ratios of the present study stayed below the recommendation of the Department of Health and Social Security (1984), which establishes the ideal value as over 0.45.

Linolenic (n-6) and alpha-linolenic (n-3) fatty acids, although at low percentages, were also found in the Gorgonzola cheese brands assessed. The values for the ratio between omega-6 and omega-3 fatty acids (n-6/n-3) were between 2:1 and 9:1, however, the vast majority of cheeses had a 4:1 ratio. According to Simopoulos et al. (1999), the recommended value for this ratio would be 2:1 or 3:1, so that, only sample E would be suitable for consumption. However, the World Health Organization (WHO, 1995) considers ratios 5:1 to 10:1 acceptable, which means that all of the assessed cheeses, with their n-6/n-3 ratios, fell within the recommendation.

Furthermore, according to Martin et al. (2006) the consumption of omega-3 should be proportional to that of omega-6, with the appropriate ratio being 4:1. According to Shetty and Shetty (2020) the proportions of n-6 to n-3 fatty acids can serve as essential predictors for diseases, especially type 2 diabetes mellitus. A review of Patel et al. (2022) showed that changes in fatty acids ratio coincide with a neurodegenerative disorder, coronary heart disease, hypertension, cancer, diabetes, obesity, rheumatoid arthritis and disease autoimmune.

Regarding the atherogenicity and thrombogenicity index, despite the lack of an established parameter for these values, the lower the result, the lower the risk of platelet
aggregation and coronary diseases due to a higher concentration of anti-atherogenic fatty
acids (monounsaturated and omega 3 and 6) (Turan et al., 2007). For the Gorgonzola
cheeses, results varied between 1.96 and 2.87 for the atherogenicity factor, and 2.76 and
4.29 for thrombogenicity.

The results presented in this study, regarding fatty acid profile and lipid quality
indexes and ratios, can have a direct correlation to the type of animal and its food
consumption (Chilliard; Ferlay, 2004), the seasonality of the pasture (Chion et al., 2010)
and the production region (Collomb et al., 2002), among other aspects that can interfere
in the quality of animal products.

CONCLUSIONS

The physicochemical characterization revealed that Gorgonzola cheese contains
high levels of proteins and lipids and, consequently, high energy values. This cheese
presented also elevated sodium level, with varied among the studied brands. With the
exception of butyric acid, all other acids showed a significant difference between the
brands. The composition in fatty acids presented principally saturated fatty acids,
resulting in low PUFA/SFA ratios.

Thus, based on the healthfulness parameters (content of sodium, fat, caloric value,
fatty acid ratios and lipid quality indexes), it can be concluded that Gorgonzola cheeses
should be consumed in moderation in order to avoid non-comunicable chronic diseases.

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