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Obtaining and characterizing "dulce de leche" prepared with sheep's and cow's milk in different proportions

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Abstract

The "dulce de leche", a viscous milk candy spread, is one of the most produced Brazilian dairy products. However, it is still mostly made from cow's milk and the sheep's milk rarely used in its production. This study aimed to obtain and physicochemically characterize the whole milks and the "dulce de leche" (DL) made from cow's (CM) and sheep's milk (SM), and CM:SM mixing rations of 100:0, 75:25, 50:50, 25:75 and 0:100. Milk's physicochemical analyzes were pH, titratable acidity, density, moisture, total solids, proteins, lactose, fats, and ash. DL physicochemical analyzes were the same of the milk plus yield, color, and texture profile analysis. From inclusion of sheep's milk, the DL presented higher values of total solids, ashes and protein, with lipids increased only from the proportion 50:50. Lactose decreased at the proportion 0:100, however, was similar to the 25:75 proportion. The moisture decreased as the inclusion of sheep's milk on the product. Regarding color, the difference from the standard sample was verified with the inclusion of higher proportions of sheep milk. The combination of sheep's milk and cow's milk is a technically viable alternative, as it considerably increases the nutritional value and yield of the product.

Keywords: ash; fat; lactose; proteins; total solids.

Practical Application: Milk processing industries.

1 Introduction

The increase in the demand for products with healthy and sustainable appeal has created opportunities for poorly exploited markets, such as the use of sheep's milk in dairy products. This raw material has been highlighted in Brazil as a sustainable alternative, with advantages for small and medium-sized rural producers, and the potential of adding value to industrialized dairy products (Peruzzi et al., 2016). Sheep's milk has great nutritional value due to its high content of proteins, lipids, minerals and essential vitamins. Physicochemical characteristics such as high viscosity, small fat globules and expressive amount of short chain fatty acids (Revers et al., 2016; Balthazar, et al., 2017b), which make it increasingly popular in the market due to its peculiar taste and higher nutrient content and functional properties. For this reason, it can be used as a raw material in cheese making (Albenzio et al., 2015), dulce de leche (Gaze et al., 2015), yogurt (Balthazar et al., 2015) and ice cream (Balthazar et al., 2017b).

The "dulce de leche" is one of the most produced dairy product in Brazil, which consists of a concentrated milk obtained by heat treatment with or without negative pressure, with the addition of ingredients, mainly sucrose, providing differential sensory and physicochemical characteristics compared to other dairy products and highly consumed in Latin America. In addition, some countries such as Brazil have exported to other economic blocs, such as the European Union and Asia Pacific Economic Cooperation, highlighting the process of expansion of the dulce de leche exportation market (Gaze et al., 2015).

"Dulce de leche" is still mostly made from cow's milk, while sheep's milk (which is commonly present in the production of cheeses) is rarely, if ever, used as a raw material (Chacón Villalobos et al., 2013). Furthermore, sheep's milk is significantly more expensive, and therefore less attractive from the standpoint of adding value to industrial products. However, combining both types of milk could be an alternative for adding nutritional value without considerably increasing the final product's price. The objective of this work was to obtain and physicochemically characterize the milks and "dulce de leche" made from the combination of cow's and sheep's whole milks, in different proportions.

2 Materials and methods

2.1 Raw material

Sheep's milk (SM) and cow's milk (CM) used in this research were purchased from regional producers and transported in thermal boxes containing ice. Both *in natura* milks were pasteurized slowly (65 °C for 30 minutes) using a Thermomix (Vorweker®), and then packed in 900 mL, duly sanitized plastic packages, for storage at -20 °C in a freezer (Consul®).

Fractions of the milks were thawed in a water bath (Tecnal®) at 60 °C, to prepare the mixtures, and standardized to 0.13 $g.L^{-1}$ sodium bicarbonate (Vetec®), followed by the production of the "dulce de leche" samples.

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2.2 Milks' characterization

The milks were individually characterized, and the mixtures were then performed according to the following CM:SM proportions: 100:0, 75:25, 50:50, 25:75, 0:100. The physicochemical analyzes were pH (method 943.02), acidity (method 945. 05), ash (method 945.46), moisture (method 990.20), total solids (method 990.20), proteins (method 991.20), lactose (by redox titration using alkaline $CuSO_4$) and lipids by the Gerber method (method 2000.18), in accordance with Association of Official Analytical Chemists (2016). Density and lactose analyses followed the methodology of the Instituto Adolfo Lutz (2008).

2.3 Milks' microbiological analyses

To confirm the efficiency of the milk pasteurization process, microbiological analyzes for mesophiles, psychrotrophs, molds, yeasts, *Salmonella*, *Staphylococcus aureus* and thermotolerant coliforms were carried out, following Association of Official Analytical Chemists (2000).

2.4 "Dulce de leche" production

"Dulce de leche" production followed a modified version of the methodology described by Chacón Villalobos et al. (2013). The proportions of CM:SM used to obtain the product were 100:0, 75:25, 50:50, 25:75, and 0:100. Using a Thermomix, each formulation went through three steps: (1) milk (1 L) and sucrose addition (crystal sugar, *União*®, 250 g.L^{-1}), heating up to 80 °C under constant stirring (800 rpm) until reaching 45 °Bx; (2) heating up to 90 °C and stirring (1100 rpm) until reaching 55 °Bx; (3) glucose (Glucosul®) addition (2 g.L^{-1}), heating up to 90 °C and stirring (1100 rpm) until reaching 70 °Bx.

2.5 Characterization of "dulce de leche" samples

Characterization of the samples followed Protocol nº 354/1997, which establishes the following parameters (g/100 g): moisture (max. 30.0); fat (6.0 to 9.0); ash content (max. 2.0) and proteins (min. 5.0). These analyses followed Association of Official Analytical Chemists (2000) methodologies. Lactose analysis was also performed by redox titration using alkaline $CuSO_4$ (Fehling's solution) (Instituto Adolfo Lutz, 2008). Color analysis was performed using a colorimeter system (Mini Scan EZ Hunterlab 4500L), in three scales: L* a* b*. The color difference between the formulations is given by ΔE*, according to Equation 1. The 100:0 (CM:SM) ratio was used as the standard. When $\Delta E^* < 1$, no color difference is perceived by the human eye; when $1 < \Delta E^* < 3$, small color differences may be perceived, depending on the tonality; and when $\Delta E^* > 3$, color differences are obvious to the human eye (Bodart et al., 2008).

$$\Delta \boldsymbol{E}^* = \sqrt{\left[(\Delta \boldsymbol{L}^*)^2 + (\Delta \boldsymbol{a}^*)^2 + (\Delta \boldsymbol{b}^*)^2\right]} \quad (1)$$

Texture profile analysis (TPA) was performed using a texture analyzer (CT3 Texture Analyzer, Brookfield®) with a 25.4 mm acrylic probe, 0.05 N trigger load, 2.00 mm/s test speed, where the following parameters were analyzed: hardness, elasticity, adhesiveness, cohesiveness and gumminess (Ferreira et al., 2012).

2.6 Statistical analysis

The "dulce de leche" formulations were performed in duplicate and the analyzes in triplicate. Samples were submitted to analysis of variance (ANOVA) and Tukey's test at 95% significance ($p \leq 0.05$), using *R* software.

3 Results and discussion

3.1 Milks' characterization

The results of the physicochemical analysis of CM, SM and their mixtures (Table 1) indicated higher values of density, titratable acidity, total solids, ashes, proteins, lipids and lactose when 0:100 CM:SM proportion was used. For instance, the analyzed parameters increased gradually along with the proportion of SM in the mixtures.

Sheep's milk has higher solid contents and, therefore, greater density values. The results showed that there were significant differences ($p \leq 0.05$) among 100:0, 25:75 and 0:100 samples. As shown, this study found a total solids value of 1.036 g.mL^{-1} in the 0:100 CM:SM mixture, in comparison to 1.028 g.mL^{-1} in the 100:0 mixture. This value is line with the current legislation, which establishes total solids values between 1.028 and 1.034 g.mL^{-1} (Brasil, 2002) for cow's milk. Our results to sheep's milk are similar to those found by Park et al. (2007) (1.034 to 1.038 g.mL^{-1}).

The higher titratable acidity found in the 0:100 CM:SM formulation may be explained by the availability of lactose in this

Table 1. Centesimal composition of the cow (CM) and sheep milks (SM) and their respective proportions (CM:SM): 100:0, 75:25, 50:50, 25:75, 0:100.

Parameters	CM:SM proportions				
	100:0	75:25	50:50	25:75	0:100
Density (g.mL^{-1})	1.028 ± 0.01 ^{d}	1.034 ± 0.17 ^{c}	1.034 ± 0.23 ^{c}	1.035 ± 0.12 ^{b}	1.036 ± 0.17 ^{a}
Titratable acidity (°D)	17.33 ± 0.58 ^{d}	21.67 ± 0.58 ^{c}	22.33 ± 0.58 ^{c}	25.67 ± 0.58 ^{b}	27.67 ± 0.58 ^{a}
pH	6.77 ± 0.02 ^{a}	6.61 ± 0.01 ^{b}	6.55 ± 0.01 ^{c}	6.46 ± 0.01 ^{d}	6.41 ± 0.01 ^{e}
Moisture (g/100 g)	88.88 ± 0.06 ^{a}	87.71 ± 0.07 ^{b}	87.45 ± 0.06 ^{c}	86.01 ± 0.12 ^{d}	80.80 ± 0.03 ^{e}
Total solids (g/100 g)	11.12 ± 0.06 ^{e}	12.29 ± 0.07 ^{d}	12.55 ± 0.06 ^{c}	13.99 ± 0.11 ^{b}	19.20 ± 0.03 ^{a}
Ashes (g/100 g)	0.71 ± 0.05 ^{b}	0.83 ± 0.03 ^{ab}	0.84 ± 0.05 ^{a}	0.88 ± 0.003 ^{a}	0.90 ± 0.06 ^{a}
Proteins (g/100 g)	3.28 ± 0.05 ^{e}	4.06 ± 0.05 ^{d}	4.52 ± 0.07 ^{c}	5.14 ± 0.07 ^{b}	5.66 ± 0.08 ^{a}
Lipids (g/100 g)	3.27 ± 0.06 ^{e}	3.95 ± 0.01 ^{d}	4.64 ± 0.03 ^{c}	5.18 ± 0.02 ^{b}	6.00 ± 0.10 ^{a}
Lactose (g/100 g)	3.74 ± 0.02 ^{e}	3.87 ± 0.03 ^{d}	4.03 ± 0.02 ^{c}	4.16 ± 0.04 ^{b}	4.26 ± 0.04 ^{a}

Values expressed as mean ± standard deviation. Different letters on the same line indicate a significant difference ($p \leq 0.05$) according to the Tukey test.

milk, given that titratable acidity is a function of the relationship between lactose availability and lactic acid production by microorganisms, leading to an increase in lactic acid (Revers et al., 2016). Results also showed that there was a significant difference ($p \leq 0.05$) among samples 100:0, 25:75 and 0:100. Park et al. (2007) reported sheep's milk had an acidity between 16 and 28 °D, while the titratable acidity of cow's milk was between 15 and 18 °D. Based on that, the values found on the present research are in accordance with published data.

The 100:0 CM:SM formulation had a higher pH when compared the 0:100 CM:SM formulation, with a gradual decrease ($p \leq 0.05$) in pH along the incorporation of SM in higher proportions. Sheep's milk's pH varies from 6.63 to 6.68, according to Park et al. (2007). The amounts of casein, phosphates and other acid components in the milk's dry matter cause this oscillation, with acidity being higher in milks with more protein content.

About moisture, there were statistical differences ($p \leq 0.05$) among the 50:50, 25:75 and 0:100 proportions. Abdelgawad et al. (2014) found moisture values close to those reported in this study regarding the 100:0 CM:SM and 0:100 CM:SM proportions (86.00% and 81.00%, respectively).

The total dry extract reflects the constituents of milk (fat, proteins, lactose and minerals), and sheep's milk has higher solids concentration than other species of milk (Fava, 2012). This explains the 19.20% of total solids (0:100 CM:SM), which was statistically different ($p \leq 0.05$) from the values found for 100:0 CM:SM proportion (11.12%).

The ash value in the 100:0 CM:SM proportion was 0.71%, which differed statistically from the others ($p \leq 0.05$). The values reported in the literature vary between 0.53-0.8 and 0.85-1.0 for cow's milk and sheep's milk, respectively (Guerra et al., 2008; Park et al., 2007).

The protein levels reported by Guerra et al. (2008) and Park et al. (2007) ranged from 3.2 to 7.0% for sheep's milk, and from 3.0 to 3.9% for cow's milk. These values were similar to those found here for the 0:100 and 100:0 CM:SM proportions. It can be noticed that as the inclusion of SM on the formulations, protein concentration increased, and all treatments were statistically different ($p \leq 0.05$).

According to the analyses, the 100:0 CM:SM proportion had a 45.5% fat value, lower than in the 0:100 CM:SM proportion. Brito et al. (2006) found 5.79% fat in milk from Lacaune sheep, which is below the values found here (6.0%). This may have occurred due to seasonal variations, lactation period and milking shift (Guerra et al., 2008).

Lactose contents in all proportions were statistically different ($p \leq 0.05$), and the values found here for cow's milk were lower than those reported in the literature: 4.3% according to Oliveira & Timm (2006). Lactose values for sheep's milk were lower than those found by Czarnobay et al. (2017), of 4.71%.

Microbiological analyses of cow and sheep's milks after slow pasteurization showed no indication of mesophiles, psychrotrophs, molds, yeasts, *Salmonella*, *Staphylococcus aureus* or thermotolerant coliforms.

3.2 Process of solids concentration for "dulce de leche" production

The yield of the 0:100 CM:SM formulation was higher when compared to the other formulations (Table 2), which can be explained by the sheep's milk solid's concentration. This amount increased together with the sheep's milk content in the mixture. Formulation 100:0 CM:SM had the lowest yield, confirming the importance of total solids for the cost-effectiveness and value conferred to this product. The 50:50, 25:75, and 0:100 CM:SM formulations showed no significant differences ($p \leq 0.05$) for yield, pointing that the substitution of up to 50% of cow's milk by sheep's milk may be advantageous in terms of product yield.

3.3 Characterization of "dulce de leche" samples

According to Table 3, moisture values decreased as the SM was included on the formulation, possibly due to the high levels of total solids present in sheep's milk, i.e., "dulce de leche"

Table 2. Yield values of "dulce de leche" formulations made from cow (CM) and sheep milks (SM) and their respective proportions (CM:SM): 100:0, 75:25, 50:50, 25:75, 0:100.

	CM:SM proportions				
	100:0	75:25	50:50	25:75	0:100
Yield (kg/L)	0.45 ± 0.21 [c]	0.48 ± 0.08 [b]	0.51 ± 0.07 [a]	0.52 ± 0.03 [a]	0.53 ± 0.02 [a]

Values expressed as mean ± standard deviation. Different letters on the same line indicate a significant difference ($p \leq 0.05$) according to the Tukey test.

Table 3. Centesimal compositions of "dulce de leche" formulations made from cow (CM) and sheep milks (SM) and their respective proportions (CM:SM): 100:0, 75:25, 50:50, 25:75, 0:100.

	CM:SM proportions				
Parameters	100:0	75:25	50:50	25:75	0:100
Moisture (g/100 g)	29.27 ± 0.21 [a]	28.61 ± 0.20 [b]	26.19 ± 0.20 [d]	27.44 ± 0.21 [c]	24.27 ± 0.19 [e]
Total solids (g/100 g)	70.73 ± 0.21 [e]	71.39 ± 0.20 [d]	73.81 ± 0.20 [b]	72.56 ± 0.21 [c]	75.73 ± 0.19 [a]
Ashes (g/100 g)	1.34 ± 0.03 [c]	1.49 ± 0.02 [b]	1.72 ± 0.05 [a]	1.70 ± 0.03 [a]	1.74 ± 0.04 [a]
Proteins (g/100 g)	6.29 ±0.07 [e]	7.58 ± 0.18 [d]	8.45 ± 0.02 [c]	9.12 ± 0.05 [b]	10.13 ± 0.07 [a]
Lipids (g/100 g)	4.00 ± 0.01 [c]	6.00 ± 0.01 [d]	9.33 ± 0.28 [b]	11.3 3± 0.28 [a]	11.50 ± 0.01 [a]
Lactose (g/100 g)	8.21 ± 0.15 [a]	8.08 ± 0.03 [a]	7.90 ± 0.22 [a]	7.75 ± 0.08 [ab]	7.36 ± 0.29 [b]

Values expressed as mean ± standard deviation. Different letters on the same line indicate a significant difference ($p \leq 0.05$) according to the Tukey test.

produced with higher amounts of sheep's milk had a lower moisture content. As the moisture content is inversely proportional to the solids content, the 0:100 CM:SM formulation, which had the highest solids value (75.73%), presented the lowest moisture. On the other hand, the 100:0 CM:SM formulation, which had the lowest solids value (70.73%), presented the highest moisture. All formulations differed statistically ($p \leq 0.05$) for moisture values and, consequently, for total solids as well. The moisture values seen in this study are in agreement with the 30% parameter determined by Normative Instruction No. 354/97 (Brasil, 1997). It is worth noting that low moisture improves the conservation of the "dulce de leche" (Demiate et al., 2001).

Concerning ashes analysis, there were statistical differences between the 100:0 CM:SM and 0:100 CM:SM formulations. These ash contents may be related to milk density, given that the higher the amount of minerals and organic substances, the greater the density. The increase in ash contents occurs during the "dulce de leche" manufacturing process, when milks loose water, leading to a higher concentration of minerals (Pellegrini et al., 2013). The protein contents of the "dulce de leche" formulations followed the same milk-composition pattern seen elsewhere, and protein values were statistically different for all formulations ($p \leq 0.05$). The 100:0 CM:SM formulation had the lowest protein content, while the 0:100 CM:SM formulation had the highest protein content.

Lipid contents in the formulations were 6.00% for the 100:0 CM:SM proportion and 11.50% for the 0:100 CM:SM proportion and differed statistically ($p \leq 0.05$). The physicochemical fat standard established by the Technical Regulation for the Identification and Quality of "dulce de leche" (Brasil, 1997) is a maximum of 9.0%. The 25:75 and 0:100 CM:SM formulations presented fat values above this standard, due to the naturally higher fat contents of the sheep's milk's, not yet regulated by Brazilian legislation. For ash, moisture and proteins, all formulations were in accordance with current legislation (Brasil, 1997).

The results obtained in this research were similar to those reported by Laguna (2000) for "dulce de leche" made from goat's milk: average moisture content (19.44%) lower than the maximum standard stipulated by the legislation for cow-milk "dulce de leche"; and average protein contents (13.14%) and lipids (11.5%) higher than official requirements (also for cow's milk), evidencing the differences between each type of milk.

The lactose concentration was higher in the 100:0 CM:SM than in the 0:100 formulation, decreasing along with the decrease in cow's milk contents. The lower lactose value of sheep's milk is possibly due to the relationship between lactose and total solids, providing better digestibility and can be considered an ideal cow's milk substitute for allergic people (Balthazar et al., 2017a).

Studies carried out by Niro et al. (2014) with different proportions of sheep's milk and cow's milk in cheese production showed that the addition of sheep's milk (18%) influenced nutritional composition, with higher protein (4.0%), fat (4.6%) and lactose (4.3%) values when compared to cheese made from 100% cow's milk (3.6% protein, 4.4% fat and 4.0% lactose). The increase in the proportion of sheep's milk in the formulations resulted in an increase of important constituents, such as proteins, and a decrease in lactose when compared to standard "dulce de leche" (100:0 CM:SM). As such, sheep's milk proves to be a viable alternative to improve the product's nutritional characteristics.

Results for the texture profile analysis of "dulce de leche" formulations are presented in Table 4. There were no significant hardness differences ($p \leq 0.05$) between the formulations, that is, the different proportions of sheep's milk did not influence this parameter. In regard to adhesiveness, only the 100:0 CM:SM formulation presented a significant difference ($p \leq 0.05$). Adhesiveness is defined as the energy required to overcome the attractive forces between the food surface and other materials in contact with it (Szczesniak et al., 1963). Formulations containing sheep's milk in different proportions led to a decrease in the adhesiveness, possibly due to the higher lipid contents of sheep's milk (Table 1).

Cohesiveness values in the studies conducted by Francisquini et al. (2016) were between 0.44 and 0.93 for commercial "dulce de leche" based on cow's milk, close to those seen in this study (0.82 to 0.96). Cohesiveness-wise, only the 75:25 CM:SM sample differed statistically from the others ($p \leq 0.05$).

Elasticity of the samples increased along with the proportion of sheep's milk in the formulations, which is probably due to the higher protein and lipid content of sheep's milk (Tables 1). Elasticity values were similar to those found by Francisquini et al. (2016), which ranged from 9.94 to 25.11 in commercial "dulce de leche".

Gumminess decreased along with the increase of sheep's milk in the mixtures (Table 4). The higher fat content (Table 1) in sheep's milk facilitates the disintegration of the sample. Thus, the formulations with the highest proportions of sheep's milk had the lowest gumminess values. Chewiness, hardness, adhesiveness, gumminess, cohesiveness and elasticity vary according to solids concentration, temperature and process time (Carvalho et al., 2017).

Table 4. Texture profile analysis of "dulce de leche" formulations made from cow (CM) and sheep milks (SM) and their respective proportions (CM:SM): 100:0, 75:25, 50:50, 25:75, 0:100.

Parameters	CM:SM proportions				
	100:0	75:25	50:50	25:75	0:100
Hardness (N)	0.27 ± 0.02 [a]	0.23 ± 0.03 [a]	0.25 < 0.01 [a]	0.22 ± 0.03 [a]	0.22 ± 0.03 [a]
Adhesiveness (mJ)	0.60 ± 0.10 [a]	0.13 ± 0.06 [b]	0.27 ± 0.06 [b]	0.17 ± 0.06 [b]	0.22 ± 0.07 [b]
Cohesiveness	0.86 ± 0.03 [b]	0.96 ± 0.02 [a]	0.83 ± 0.04 [b]	0.82 ± 0.03 [b]	0.82 ± 0.03 [b]
Elasticity (mJ)	7.30 ± 0.30 [c]	9.87 ± 0.43 [bc]	9.49 ± 0.72 [bc]	11.77 ± 0.73 [ab]	13.67 ± 0.21 [a]
Gumminess (N)	0.24 ± 0.01 [a]	0.21 ± 0.01 [b]	0.18 < 0.01 [c]	0.16 < 0.01 [c]	0.17 < 0.01 [c]

Values expressed as mean ± standard deviation. Different letters on the same line indicate a significant difference ($p \leq 0.05$) according to the Tukey test.

The 75:25 CM:SM formulation had the smallest amount of color deviation (ΔE^*) from the standard formulation (100:0 CM:SM), with a barely perceivable tonal difference. The 0:100, 50:50 and 25:75 formulations, however, presented greater, easily perceived differences. Among the formulations, none had color differences imperceptible to the naked eye ($\Delta E^* < 1$) when compared to the standard. This was due to the distinct characteristics of the different milk compositions, which influence color, flavor and aroma of the final product.

According to Shibao & Bastos (2011), among the existing browning reactions, the Maillard reaction, which occurs in a wide variety of foods including "dulce de leche", causes the most important changes in color, taste, nutritional value, antioxidant properties, and food texture. In fact, determinants for the course of the reaction are processing at temperatures above 40 °C, pH between 6 and 8, and water activity between 0.4 and 0.7. Sheep's milk has a higher lactose content than cow's milk, probably hindering the development of the Maillard reaction during the concentration stages. According to Fennema et al. (2010), the Maillard's start depends on the reaction of the aldehyde group of lactose with the ε-amino group of lysine. The light color is due to the low contents of reducing sugars (glucose) in the formulations used in this study.

Mixtures of milk from different species have a potential for dairy market, because together, they can improve the sensory and physicochemical properties, adding value to these products (Gomes et al., 2013; Queiroga et al., 2013). Further sensory analysis of "dulce de leche" formulations is recommended by qualitative methods. In the study performed by Pinto et al. (2018), with different fermented milk formulations, the authors demonstrated viable and effective application of sensory analysis, to clarify consumer perceptions and commercial viability.

4 Conclusion

The results obtained in this study show the greater nutritional richness of sheep's milk in comparison to cow's milk, pointing to the potential of this milk's use, both as exclusive raw material and mixture component, in the manufacturing of "dulce de leche". The combination of sheep's milk and cow's milk is a technical and nutritionally viable alternative, as it considerably increases the yield of the product. However, sensorial properties and the cost-effectiveness of this product should be considered.

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