Journal of Agricultural Science; Vol. 11, No. 17; 2019
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Microbiological and Microscopic Analysis of Sugarcane Syrup

Jhéssica Samara A. H. S. Belé[1], Carolina M. Vicentini-Polette[1], Sandra Regina Ceccato-Antonini[2], Marta Helena F. Spoto[3], Valdinei Luis Belini[4] & Marta Regina Verruma-Bernardi[2]

[1] Programa de Pós Graduação em Agroecologia e Desenvolvimento Rural, Centro de Ciências Agrárias, Universidade Federal de São Carlos, Araras, SP, Brazil

[2] Departamento de Tecnologia Agroindustrial e Socioeconomia Rural, Centro de Ciências Agrárias, Universidade Federal de São Carlos, Araras, SP, Brazil

[3] Departamento de Agroindústria, Alimentos e Nutrição, Universidade de São Paulo, Piracicaba, SP, Brazil

[4] Departamento de Engenharia Elétrica, Universidade Federal de São Carlos, São Carlos, SP, Brazil

Correspondence: Jhéssica Samara A. H. S. Belé, Centro de Ciências Agrárias, Universidade Federal de São Carlos, Rod. Anhanguera, km 174-SP-330, Araras, SP, CEP 13600-970, Brazil. Tel: 55-19-3543-2673. E-mail: jhessica_samara@hotmail.com

Received: July 20, 2019 Accepted: August 22, 2019 Online Published: October 15, 2019

doi:10.5539/jas.v11n17p210 URL: https://doi.org/10.5539/jas.v11n17p210

Abstract

Sugarcane syrup is defined as the product obtained by the concentration of sugarcane juice (*Saccharum officinarum* L.) or from melted cane rapadure. This product has good acceptance in the Brazilian market and can be used as a sweetener in substitution of refined sugar, besides containing important minerals. This study analyzed samples of sugarcane syrup based on its microbiological and microscopic properties. In total, 15 commercial brands of sugarcane syrups were analyzed. No brands had the presence of flat-sour thermophilic bacteria, total coliforms or *Escherichia coli*, while five brands were contaminated with mesophilic bacteria, molds, and yeasts. Microscopic analysis, performed under optical light transmission microscopy, revealed that 14 (93%) brands contained some kind of dirt or foreign material, with only one brand (M) according to the standards. The Brazilian standard in force (RDC, 2001) specifies the microbiological standards for food but does not contain important information for sugarcane syrup, and an update is required.

Keywords: food, food safety, contamination, microscopy

1. Introduction

Sugarcane syrup is defined as the product obtained by the concentration of sugarcane juice (*Saccharum officinarum* L.) or from the melted rapadure and presents as sensorial characteristics the syrupy and dense aspect (viscous), yellowish-amber, with its scent, and sweet taste (Brasil, 2005). However, this term cannot be confused with the molasses, a by-product resulting from the production of crystal sugar.

This product has good acceptance in the Brazilian market and contains important minerals such as iron, calcium, phosphorus, and magnesium (Taco, 2011). Sugarcane syrup is a food that can be used as a sweetener instead of refined sugar in culinary recipes, as well as being consumed pure or in mixtures with diverse types of cheese, or with flour, cookies, cakes or even served with yam or cassava. There are also suggestions for use in animal feeding (Chaves et al., 2003).

The production of sugarcane syrup in Brazil is an agro-industrial process, either traditional or artisanal, available in the market in the industrialized, natural and organic types (Braun, 2015). For small rural producers, the syrup has been a lucrative way of benefiting from the sugarcane by the use of affordable equipment and family labor (A. A. Delgado & A. P. Delgado, 1999), but broth sanitation procedures and quality control in the production process are often not utilized.

For Chaves et al. (2003), some of the most common problems that affect the quality of sugarcane syrup are the presence of fragments or whole insects, animal hair, soil, total and fecal coliforms, and fungi and yeasts, reflecting the lack of hygiene and care in the production process.

Sugarcane syrup often comes from non-standard techniques that can expose the final product to chemical and/or microbiological contamination, like the lack of adequate technology and equipment to remove impurities such as sand or soil that may be present in the sugarcane juice at the time of grinding, especially in small artisanal production facilities where there is no adequate process for cane washing with potable water, for example (Braun, 2015).

However, small adaptations in the production process such as cane washing with potable water and broth filtration, and some investment such as the acquisition of own and suitable cooking pot and insulated and sanitized place for the manufacture of sugarcane syrup would be enough to improve the quality of the product.

Microorganisms in the sugar manufacturing environment, such as bacteria, filamentous fungi, and yeasts, should be identified and monitored as they may affect the quality of the product and, in addition to losses in production, cause health hazards due to diseases caused by some of these microorganisms (Carvalho, 2010).

The sugarcane syrup has interesting attributes both in the food aspect because it is considered a nutritious and energetic food and has important nutrients, as in the economic and social issue, since it generates income for small and medium producers. However, there are few documents recording how this food is produced and monitored in terms of its nutritional, microbiological and microscopic quality.

In this context, it is important to develop studies on the product to increase knowledge and therefore its consumption potential, achieving greater reliability, raising the demand for the product and eventually increasing the income and technical development from small producers.

This study aimed to evaluate the hygienic-sanitary quality of commercial brands of sugarcane syrups by microbiological and microscopic evaluation, as well as to verify if there is specific legislation about the product in Brazil and if they are sufficient to maintain sugarcane syrup standard quality.

2. Method

2.1 Raw Material

The study was carried out at the Agrarian Sciences Center of the Federal University of São Carlos (UFSCar), Araras/SP, Brazil. Fifteen commercial brands of sugarcane syrup were evaluated, containing in their labels: product designation, net weight, ingredients and date of manufacture. Three samples of each brand from the same batch were collected.

2.2 Microbiological Evaluation

The analyses were carried out at the Agricultural and Molecular Microbiology Laboratory (LAMAM) of the DTAiSeR/CCA/UFSCar, following the methodology of the American Public Health Association and Official Methods of Analysis [APHA] (2017); AOAC (2016). The samples were analyzed in terms of the amount of total aerobic mesophilic bacteria (APHA 08: 2015), molds and yeasts (APHA 21: 2015), amount of spores of flat-sour thermophilic bacteria (APHA 25: 2015), total coliforms and *Escherichia coli* (Petrifilm-AOAC 991.14-3M Microbiology). Duplicates were performed on the three samples of each brand.

2.3 Microscopic Analysis

The observation of dirt and foreign matter followed the guidelines of AOAC method 945.79 (2005) with adaptations. 10 g of the sample were diluted in 125 mL of distilled water acidified with 2 mL of nitric acid (HNO_3), followed by vacuum filtration on a Buchner funnel with a filter paper, and dried at 105 °C. The sediments were observed by a magnifying glass (1.5× magnification factor) and a transmission optical microscope (model Motic BA210) equipped with a 10× objective. The identification of the elements found was recorded by photographs captured by a digital camera (model 2300, Moticam) coupled to the microscope. The program employed for image capturing was Motic images plus 2.0 from the Laboratory of Alcohol and Spirits Distillery (UFSCar/CCA). The procedure was performed on the three samples of each brand.

2.4 Analysis of the Results

The data obtained by the microbiological analysis were compared with references documented in the standard document of the National Agency of Sanitary Surveillance-ANVISA (Brasil, 2001), International Commission for Uniform Methods of Sugar Analysis (ICUMSA, 2004) and the National Canners Association (RTCM, 2008). The data on dirt analysis followed the national guidelines according to the standards of the National Agency of Sanitary Surveillance-ANVISA (Brasil, 2005).

3. Results and Discussion

3.1 Microbiological Analysis

According to the Brazilian legislation (Brasil, 2001), sugarcane syrup must present a maximum coliform limit of 10^2 Colony Forming Unit [CFU]/g at 45 °C and the absence of *Salmonella* in 25 g. The analysis of *Salmonella* was not performed considering its high cost and the fact that this microorganism would not survive during syrup cooking, which reaches temperatures up to 108 °C. The standard values used to compare the results obtained in the microbiological analysis (Table 2) are shown in Table 1.

Table 1. Reference values of the microbiological standards used for sugars

	ANVISA	National Canners Association	ICUMSA
Mesophilic bacteria	-	50 CFU/g	200 CFU/10 g
Molds/yeasts	-	50 CFU/g	20 CFU/10 g
flat-sour spores	-	50 sp/10 g	50 sp/10 g
Fecal coliforms	10^2 CFU/g	Absence	Absence

Of the 15 brands evaluated, five were not following the norms established by the international standards National Canners Association (RTCM, 2008) and ICUMSA (2004) (Table 2). About the count of total mesophilic bacteria, values of 5.4×10^2 CFU/g, 1.5×10^3 CFU/g, 6×10 CFU/g, 1.5×10^2 CFU/g and 9×10 CFU/g were obtained, indicated respectively by the letters A, B, C, F, and K, which are therefore not suitable for the international commercial standard.

Table 2. Results of the analysis of total aerobic mesophilic bacteria and molds and yeasts of commercial brands of sugarcane syrup

Sugarcane Syrup	Total aerobic mesophilic bacteria (CFU/g)			Fungi (molds and yeasts) (CFU/g)		
	A1	A2	A3	A1	A2	A3
A	4×10	5.4×10^2	4×10	4×10^2	2×10^2	9×10^2
B	4×10	<10	1.5×10^3	<10	<10	<10
C	6×10	<10	3×10	<10	2×10^2	<10
D	<10	<10	<10	<10	<10	<10
E	2×10	<10	2×10	<10	<10	<10
F	2×10	9×10	1.5×10^2	<10	<10	<10
G	<10	<10	<10	<10	<10	<10
H	<10	<10	<10	7×10^2	3×10^2	5×10^2
I	<10	<10	<10	<10	<10	<10
J	<10	<10	<10	<10	<10	<10
K	9×10	<10	<10	<10	3×10^2	3×10^2
L	<10	<10	<10	8.5×10^3	1.6×10^4	7×10^2
M	2×10	2×10	<10	<10	<10	<10
N	<10	<10	<10	<10	<10	<10
O	<10	<10	<10	<10	<10	<10

Note. *A1, A2, A3 = Samples. Means in the same column, followed by equal letters, did not differ significantly ($p \geq 0.05$) by the Tukey test.

Mesophilic aerobic microorganisms are those that multiply at optimal temperatures between 25 and 40 °C, minimum between 5 and 25 °C, and maximum between 40 °C and 50 °C. Total mesophilic aerobic count indicates bacterial populations in food, but it does not differentiate bacterial types and it is used to obtain general information on product quality, manufacturing practices, raw-materials used, processing conditions, handling, and shelf life (Tortora et al., 2012; Silva et al., 2017). It is not a safety indicator as it is not directly related to the presence of pathogens or toxins (Silva et al., 2017).

According to Franco and Landgraf (2003), the high count of this group of bacteria in perishable foods may indicate abuse during storage concerning the time/temperature binomial. This group of bacteria does not survive at elevated temperatures above 50 °C, so it would not survive during the production process of sugarcane syrup, that reaches temperatures up to 108 °C; thus, the brands (A, B, C, F, and K) that showed values above the maximum allowed for these bacteria were contaminated during potting, either by the use of contaminated packaging or by poor storage.

Although the different samples for each sugarcane syrup brand in this present study are from the same manufacturing batch, different degrees of contamination among them are noticed. This may be due to quality control failures during the manufacturing, bottling, or storage processes, indicating a need for greater quality control of the process.

Verruma-Bernardi et al. (2007), analyzing mesophilic bacteria in nine brands of brown sugar, observed that only three presented values within those recommended by the international standard of the National Canners Association and ICUMSA. Generoso et al. (2009), studying 31 brands, verified that 10 of them did not present good quality conditions in terms of mesophilic bacteria, indicating values higher than 50 CFU/g.

Regarding international standards, brand B (A3) (Table 2) presented about 30 times the maximum value allowed for mesophilic bacteria, therefore unacceptable to be consumed. As for the national standard, there is no standard that establishes the maximum amount of total aerobic mesophilic bacteria in sugarcane syrup, which would be of great importance, since considerable amounts of these bacteria indicate unsatisfactory hygienic-sanitary conditions and failure in the manufacturing process, since they only grow under specific temperature conditions (20 to 40 °C) (Franco & Landgraf, 2003).

About the analysis of molds and yeasts, five of the 15 brands analyzed (A, C, H, K, and L) did not conform to the maximum values allowed by international standards (RTCM, 2008; ICUMSA, 2004), once the lowest value found was 200 CFU/g (Table 2). The current national standard (Brasil, 2001) also does not establish an upper limit for the number of molds and yeasts. Brands A, H, and L showed contamination by molds and yeasts in the three samples analyzed, proving the lack of quality control and hygiene in the product manufacturing process.

Brand L showed high contamination in the three samples analyzed. This brand was noticeably more liquid and less viscous compared to the others, that is, it is likely that the sugarcane syrup did not reach its ideal cooking point until the time of filling, and it may contain high moisture content in the final product. According to Generoso et al. (2009), high humidity allows the development of microorganisms that cause the deterioration of the product. Silva; Parazzi (2003), performing a study with brown sugar, observed that samples with higher moisture content were those that contained the highest growth of mold and yeast.

Another possible cause for high contamination in brand L would be a failure during the potting or filling process. Reyes and Ortiz (2007) determined the minimum quality requirements for hydrolyzed honey by microbiological analysis and found that microbiologically it showed no contamination due to hot filling. Thus, it is believed that if the sugarcane syrup is potted still hot, soon after the end of cooking, much of the contamination by mold and yeast can be prevented.

Parazzi et al. (2009) conducted a microbiological study with brown sugar and found that only one of 12 samples studied was contaminated with 119 CFU/g and that mold contamination was considered a potential deterioration in food, mainly related to problems in the conservation and storage of products.

For the analysis of total coliforms and *Escherichia coli*, all brands comply, both nationally and internationally (Brasil, 2001; ICUMSA, 2004; RTCM, 2008). The presence of total coliforms and *Escherichia coli* in food is usually considered an indication of contamination due to inadequate hygiene and sanitation during food processing (Generoso et al., 2009). Coliform counts are widely used in the analysis of heat-treated foods. In this context, the presence of *E. coli*, for example, is indicative of inadequate heat treatments or likely subsequent contamination (Sousa, 2006), which was not verified in the present study. Generoso et al. (2009) and Parazzi et al. (2009) did not observe the presence of total coliforms and *E. coli* in brown sugar.

Brasil (2001) does not establish microbiological standards for the analysis of *flat-sour* bacteria (Table 1). For the international guidelines (ICUMSA, 2004; RTCM, 2008), only brands B, D, and K contained *flat-sour*, with amounts of 5, 10 and 5 spores/10 g, respectively. However, these values are within the permitted range (50 esp/10 g), indicating that all brands met the required standards. These results indicate that the quality of the sugarcane syrup analyzed was satisfactory in relation to this type of microorganism. The species of thermophilic bacteria are sporogenic and can survive during the sugar manufacturing process by withstanding high temperatures. Therefore, their low concentration indicates the quality and efficiency of the manufacturing process, as well as a good state of hygiene of the manufacturing site (Parazzi et al., 2009).

Jesus (2010) observed the presence of flat-sour bacteria in brown sugar. The author found that seven out of 10 samples were within the limits tolerated by international standards (ICUMSA, 2004; RTCM, 2008), whereas 3 samples did not meet established standards. Parazzi et al. (2009) observed that six samples from four commercial brands of brown sugar showed contamination by deteriorating flat-sour bacteria.

Brasil (2001, 2005) mentions the technical standards and some microbiological standards for sugarcane syrup, important characteristics to be observed in a food. However, the legislation still appears to be inadequate relative to other information such as the standard values for total aerobic mesophilic bacteria and molds and yeasts. These data are important because it allows verifying the food quality and its control guarantees to the manufacturer relevant information on the microbiological safety, providing better control to the processed food (Vasconcelos & Melo Filho, 2010).

3.2 Microscopic Analysis

The Association of Analytical Chemists International-AOAC (2005) defines as "foreign material" any foreign matter in the product, associated with questionable conditions or practices in production, storage or distribution, which include soils (light, heavy, screened) decomposed material, sand, earth, and glass.

Brasil (2014) defines "foreign material" as any non-constituent material associated with inappropriate conditions or practices in production, handling, storage or distribution and classifies it in:

- *Macroscopic*: detected by direct observation (to the naked eye), and can be confirmed with the aid of optical instruments.

- *Microscopic*: detected with the aid of optical instruments with a minimum optical magnification of 30 times.

- *Inevitable*: when they occur in the food even with the application of good practices.

- *Indicative of failures of good manufacturing practices*: live or dead arthropods, whole or in parts, exuviae, webs and excrement, undesirable parts of the raw material, human and other animal hair, sand, earth and other macroscopic particles, filamentous fungi and yeasts that are not characteristic of products, vertebrates or invertebrates not mentioned above, and other materials not related to the production process.

- *Indications of risks to human health*: insects such as cockroaches, ants, flies that reproduce or habitually contact feces, corpses, and trash, as well as the "kissing bug", at any stage of development, alive or dead, whole or in parts; rodents: whole or in pieces; excrement of animals, other than those of arthropods considered appropriate to the crop and storage; parasites: helminths and protozoa, at any stage of development, associated with human health problems; rigid, pointed, or sharp objects, equal to or greater than 7 mm, which may cause injury to the consumer, such as: bone and metal fragments; sliver; and hard plastic; rigid objects with diameters equal to or greater than 2 mm that can cause injury to the consumer, such as: stone, metal, teeth, whole or fragmented stone; fragments of glass of any size or shape and plastic films that may cause harm to the consumer's health.

- *Undesirable parts or impurities*: parts of plants or animals that interfere with the quality of the product, such as peels, peduncles, petioles, cartilage, aponeurosis, bones, feathers, and animal hair and carbonized particles of the food from processing or not removed by the same.

- *Risk*: a function of the likelihood of an adverse effect on health and the severity of such an effect, resulting in food hazards.

- *Vectors*: animals that carry pathogens from a host, from an origin or a place, carrying them to food, which can cause human health problems by eating contaminated food (Brasil, 2014).

Figure 1 shows images visualized in the samples of the sugarcane syrup analyzed.

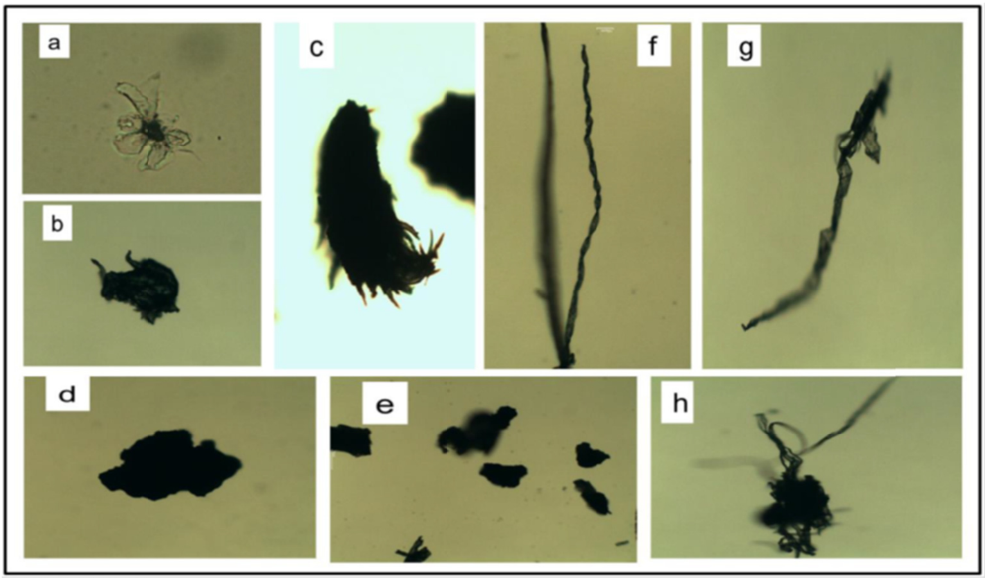

Figure 1. Photos of foreign objects visualized in samples of sugarcane syrup by light microscopy (10× objective): Fragments of insects (a, b, c), carbonized particles (d, e), unidentified objects (f, g, h)

Brands C, D and I presented objects that were not identified (Figure 1) due to the insufficiency of a recommended methodology of analysis for the sugarcane syrup and the lack of references available in the literature for comparison. Prado et al. (2010), performing dirt analysis with sugarcane juice, described unidentified materials in 2.2% of the 90 samples analyzed. According to Brasil (2014), any substances or agents of biological, chemical or physical origin, foreign to food, that are considered harmful to human health or that compromise its integrity, are considered contaminants.

Among the 15 brands of sugarcane syrup analyzed, 14 (93%) presented some type of dirt, and only brand M complied with the current Brazilian legislation (Brasil, 2014), with none occurrence of foreign matter in the sample (Table 3).

Table 3. Dirt occurrences in sugarcane syrup samples

Occurrence	Sugarcane syrup														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Insect fragments		X					X					X			
Carbonized particles	X			X	X	X	X	X		X	X	X		X	X
Unidentified			X	X					X						

The highest incidence of contamination occurred due to the presence of carbonized particles, appearing in 73.3% of the brands analyzed. This fact can be explained by the burning of the wood used in the manufacturing process when the food is handled in an artisanal manner, particularly in small properties, and the use of sugarcane harvested after burning.

Brands B, G, and L showed fragments of insects, which presuppose a lack of care with the hygiene during the production process. Insects, in addition to depositing their excrements on food, can contaminate the products with microorganisms that are adhered to their body and legs, which corroborates with the observed in the microbiological analysis (Table 2) in which brands B and L presented contamination by microorganisms.

Prado et al. (2010) verified that from the 90 samples of sugarcane juice analyzed, 29 (32.2%) were in disagreement with the microscopic parameters, presenting dirt as fragments of insects, rodents, synthetic fibers, sand, earth, and carbonized particles.

Matos (2018) analyzed the foreign matter and dirt in 27 samples of brown sugar and observed that 55.56% of the samples contained some kind of foreign matter or dirt.

The high amounts of dirt found in the analysis may be associated with several factors, such as the use of sugarcane harvested with burning, inefficient cleaning of sugarcane after cutting, as a lack of washing with potable water, for example, and little or no filtration of the broth after milling. Even with all the taken care during harvest, transport, and storage, the extracted broth still contains several coarse impurities, especially bagasse and soil, which must be removed.

Products such as sugarcane and açaí, for example, may serve as oral transmission vehicles for diseases, such as acute Chagas disease transmitted by the protozoan *Trypanosoma cruzi* by fecal material from hematophagous insects such as the "kissing bug", for example (Prado et al., 2010).

Studies that assess contamination by foreign matter, pests and vectors contribute significantly to improve the quality of food and vegetable-based beverages (Prado et al., 2010).

In conclusion, it was verified that of the 15 brands of sugarcane syrup evaluated, only one (M) met all the analyzed standards adopted by the national and international legislations used in the research. The other brands did not comply with the national and international legislation in force in the number of mesophilic bacteria, mold/yeast, and foreign matters. Also, the presence of dirt was visible in 93% of the analyzed brands, indicating that the quality control is deficient, being necessary more orientations for the producers of sugarcane syrup.

The RDC standard 12 of January 2nd, 2001, which specifies the microbiological standards for food, does not contain enough information to indicate the microbiological quality of the sugarcane syrup, such as the maximum value for quantities of mesophilic bacteria and molds and yeasts; therefore, an update is required, and this study indicates the need to develop adequate technical standards for the production of sugarcane syrup in Brazil, so that it has an identity and a quality standard and can be safely consumed, thus promoting food safety, consumer confidence and valorization of the product.

Acknowledgements

This study was performed with the support of the Coordination of Improvement of Higher Education Personnel-Brazil (CAPES)-Financing code 001.

References

AOAC International. (2016). *Official Methods of Analysis of AOAC International (OMA) Online*. AOAC International. Retrieved from http://www.eoma.aoac.org

AOAC. (2005). *Official methods of analysis of AOAC international*. Gaithersburg: Association of Official Analytical Chemists.

APHA (American Public Health Association). (2015). In N. Silva, V. C. A. Junqueira, N. F. A. Silveira, M. H. Taniwaki, R. A. A. Gomes, & M. M. Okazaki (Eds.), *Manual de métodos de análise microbiológica de alimentos e água* (p. 535). São Paulo: Blucher.

Brasil. (2001). Agência Nacional de Vigilância Sanitária (ANVISA). *Resolução RDC nº 12, de 02 de Janeiro de 2001*. Retrieved from http://www.anvisa.gov.br

Brasil. (2005). Agência Nacional de Vigilância Sanitária (ANVISA). *Resolução RDC nº 271, de 22 de Setembro de 2005*. Retrieved from http://www.anvisa.gov.br

Brasil. (2014). Agência Nacional de Vigilância Sanitária (ANVISA). *Resolução RDC nº 14, de 28 de Março de 2014*. Retrieved from http://www.anvisa.gov.br

Braun, C. L. K. (2015). *Avaliação físico-química, microbiológica e de sujidades de rapaduras artesanais produzidas na baixada cuiabana, Mato Grosso, Brasil*. Cuiabá (MT): Instituto Federal de Ciência e Tecnologia do Mato Grosso.

Carvalho, I. T. (2010). *Microbiologia dos alimentos*. Retrieved January 8, 2019, from http://pronatec.ifpr.edu.br/wpcontent/uploads/2013/06/Microbiologia_dos_Alimentos.pdf

Chaves, J. B. P., Fernandes, A. R., & Silva, C. A. B. (2003). *Projetos de Empreendimentos Agroindustriais—Produtos de origem Vegetal* (1st ed., Chapter 4, pp.119-169). Editora UFV. https://doi.org/10.13140/rg.2.1.3198.5524

Delgado, A. A., & Delgado, A. P. (1999). *Produção do açúcar mascavo, rapadura e melado*. Piracicaba (SP): Alves.

Franco, B. D. G. M., & Landgraf, M. (2003). *Microbiologia dos alimentos*. São Paulo: Atheneu.

Generoso, W. C., Borges, M. T. M. R., Ceccato-Antonini, S. R., Marino, A. L. F., Silva, M. V. M., Nassu, R. T., & Verruma-Bernardi, M. R. (2009). Avaliação microbiológica e físico-química de açúcares mascavos comerciais. *Revista Instituto Adolfo Lutz, 68*(2), 259-268.

IAL (Instituto Adolfo Lutz). (2008). *Normas analíticas do Instituto Adolfo Lutz: Métodos químicos e físicos de composição de alimentos*. São Paulo: Instituto Adolfo Lutz.

ICUMSA. (2004). *International Commission for Uniform Methods of Sugar Analysis*. England.

Jesus, D. A. (2010). *Qualidade microbiológica de amostras de açúcar mascavo*. Piracicaba (SP): Universidade de São Paulo.

Matos, J. A. M. (2018). *Análise de matérias estranhas e sujidades no açúcar mascavo*. Campo Mourão (PR): Universidade Tecnológica Federal do Paraná.

Melo Filho, A. B., & Vasconcelos, M. A. S. (2011). *Química de alimentos*. Recife: UFRPE.

Parazzi, C., Jesus, D. A., Lopes, J. J. C., & Valsechi, O. A. (2009). Análises microbiológicas do açúcar mascavo. *Bioscience Journal, 25*(3), 32-40. Retrieved from http://www.seer.ufu.br/index.php/biosciencejournal/article/view/6906

Prado, S. D. P. T., Bergamini, A. M. M., Ribeiro, E. G. A., Castro, M. D. C. S., & Oliveira, M. A. D. (2010). Avaliação do perfil microbiológico e microscópico do caldo de cana in natura comercializado por ambulantes. *Revista Instituto Adolfo Lutz, 69*(1), 58-59.

Reyes, A. E. F., & Ortiz, R. K. (2007). Determinación de Requisitos Mínimos de calidad panela para, azúcar orgánico, y miel hidrolizada en la provincia de Imbabura (9f., Artículo Científico (Tesis Previa Ingeniero Agroindustrial), Universidad Técnica del Norte, Ibarra, Ecuador).

RTCM. (2008). *Roteiro para treinamento de controle microbiológico do açúcar*. Piracicaba: Fermentec.

Silva, A. R., & Parazzi, C. (2003). *Monitoramento microbiológico do açúcar mascavo*. Congresso de Iniciação Científica, 11, São Carlos, São Paulo. *Anais...* São Carlos: UFSCar.

Silva, N., Junqueira, V. C. A., Silveira, N. F. A., Taniwaki, M. H., Gomes, R. A. R., & Okazaki, M. M. (2017). *Manual de métodos de análise microbiológica de alimentos e água* (p. 535). São Paulo: Blucher.

Sousa, C. P. (2006). Segurança alimentar e doenças veiculadas por alimentos: Utilização do grupo coliforme como um dos indicadores de qualidade de alimentos. *Revista APS, 9*(1), 83-88.

Steffens, C., Orsolin, D., Rosa, C. D., & Steffens, J. (2015). Redução do tempo no processo de cozimento de mortadela e avaliação da qualidade final do produto. *Ciência Animal Brasileira, 16*(4), 589-597. https://doi.org/10.1590/1089-6891v16i430548

TACO. (2011). *Tabela Brasileira de composição de alimentos* (pp. 57-60). NEPA-UNICAMP. Retrieved from http://www.cfn.org.br/wp-content/uploads/2017/03/taco_4_edicao_ampliada_e_revisada.pdf

Tortora, G. J., Funke, B. R., & Case, C. L. (2012). Eucariotos: Fungos, algas, protozoários e helmintos. In A. M. Silva et al. (Ed.), *Microbiologia* (10th ed., pp. 329-366). Porto Alegre: ARTMED.

Vasconcelos, M. A. S., & Melo Filho, A. B. (2010). Conservação de alimentos. *Escola Técnica Aberta do Brasil, 1*(1), 11-115. Retrieved from http://redeetec.mec.gov.br/images/stories/pdf/eixo_prod_alim/tec_alim/181012_con_alim.pdf

Verruma-Bernardi, M. R., Borges, M. T. M. R., Lopes, C. H., Della-Modesta, R. C., & Ceccato-Antonini, S. R. (2007). Avaliação microbiológica, físico-química e sensorial de açúcares mascavos comercializados na cidade de São Carlos-SP. *Brazilian Journal of Food Technology, 10*(3), 205-211. Retrieved from http://www.ital.sp.gov.br/bj/artigos/html/busca/PDF/v10n3293a.pdf

Vilela, D. C. (2016). *Avaliação da qualidade físico-química de amostras de melado*. Campo Mourão: Universidade Tecnológica Federal do Paraná.