Short communication: Characterization of molasses chemical composition

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ABSTRACT

Beet and cane molasses are produced worldwide as a by-product of sugar extraction and are widely used in animal nutrition. Due to their composition, they are fed to ruminants as an energy source. However, molasses has not been properly characterized in the literature; its description has been limited to the type (sugarcane or beet) or to the amount of dry matter (DM), total or water-soluble sugars, crude protein, and ash. Our objective was to better characterize the composition of cane and beet molasses, examine possible differences, and obtain a proper definition of such feeds. For this purpose, 16 cane and 16 beet molasses samples were sourced worldwide and analyzed for chemical composition. The chemical analysis used in this trial characterized 97.4 and 98.3% of the compounds in the DM of cane and beet molasses, respectively. Cane molasses contained less DM compared with beet molasses (76.8 ± 1.02 vs. 78.3 ± 1.61%) as well as crude protein content (6.7 ± 1.8 vs. 13.5 ± 1.4% of DM), with a minimum value of 2.2% of DM in cane molasses and a maximum of 15.6% of DM in beet molasses. The amount of sucrose differed between beet and cane molasses (60.9 ± 4.4 vs. 48.8 ± 6.4% of DM), but variability was high even within cane molasses (39.2–67.3% of DM) and beet molasses. Glucose and fructose were detected in cane molasses (5.3 ± 2.7 and 8.1 ± 2.8% of DM, respectively), showing high variability. Organic acid composition differed as well. Lactic acid was more concentrated in cane molasses than in beet molasses (6.1 ± 2.8 vs. 4.5 ± 1.8% of DM), varying from 1.6 to 12.8% of DM in cane molasses. Dietary cation-anion difference showed numerical differences among cane and beet molasses (7 ± 53 vs. 66 ± 45 mEq/100 g of DM, on average). It varied from −76 to +155 mEq/100 g of DM in the cane group and from +0 to +162 mEq/100 g of DM in the beet group. Data obtained in this study detailed differences in composition between sources of molasses and suggested that a more complete characterization could improve the use of molasses in ration formulation.

Key words: molasses, chemical composition, variability

Short Communication

Beet and cane molasses are produced worldwide as a by-product of sugar extraction and are widely used in animal nutrition. Due to their composition, they are fed to ruminants as an energy source, and interest in their use is still current (Martel et al., 2011; Siverson et al., 2014). Previous studies showed positive effects of molasses addition on milk fat, FCM, ruminal ammonia, MUN, and fiber digestibility (Broderick and Radloff, 2004; Brito et al., 2015; de Ondarza et al., 2017). Addition of molasses could also be associated with nonnutritive and dietetic benefits: animals prefer sweetened diets (Murphy et al., 1997); thus, molasses generally stimulates DMI. Related to this, field observations suggest that molasses or molasses-based liquid feeds could affect animal sorting behavior, with a positive effect on the consumption of longer particles in TMR (DeVries and Gill, 2012). A frequent alternative is to add water whenever the diet is considered too dry (i.e., where hay instead of silage represents the main forage source). During warmer months, however, addition of water could lead to spoilage, thus decreasing the palatability of the diet and causing health problems in the animal. In such conditions, molasses would act positively because it is not associated with spoilage or molds. From a composition standpoint, sugars represent the main component of molasses. Sugars are rapidly fermented in the rumen, but the end products differ from those obtained by starch fermentation (Penner and Oba, 2009). Previous studies indicate that replacing starch

with molasses or molasses-based liquid feeds would result in positive effects on rumen pH (Broderick and Radloff, 2004; Oelker et al., 2009; Brito et al., 2017). However, in general, molasses is not properly characterized in the literature, and its description is related to the type (sugarcane or beet) or the amount of DM, total or water-soluble sugars, CP, and ash (Broderick and Radloff, 2004; Brito et al., 2015). Other studies have better described molasses, but the final results still lack in several components, such as organic acids or DCAD (Olbrich, 1963; Bortolussi and O'Neill, 2006). Consequently, a representative part of the DM of molasses remains unknown because sugars, CP, and ash are scarcely sufficient to reach 80% DM on average.

The objective of this study was to better characterize the composition of cane and beet molasses, underline possible differences, and obtain a proper definition of such feeds. For this purpose, 16 cane and 16 beet molasses samples were sourced worldwide and analyzed for chemical composition. In particular, 7 cane molasses samples were from Central and North America, 5 were from Asia, 2 were from Africa, and 1 each was from Europe and Australia. Beet molasses was sampled in Europe (12), North America (2), and Africa (2). Dry matter was determined according to AOAC International (1990) official method 934.01, except for dried quartz sand, which was added to each vessel. Ash content was calculated as reported in AOAC International (1990) official method 900.02 for this specific feed. Crude protein determination was carried out following AOAC International (1990) official method 990.03. Starch and other carbohydrates, such as dextran, levan, and araban, were determined with polarimetric procedure 10520:1997(en) (ISO, 1997). For sugar determination, samples were clarified using a commercial kit based on Carrez reagents (Sigma-Aldrich S.r.l, Milan, Italy). After this procedure, glucose, fructose, sucrose, galactose, raffinose, arabinose, and xylose were extracted and quantified using an enzymatic method according to the manufacturer's manual (Megazyme International Ltd., Bray, Ireland). Ash was recovered to quantify Ca, Mg, Na, and K with inductively coupled plasma spectrometry, whereas organic acids (lactic, acetic, butyric, propionic, citric, malic, formic, aconitic, glycolic, and oxalic) and other components (sulfates, phosphates, chlorides, and nitrates) were measured using ionic HPLC (Metrohm Italiana Srl, Origgio, Italy) according to methods UNI EN ISO 10304-1 and 14911-1998 (ISO, 1998, 2007).

Statistical analysis was performed using JMP software (version 12.0 Pro, SAS Institute Inc., Cary, NC). Then, a principal component analysis was carried out using the FACTOR procedure of SAS (version 9.13,

SAS Institute Inc.) as described by Gallo et al. (2016). The analysis was conducted to evaluate variability among and across cane or beet molasses.

Overall, determinations of the different components characterized, on average, 97.4 and 98.3% of the DM in cane and beet molasses, respectively. Analytical results are reported in Tables 1 and 2. Within the cane molasses group, DM ranged from 75.72% to 79.56%, with an average of 76.8%. An average of 78.3% DM was observed in beet molasses, with a minimum of 74.1% and a maximum of 78.9%. Ash was numerically higher in cane molasses (13.1% of DM) than in beet molasses (11.7% of DM), with a maximum value of 18.5% of DM and a minimum value of 6.5% of DM in beet molasses. The CP concentration differed among and within groups, being 6.7 ± 1.8 and $13.5 \pm 1.4\%$ of DM in cane and beet molasses, respectively, and ranged from a minimum of 2.2% of DM in cane molasses to a maximum of 15.6% of DM in beet molasses. This difference could be related to specific molecules occurring in sugar beet, such as betaine. Betaine, a nitrogen compound that is widely used in the cosmetic, health, and pharmaceutical industries as well as in animal nutrition (Fernández-Fígares et al., 2002; Escudero and Olga Ruiz, 2011), promotes growth and modulates lipid accumulation.

Sugar profile differed among samples. Sucrose was the most represented in both cane and beet molasses, although its concentration varied even within group. In cane molasses, an average of 48.8% of DM was observed, ranging from 39.2% to 67.3%. Beet molasses showed a numerically higher sucrose concentration (60.9% of DM on average), with a minimum of 46.5% and a maximum of 66.1%. Glucose and fructose had an average concentration of 8.1 and 5.3% of DM, respectively, in cane molasses and were barely detectable in beet molasses (0.3% of DM on average for both). The ranges for cane molasses were wide, with maximum values of 14.3 and 12.1% of DM and minimum values of 2.3% and 1.3% of DM for fructose and glucose, respectively. Other analyzed sugars (galactose, raffinose, arabinose, and xylose) were almost undetectable, and even the sum of maximum values was lower than 1% of DM in cane molasses. The only exception was raffinose in beet molasses, which was 0.6% of DM on average, but with a maximum value of 2.2% of DM. This finding is in line with that observed by Olbrich (2006), who identified sucrose and raffinose as the 2 major sugars in German beet molasses. Reasons for these differences could be related to the extraction process applied as well as the origin of the molasses. Sucrose is a disaccharide that comprises glucose and fructose. Uptakes of these 2 sugars are usually associated, and both represent a major

Table 1. Descriptive statistics of the chemical composition (% of DM unless otherwise noted) of cane molasses

Item	Average	SD	Minimum	Maximum
DM	76.8	1.0	75.7	79.6
CP	6.65	1.79	2.22	9.31
Total sugars	62.3	4.7	57.0	71.0
Sucrose	48.8	6.4	39.2	67.3
Glucose	5.29	2.69	1.30	12.07
Fructose	8.07	2.83	2.30	14.28
Raffinose	0.03	0.00	0.02	0.03
Galactose	0.04	0.00	0.04	0.04
Arabinose	0.01	0.02	0.00	0.04
Xylose	ND[1]	ND	ND	ND
Starch	0.33	0.25	0.06	1.07
Levans	0.86	0.26	0.26	1.21
Dextrans	0.79	0.42	0.27	1.63
Arabans	0.20	0.05	0.06	0.28
Aconitic acid	1.42	0.85	0.24	3.78
Lactic acid	6.10	2.82	1.62	12.75
Malic acid	0.10	0.05	0.03	0.21
Citric acid	0.13	0.04	0.08	0.22
Pyrocarbonic acid	0.34	0.13	0.18	0.62
Oxalic acid	0.06	0.02	0.04	0.09
Glycolic acid	0.00	0.00	0.00	0.00
Acetic acid	0.44	0.28	0.16	1.04
Ash	13.1	1.5	10.2	16.3
Ca	1.39	0.55	0.82	3.13
Mg	0.43	0.14	0.19	0.63
Na	0.08	0.10	0.01	0.42
K	1.82	1.91	0.31	7.99
Sulfates	2.09	0.88	0.81	4.09
Sulfur[2]	0.69	0.29	0.27	1.36
Phosphates	2.03	0.77	0.70	2.97
Nitrates, mg/kg	464	337	17	999
Chlorides, mg/kg	60	86	1	340
DCAD,[3] mEq/100 g	7	53	−76	155

[1]ND = not detectable.

[2]Sulfur obtained from sulfates considering their respective molecular weights.

[3]Calculated as DCAD, mEq/100 g = [K (% of DM)/0.039 + Na (% of DM)/0.023] − [Cl (% of DM)/0.0355 + S (% of DM)/0.016].

substrate for microbial fermentations. However, glucose and fructose could undergo different fermentation pathways (Luick et al., 1957). Thus, considering the variability observed within group (cane or beet), these data suggest that a more accurate analysis is required to properly characterize the molasses.

Differences were also observed in organic acids. Lactic acid was more concentrated in cane molasses than in beet molasses (6.1 and 4.5% of DM, respectively), varying from a minimum of 1.6% to a maximum of 12.8% of DM among cane molasses. Aconitic acid was found only in cane molasses (1.4% of DM on average), whereas glycolic acid was found in beet molasses (0.25% of DM on average). Other analyzed acids (acetic, butyric, propionic, citric, malic, formic, glycolic, and oxalic) were poorly represented in both cane and beet molasses. The total sum of acids ranged from 2.4% to 18.7% of DM in cane molasses, whereas it was a minimum of 4.1% and a maximum of 11.9% of DM in beet molasses. Organic acids are not so frequently quantified when molasses is

added to a diet. However, considering their variability, such fractions should be determined because organic acids could affect rumen metabolism, leading to different consequences in terms of animal health and performance, as underlined by other authors in respect to silages (Kung et al., 2018).

Starch, dextran, levan, and araban were 2.2% of DM on average in cane molasses and <1% of DM in beet molasses. Due to the low concentration, the variability range was also narrow. Sulfates, phosphates, and chlorides had a higher concentration in cane molasses, which showed a numerically lower DCAD compared with beet molasses (7 ± 53 vs. 66 ± 45 mEq/100 g of DM). Within the cane group, DCAD varied from −76 to +155 mEq/100 g of DM in the cane group and from 0 to +162 mEq/100 g of DM in the beet group. The observed DCAD variability across samples underlines the importance of this determination when molasses is added to the diet. Even with a similar amount of total sugars, different molasses types could have a completely

Table 2. Descriptive statistic of the chemical composition (% of DM unless otherwise noted) of beet molasses

Item	Average	SD	Minimum	Maximum
DM	77.6	3.2	67.0	80.9
CP	13.5	1.4	10.7	15.6
Total sugars	62.1	3.9	50.6	68.4
Sucrose	60.9	4.4	46.5	66.1
Glucose	0.28	0.48	0.02	1.96
Fructose	0.29	0.30	0.01	0.87
Raffinose	0.60	0.56	0.12	2.18
Galactose	0.03	0.00	0.02	0.03
Arabinose	0.01	0.01	0.00	0.05
Xylose	0.01	0.00	0.00	0.01
Starch	0.08	0.04	0.02	0.17
Levans	0.47	0.16	0.15	0.71
Dextrans	0.09	0.04	0.02	0.19
Arabans	0.06	0.02	0.03	0.10
Aconitic acid	ND[1]	ND	ND	ND
Lactic acid	4.51	1.83	1.77	7.13
Malic acid	0.08	0.04	0.02	0.13
Citric acid	0.30	0.12	0.11	0.50
Pyrocarbonic acid	2.77	0.52	1.74	3.76
Oxalic acid	0.03	0.01	0.02	0.05
Glycolic acid	0.25	0.04	0.18	0.32
Acetic acid	0.42	0.12	0.20	0.60
Ash	11.7	2.5	6.5	18.5
Ca	0.30	0.35	0.02	1.24
Mg	0.02	0.02	0.00	0.09
Na	0.62	0.43	0.05	1.45
K	2.44	1.33	0.65	5.54
Sulfates	0.61	0.41	0.17	1.84
Sulfur[2]	0.20	0.14	0.06	0.61
Phosphates	0.76	0.38	0.31	1.65
Nitrates, mg/kg	55	29	16	116
Chlorides, mg/kg	3,974	2,236	411	8,056
DCAD,[3] mEq/100 g	66	45	0	162

[1]ND = not detectable.

[2]Sulfur obtained from sulfates considering their respective molecular weights.

[3]Calculated as DCAD, mEq/100 g = [K (% of DM)/0.039 + Na (% of DM)/0.023] − [Cl (% of DM)/0.0355 + S (% of DM)/0.016].

different anion:cation ratio, with possible effects on animal health and performance. For example, given a ration for close-up cows (270 d of pregnancy) formulated with corn silage, grass hay, corn meal, soybean meal, and a mineral-vitamin supplement, such ration would result in a DCAD of ~39 mEq/100 g. Substituting corn meal with the molasses at opposite values (−76 and +155 mEq/100 g), the final DCAD would be +38 and +48 mEq/100 g. As reported in the literature, a proper balance is required to avoid the occurrence of disease in different stages of lactation (Block, 1994; Goff and Horst, 1997; Hu and Murphy, 2004) or in animals under stressful environmental conditions (West et al., 1991, 1992; Sanchez et al., 1994).

Sample distribution resulting from the principal component analysis is reported in Figure 1. Range of variability was wide among the samples and even within the same group, especially in cane molasses. In conclusion, the obtained results demonstrate that differences in composition could occur among molasses.

Defining a molasses as cane or beet is important but not sufficient to properly evaluate its potential nutritional role. As reported in several studies in which molasses was added to the diets, determination of ash, CP, total sugars, and few other components represents partial identification and does not seem appropriate for characterizing such feeds. Molasses is a good source of fermentable sugars, but other components are present as well, with potential effects on animal health status or production performance. Moreover, from a scientific standpoint, use of molasses, which can be similar in terms of amount of total sugars or protein but different in organic acids or in minerals, could lead to different results across studies, as observed by other authors (Firkins et al., 2008; Baurhoo and Mustafa, 2014; Ghedini et al., 2018). Thus, this study underlines that a more accurate description and characterization of molasses is possible and strictly required, especially if its use in animal feed has to be fully optimized.
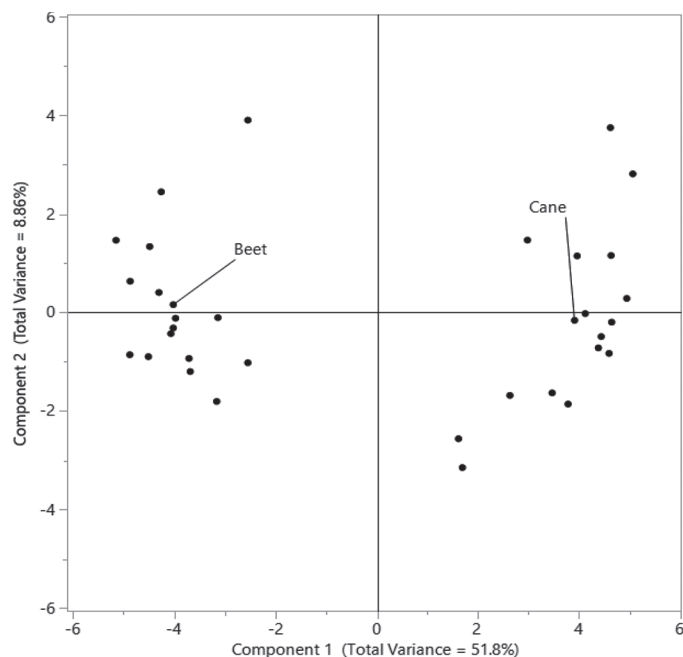
Figure 1. Samples distribution. Principal component analysis results for molasses variability. Distance between dots is inversely proportional to the similarity among samples.

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