Foods 2024 Conference

The 5th International Electronic Conference on Foods

28–30 October 2024 | Online

STUDY OF THE PRESENCE OF BIOGENIC AMINES IN SILAGE DESTINED FOR ANIMAL FEED

Vilar Cabrera, P.; Tomás Fornés, D.; Barat Baviera, J. M.; Fuentes López, A.

INTRODUCTION & OBJECTIVE

Silage is a technique that preserves animal fodder via fermentation. The health hazard associated with silage is the presence of pathogenic microorganisms and/or their metabolites, including mycotoxins or biogenic amines (BAs). BAs can also occur in the rumen produced by microbial flora during normal fermentation. Then ruminants could receive these substances from both dietary and microbial sources. High exposure to BAs provokes lowered intake and is also linked to acute and subacute toxicity. Moreover, several studies have demonstrated that low levels of BAs have adverse effects on both growth performance and meat quality.

This work aimed to evaluate the presence of 6 BAs in different types of silage destined for animal feed.

MATERIAL & METHODS

- **ANALYZED SAMPLES**: 18 different silages (maize, grass, *ryegrass*, unifeed…)
- **BIOGENIC AMINES (BAs) DETERMINED**: Putrescine (Put), Cadaverine (Cad), Histamine (His), Tyramine (Tyr), Spermidine (Spd), Spermine (Spm)

DETERMINATION OF BIOGENIC AMINES IN SILAGES

- **SAMPLE PREPARATION** (Jia & Yu, 2022)

LIOFILIZATION → EXTRACTION (TCA) → CENTRIFUGATION /FILTRATION

- **DERIVATIZATION** (Pekcici et al., 2021)

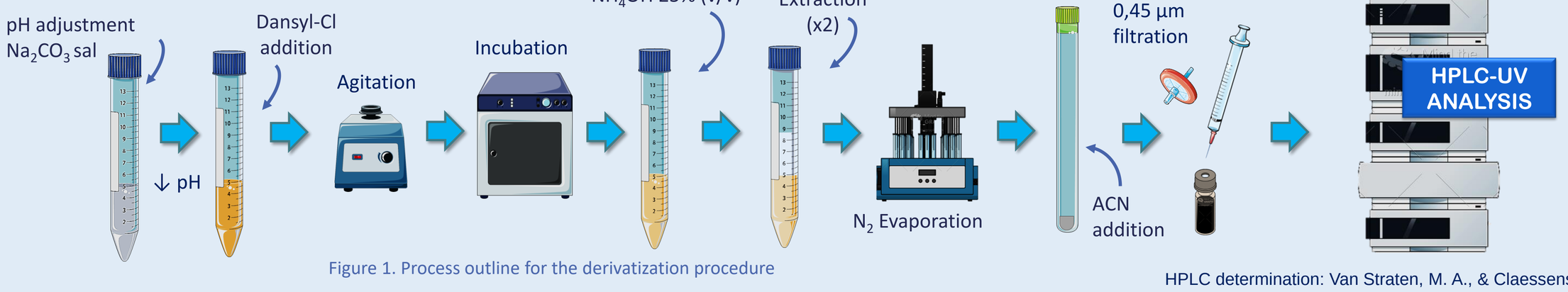
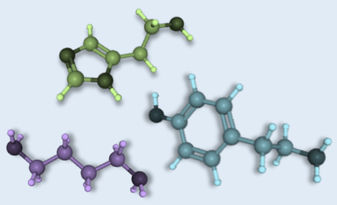


Figure 1. Process outline for the derivatization procedure

PREVIOUS CHARACTERIZATION OF SILAGES



- pH measurement
- Dry Matter determination (DM)
- Organic Acids determination

Sample preparation: Jiang et al., (2020)

SILAGE

HPLC determination: Van Straten, M. A., & Claessens, H. A. (2004)

RESULTS & DISCUSSION

The **pH range was wide**, from 3.8 to 8.3. Silage pH value is related to the fermentation process.

Table 1. Sample, type of silage and characterization parameters

Sample Code	Type of Silage	pH	DM (%)
1	Maize	7.00 (0.02)^{j}	29.60^{d}
2	Maize	7.50 (0.15)^{k}	34.42^{a}
3	Special mix for ruminants	5.00 (0.04)^{h}	44.14^{a}
4	Maize	3.95 (0.04)^{b}	33.46^{a}
5	Grass	4.60 (0.03)f	31.84^{i}
6	Maize	6.50 (0.06)^{i}	31.56^{h}
7	Maize	3.80 (0.03)^{j}	31.56^{h}
8	Grass	8.30 (0.09)^{m}	30.60^{f}
9	Maize	8.00 (0.10)^{l}	36.38^{a}
10	Ryegrass	4.50 (0.04)^{e}	31.23^{g}
11	Maize	4.20 (0.02)^{c}	25.90^{b}
12	Maize	8.30 (0.05)^{m}	27.44^{c}
13	Unifeed mix	6.50 (0.07)^{i}	58.43^{a}
14	Grass	4.30 (0.06)^{d}	21.06^{a}
15	Maize	3.80 (0.03)^{a}	35.78^{a}
16	Maize	3.90 (0.16)^{a}	30.23^{e}
17	Unifeed mix	4.90 (0.17)^{g}	44.14^{a}
18	Grass	5.00 (0.04)^{h}	53.43^{a}
p-value		***	***

Mean value (standard deviation). Different letters indicate significant differences ($p < 0.001$)

Tables 2 comprises the BAs values (calculated as ppm of DM) **and the organic acid content** (expressed as mg/ Kg of DM). The results obtained show the **presence of BAs in all the samples analysed**.

Spermidine was either under the Limit of Detection (LOD) or the Limit of Quantification (LOQ) in all samples. The BA that was found **at higher levels** was **Tyramine**, followed by Cadaverine and Putrescine.

Table 2. Biogenic amine and organic acid content of silage samples

Sample Code	Put (ppm DM)	Cad (ppm DM)	His (ppm DM)	Tir (ppm DM)	Spd (ppm DM)	Spm (ppm DM)	Lactic Acid (g/kg DM)	Acetic Acid (g/kg DM)
1	15.00 (0.57)^{a}	29.53 (3.32)^{a}	18.69 (1.89)^{abc}	96.08 (0.24)^{bcd}	< LOQ	< LOQ	7.62 (0,25)^{c}	28.81 (0.06)^{i}
2	13.82 (2.85)^{a}	18.36 (2.62)^{a}	18.89 (3.89)^{abc}	99.26 (20.05)^{bcd}	< LOQ	< LOD & LOQ	0.54 (0.01)^{a}	0.79 (0.03)^{a}
3	195.97 (12.19)^{ef}	264.99 (14.56)^{def}	146.38 (5.39)^{h}	245.96 (6.62)^{fgh}	< LOQ	35.16 (2.61)^{ijk}	40.99 (0.40)^{i}	7.73 (0.32)^{d}
4	148.25 (24.97)^{de}	199.18 (24.78)^{bc}	43.81 (6.61)^{cd}	355.60 (34.75)^{ij}	<LOQ	< LOQ	78.82 (1.59)^{a}	26.50 (0.73)^{h}
5	230.71 (27.66)^{fg}	283.58 (25.77)^{ef}	116.70 (12.48)^{g}	240.84 (30.13)^{fgh}	< LOQ	< LOD & LOQ	7.05 (0.03)^{c}	13.90 (0.55)^{f}
6	105.87 (20.36)^{cd}	142.00 (23.75)^{b}	119.04 (13.78)^{g}	167.90 (25.86)^{def}	< LOQ	24.52 (3.87)^{ghi}	2.66 (0.08)^{b}	3.42 (0.35)^{bc}
7	157.22 (3.27)^{e}	177.53 (8.80)^{bc}	34.16 (0.21)^{bcd}	312.75 (7.18)hi	< LOQ	31.43 (2.55)^{ij}	46.48 (0.94)^{k}	9.75 (0.97)^{e}
8	10.85 (1.81)^{a}	10.23 (1.13)^{a}	6.43 (1.01)^{a}	27.99 (3.06)^{ab}	< LOQ	< LOD & LOQ	1.11 (0.07)^{a}	3.08 (0.24)^{bc}
9	32.59 (0.75)^{ab}	37.94 (1.01)^{a}	39.23 (0.75)^{bcd}	135.75 (0.81)^{de}	< LOQ	21.40 (1.78)^{fgh}	1.33 (0.02)^{ab}	2.35 (0.40)^{ab}
10	67.56 (3.47)^{bc}	187.00 (13.62)^{bc}	58.44 (2.29)^{de}	235.14 (17.80)^{fgh}	< LOQ	32.95 (2.19)^{ij}	19.55 (1.54)^{d}	54.37 (1.69)^{a}
11	262.99 (3.44)^{g}	320.63 (2.06)^{f}	196.90 (4.10)^{i}	427.36 (9.55)^{i}	< LOQ	29.10 (1.26)^{hij}	25.03 (0.91)^{e}	33.66 (1.89)^{j}
12	6.95 (0.46)^{a}	4.50 (0.17)^{a}	4.22 (0.60)^{a}	5.11 (0.50)^{a}	< LOQ	< LOD & LOQ	0.62 (0.02)^{a}	4.24 (0.18)^{c}
13	23.58 (0.21)^{ab}	32.94 (1.65)^{a}	15.46 (0.60)^{ab}	33.07 (2.01)^{ab}	< LOQ	< LOD & LOQ	8.19 (0.67)^{c}	2.39 (0.31)^{ab}
14	266.73 (21.17)^{g}	503.88 (29.00)^{h}	136.24 (14.95)^{gh}	190.37 (10.18)^{efg}	<LOQ	42.28 (1.50)^{ab}	159.18 (0.15)^{a}	111.16 (0.93)^{a}
15	158.55 (5.93)^{e}	209.94 (8.75)^{cd}	51.16 (2.14)^{de}	257.31 (33.96)^{gh}	< LOQ	67.47 (7.90)^{a}	44.39 (0.42)^{j}	9.87 (0.72)^{e}
16	188.06 (6.18)^{ef}	220.47 (14.98)^{cde}	87.69 (2.01)^{f}	256.60 (26.36)^{gh}	< LOQ	46.71 (5.61)^{a}	34.76 (0.29)^{g}	42.28 (0.37)^{k}
17	326.35 (2.96)^{h}	414.21 (1.75)^{g}	150.62 (3.57)^{h}	256.68 (12.55) ^{gh}	< LOQ	24.73 (1.22)^{ghi}	30.32 (0.21)^{f}	13.25 (0.35)^{f}
18	192.25 (16.91)^{ef}	175.43 (17.81)^{bc}	71.62 (7.92)^{ef}	117.51 (14.08)^{cde}	< LOQ	< LOD & LOQ	36.12 (0.07)^{h}	16.69 (0.36)^{g}
p-value	***	***	***	***		***	***	***

Mean value (standard deviation). Different letters indicate significant differences ($p < 0.001$)

Lactic acid values ranged from 0.54 to 159.17 mg/Kg DM. Acetic acid values ranged from 0.79 to 111.16 mg/Kg DM.

CONCLUSION

The results obtained show the presence of BAs in all the samples analysed. The concentration of each amine varied between samples, indicating that factors such as the raw material used and the fermentation process could determine their accumulation. Due to the effect of BAs on animal performance and meat quality, further studies are needed to characterise this chemical hazard and to establish control strategies to prevent their presence in silage.

REFERENCES

Jia, T., & Yu, Z. (2022). Effect of Temperature and Fermentation Time on Fermentation Characteristics and Biogenic Amine Formation of Oat Silage. Fermentation, 8(8), 352. https://doi.org/10.3390/fermentation8080352

Jiang, F., Cheng, H., Liu, D., Wei, C., An, W., Wang, Y., Sun, H., & Song, E. (2020b). Treatment of Whole-Plant Corn Silage With Lactic Acid Bacteria and Organic Acid Enhances Quality by Elevating Acid Content, Reducing pH, and Inhibiting Undesirable Microorganisms. *Frontiers In Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.593088

Pekcici, M. E., Guler, E., & Topkafa, M. (2021). Biogenic amine contents in Turkish dairy products: determination and comparison. *Journal Of Food Measurement & Characterization*, *15*(5), 4119-4127. https://doi.org/10.1007/s11694-021-00996-6

Sketches including 15 mL centrifuge tubes (with modifications), vortex, incubator, syringe and filter. [Image]. (n.d.) Retrieved from https://smart.servier.com/

Sketches including the N2 concentrator-evaporator, the HPLC vial and the HPLC. [Image]. (n.d.) Retrieved from https://mindthegraph.com/es/

Van Straten, M. A., & Claessens, H. A. (2004, 26 july). *Analysis of Organic Acids in Aqueous Samples. Application.* Agilent. Retrieved July 1st of 2024, from https://www.agilent.com/cs/library/applications/5989-1265EN_low.pdf

ACKNOWLEDGEMENTS

Research reported in this *poster* was supported by *the "INVESTIGO" PROGRAMME subsidised by the European Recovery Instrument (Next Generation EU) in the framework of the Spanish Recovery, Transformation and Resilience Plan*.

Funded by the European Union NextGenerationEU

Plan de Recuperación, Transformación y Resiliencia