5. Due to the evidential detection of the target gas cross sensitivities are reduced to a minimum and can be evaluated and analysed even after the measurement.
6. Generating of ct-diagrams is a crucial part of the whole FTIR measurement.
7. Ready to measure new fumigants
8. High concentrations of fumigants during fumigations and low concentrations for clearance /entry permits can be measured with one instrument

A lot of gases can be detected, qualitative and quantitative

	Gas	Fumigation procedure	Clearance/ entry permit	LDL in N2
1.	Methyl Bromide	X		0,4 ppm
2.	Phosphine	X		0,2 ppm
3.	Sulfuryl Fluoride (Vikane®, Profume®)	X	X	0,03 ppm
4.	HCN	X	X	0,35 ppm
5.	EDN	X	X	0,9 ppm
6.	COS	X	X	0,004 ppm
7.	Ethyl Formate	X	X	0,1 ppm
8	Propylene Oxide	X	X	0,1 ppm
9.	Methyl Iodide	X	X	0,1 ppm
10.	Chloropricrin	X	X	0,08 ppm
11.	Formaldehyde	X		0,09 ppm
12.	Ethylene Oxide	X	X	0,2 ppm

A real big advantage is to detect several gases (up to 50) parallel. By using the entire spectrum between 850 and 4200 waves/cm.

Examples for parallel evaluations are:

1. HCN and CN2
2. SO2F2 and Chloropricrin

This options enables the user to check for interactions with the fumigated material, metabolisms and other tasks where more than one gas has to be evaluated.

Determination of safe storage moisture content of commercial maize (*Zea mays*) seeds during hermetic storage

Bernadette Abadía[1,*], Ricardo Bartosik[1,2]

[1] Researcher, National Institute of Agricultural Technology (INTA), Balcarce Research Station, Argentina
[2] Researcher, National Scientific and Technical Research Council (CONICET), Argentina
* Corresponding author: abadia.maria@inta.gob.ar
DOI 10.5073/jka.2018.463.132

Abstract

Germination declines during storage and meeting official standards (90% limit) can be challenging for the seed industry. Hermetic storage, through the establishment of self-modified atmospheres has shown to preserve germination in high-moisture maize seeds, but in the range of the low-moisture contents (m.c.) used by the seed industry, the relationship between hermetic storage and seed quality has not been fully studied. The aim of this work was to determine the safe storage m.c. of commercial maize seeds during hermetic storage considering both germination and microbiological aspects. Maize seeds with 95% initial germination were conditioned to m.c.s. between 11.5 and 14.5% and stored hermetically at 25ºC for 6 months. Germination, % oxygen, % infected grains, and colony forming units (CFU) were evaluated. Germination declined with increasing m.c.s, dropping to 50% at 14.5% m.c. Microflora respiration started to be detected at 13.5% m.c. and an anaerobic self-modified atmosphere was reached at 14.5% m.c. Despite the higher relative humidity, % infected grains and CFU count at 14.5% m.c. were lower than at 13.5%, probably due to the suppressive effect of the anaerobic atmosphere. In conclusion, 11.5% was a safe storage m.c. as it preserved germination above marketing requirements without microbiological risk. Hermetic storage was useful to generate self-modified atmospheres for m.c.s above 13.5%, but these self-modified atmospheres were not effective to protect germination. Further research on the effects

of controlled and self-modified atmospheres on the quality of different maize genotypes is needed to evaluate the benefit of hermetic storage of commercial seeds.

Keywords: seed germination, microbial activity, modified atmosphere, controlled atmosphere, respiration.

Introduction

Relative humidity and temperature are two major factors that must be controlled during seed storage to preserve germination (Krishnan et al., 2004; Kong et al., 2015; Mansouri-far et al., 2015). At an equilibrium relative humidity (e.r.h) above 75%, the seed associated microflora will develop rapidly during storage affecting seed quality (Pixton, 1967; Navarro and Donahaye, 2005). For most grains and oilseeds, a threshold of 70% e.r.h is used to determine the microbiological safe storage moisture content (Pixton, 1967; Giner and Gely, 2005). Also, degenerative reactions associated with seed ageing are hastened above 70% e.r.h. (Bewley et al., 2013). Because seed deterioration increases with temperature (Barzali et al., 2005), commercial maize seeds must be stored in refrigerated (5-10ºC) and controlled e.r.h chambers at the expense of high energetic costs (Robertson et al. 1984; Chiu et al. 2003; Sun et al. 2007; Abreu et al. 2013).

Besides e.r.h. and temperature, low oxygen concentrations during storage have shown to benefit germination in many species (Chiu et al., 2003). Low-oxygen concentrations limit the development of various aerobic genera of seed fungi (Hocking, 1990; Weinberg et al., 2008; Taniwaki et al., 2009; Marcos Valle, 2015) and also impair oxidative aging reactions (Chiu et al., 2003; Yeh et al., 2005; Groot et al., 2015). Exploring the potential of low-oxygen modified atmospheres as an alternative technology to refrigeration is of great interest for the seed industry, as it could help to save energy and reduce storage costs.

Low-oxygen atmospheres can be easily obtained storing seeds hermetically. During hermetic storage, the respiration of the seed associated microflora will consume the oxygen and release carbon dioxide originating a self-modified atmosphere (Navarro and Donahaye, 2005). Seed respiration starts to be detected above 90% relative humidity (Bewley et al. 2013), and thus it is not expected to occur in the range of low-moisture contents used in the seed industry.

Hermetic storage of high-moisture maize (above 14%) has proved to benefit germination compared to open-air storage (Moreno et al. 1988; Cardoso et al. 2016). However, in the range of the low-moisture contents used by the seed industry, the relationship between hermetic storage and maize seed quality has been less studied. The aim of the present work was to determine the safe storage moisture content (s.s.m.c.) of commercial maize seeds under hermetic storage considering not only microbiological risk but also germination decay. Therefore, the s.s.m.c. was defined as the maximum moisture content (m.c.) that preserves germination above 90% (official requirement in Argentina for maize seed marketing, SAGyP (1993) with no evidence of microbial activity after six months of storage at 25ºC.

Materials and methods

Maize samples preparation and experimental procedure

Maize seeds harvested in February 2016 (GLStack 4500, KWS) were divided into four batches and put into plastic bags for moisture conditioning to four target m.c.s. (11.5, 12.5, 13.5, and 14.5%). Calculated amounts of distilled water were poured into the bags and seeds were thoroughly mixed after wetting. The four bags were stored for 7 days at 4ºC with daily mixing of the seeds for moisture stabilization.

The maize of each batch was divided into three and placed in glass jars, each with a rubber septum on the lid for gas sampling. Maize samples were collected from the jars before closing the lid for initial measurements of germination, colony forming units (CFU), and infected grains. The sealed jars were stored at 25ºC for 6 months.

Analytical procedure

The germination percentages were determined following ISTA guidelines (ISTA, 2015). Briefly, three replicates of 50 seeds per jar were placed on extended wet paper towels, rolled, and introduced into plastic bags to be incubated at 25°C for 7 days. Results were expressed as percentages of normal seedlings.

The m.c. of the maize samples was determined by forced-air oven drying at 130ºC for 72 h according to ASAE (2003). Equilibrium relative humidity (e.r.h.) of the headspace was determined at 25ºC using relative humidity sensors (I-button, Hygrochrom, EEUU). Oxygen and carbon dioxide concentrations in the headspace were determined using a gas analyzer (CheckPoint, DanSensor, Denmark).

Microbiological analysis

Colony forming units (CFU) were determined by homogenizing 10g of maize seeds in 90 ml of peptone water, serial diluting 1ml in 9ml of the same diluent, and spreading 0.1ml aliquots on potato dextrose agar (PDA) plates. Plates were incubated at 28ºC for 5-7 days, when colonies were counted (Castro et al., 2002). Results were expressed as logCFU/g of maize.

Infected grains were determined by the direct plating technique. Fifty kernels were randomly sampled from each jar, and they were surface-disinfected in a 1% sodium hypochlorite solution for 2 min. The samples were plated on potato dextrose agar (PDA) plates (10 seeds by plate) under a sterile hood, and incubated at 28ºC for 5-7 days, when the percentage of fungi contaminated seeds was determined for each sample (Berardo et al., 2005).

Statistical analysis

Linear or Generalized Linear Models were fitted to the data after checking model´s assumptions by means of residual plots. The statistical analysis included analysis of variance and Tukey´s multiple comparisons test. The packages nlme (Pinheiro et al., 2017), lattice (Sarkar, 2008), and emmeans (Lenth, 2018) of the statistics software R version 3.4.3 were used.

Results

At the beginning of the hermetic storage period, the m.c.s. of the maize in the 11.5%, 12.5%, 13.5%, and 14.5% levels were (11.64±0.01)%, (12.68±0.06)%, (13.70±0.05)%, and (14.70±0.10)%, respectively. The corresponding initial e.r.h.s. were (63.0±1.6)%, (70.5±2.5)%, (75.2±2.7)%, and (77.2±4.2)%, respectively. M.c.s. showed a slight increment during the storage period, because of respiration activity. The final m.c.s. were (12.2±0.4)%, (13.7±0.2)%, (14.8±0.1)%, and (14.9±0.8)% and the corresponding final e.r.h.s. were (63.2±1.1)%, (72.3±0.1)%, (77.1±0.5)%, and (80.7±0.8)%.

Table 1 summarizes the germination percentages of the maize seeds at the beginning and after 6 months of hermetic storage. The initial germination percentage was higher than 95% in all the four seed lots conditioned to the different m.c.s. Final germination remained unchanged at 11.5% m.c. and started to decrease at 12.5% m.c., dropping by half at 14.5% m.c. However, due to variability in the data, no significant differences were found between final germination for 11.5, 12.5, and 13.5% m.c.s.

Tab. 1. Estimated marginal means ± model standard error of germination percentage under hermetic storage.

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
0	95.7±2.9 Aa	97.7±2.9 Aa	97.0±2.9 Aa	99.0±2.9 Aa
6	94.0±2.9 Aa	83.7±2.9 Aa	81.3±2.9 Ba	48.0±2.9 Bb

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$).

As a Linear Model was fitted to the germination data, the model standard error is the same for all groups.

Table 2 shows the concentration of oxygen within the sealed containers of maize seeds after 3 and 6 months of storage. At 11.5% m.c. oxygen remained constant during the whole storage period and similar to the normal atmospheric concentration, indicating there was no respiration at this m.c. At 12.5% m.c., a slight self-modification of the atmosphere occurred by 3 months, indicating the onset of respiration at this m.c. At 13.5% and 14.5% m.c.s., oxygen dropped markedly by 3 months revealing a more intense respiration. An anaerobic self-modified atmosphere was reached only at 14.5% m.c. The carbon dioxide concentration always remained below 21% (data not shown), suggesting that aerobic respiration prevailed in the range of m.c.s. studied.

Tab. 2. Estimated marginal means ± model standard error of oxygen concentration during hermetic storage of maize seeds. Normal atmospheric oxygen content: 20.9%

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
3	20.0 ±0.2 Ac	19.4 ±0.4 Ab	1.3±1.1 Aa	0.0±0.1 Aa
6	20.1 ±0.2 Ac	18.3±0.4 Ab	2.6±1.1 Aa	0.5±0.1 Ba

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Tab. **3** shows the results of the microbiological analyses before and after 6 months of hermetic storage. The initial percentages of infected seeds were similar for the different m.c.s. After the storage period, the percentages of infected seeds were similar at 11.5% and 12.5% m.c.s., and remained on average below 40%. The final percentage of infected seeds increased abruptly at 13.5% m.c., where practically all the seeds were infected by molds. The percentage of infected seeds at 14.5% was near 60%, and was lower than at 13.5% m.c. despite the higher e.r.h. However, due to variability in the data, the differences were not significant.

The initial CFU counts were similar between the four m.c. level seeds (Tab. **3**). Only the seeds stored at 13.5% m.c. showed a significant increment in the CFU counts after the hermetic storage. There were no differences between the final CFU counts at 11.5%, 12.5%, and 14.5% m.c.s., which remained similar to the initial counts.

Tab. 3. Estimated marginal means ± model standard error of percentage of infected seeds (Inf) and CFU counts (expressed as log10CFU/g) in maize seeds under hermetic storage.

Time (months)	Moisture content (%)							
	11.5		12.5		13.5		14.5	
	Inf	CFU	Inf	CFU	Inf	CFU	Inf	CFU
0	20±8.4 Aa	3.5±1.1 Aa	22.7± 2.3 Aa	2.7±0.8 Aa	14.7± 3.6 Aa	3.5±0.2 Aa	16.5± 13.1 Aa	2.0±1.1 Aa
6	39.5±8.4 Aa	2.6±1.1 Aa	26± 2.3 Aa	2.3±0.8 Aa	98.7± 3.6 Bb	5.2±0.2 Bb	62.8± 13.1 Aa,b	1.8±1.1 Aa

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Discussion

Low-oxygen atmospheres are a promising alternative to refrigeration for seed storage. This technology could help to reduce costs and is friendly with the environment (Weinberg et al., 2008). The availability of new plastic liners with oxygen barrier (Cardoso et al., 2016) and of portable gas analyzers that provide quick results makes it feasible to implement modified atmospheres in the commercial scale. Hermetic storage may serve as a practical, simple way for obtaining low-oxygen atmospheres.

The results of this work show that, under hermetic storage, maize seeds with up to 11.5% m.c. can be stored safely for six months at 25ºC (Tab.4). At this m.c., oxygen concentration and microbial indicators remained unchanged (Tab. **2.** Estimated marginal means ± model standard error of oxygen concentration during hermetic storage of maize seeds. Normal atmospheric oxygen content: 20.9%

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
3	20.0 ±0.2 Ac	19.4 ±0.4 Ab	1.3±1.1 Aa	0.0±0.1 Aa
6	20.1 ±0.2 Ac	18.3±0.4 Ab	2.6±1.1 Aa	0.5±0.1 Ba

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Tab. **3** shows the results of the microbiological analyses before and after 6 months of hermetic storage. The initial percentages of infected seeds were similar for the different m.c.s. After the storage period, the percentages of infected seeds were similar at 11.5% and 12.5% m.c.s., and remained on average below 40%. The final percentage of infected seeds increased abruptly at 13.5% m.c., where practically all the seeds were infected by molds. The percentage of infected seeds at 14.5% was near 60%, and was lower than at 13.5% m.c. despite the higher e.r.h. However, due to variability in the data, the differences were not significant. andTab. **3**) indicating that there was no microbial activity. This result was expected due to the low e.r.h. (63.1% e.r.h. on average for the whole storage period). Final germination was 94%, higher than the official requirements for maize seed marketing (90%, Tab. **1**) and therefore 11.5% m.c. resulted a safe storage moisture content. Additionally, since the absence of microbiological activity was related to the low m.c. (oxygen was not a limiting factor), storing 11.5% m.c. corn seeds in non-hermetic conditions would have the same results.

At 12.5% m.c., the microbial activity was also undetectable what can be attributed to the still low e.r.h. (71.4% on average for the storage period). However, germination dropped to 84%. Hence, other mechanisms rather than microbial damage must have been involved in the germination loss observed at 12.5% m.c., i.e. intrinsic aging of the seed. Because it failed to meet the germination criterion, 12.5% m.c. was not a safe storage moisture content at least for the quite challenging temperature used in this work (25ºC).

Because no appreciable modification of the atmosphere occurred at 11.5 or 12.5% m.c., it is not expected to find differences in germination or microbial charge between hermetic and open-air storage at these m.c.s. In contrast, removing the oxygen from the beginning of storage at 12.5% m.c. by means of a controlled atmosphere could have an impact on final germination that remains to be studied.

At 13.5% m.c., final germination dropped to 81%. The higher e.r.h. (76.2% on average through the whole storage period) favored fungal growth. This was reflected in the oxygen depletion and the significant increase of infected grains and CFU counts. Because it failed to meet both the germination and microbiological criteria, 13.5% is not a safe storage moisture content (Tab.4).

It is noteworthy that, despite the higher e.r.h. and microbial charge, the final germination at 13.5% m.c. was similar to the observed at 12.5% m.c. The low-oxygen atmosphere at the higher m.c. might have prevented a larger germination loss. Indeed, the higher respiration rate consumed the available oxygen (less than 2% of oxygen by 3 months of storage, Tab. **2.** Estimated marginal means ± model standard error of oxygen concentration during hermetic storage of maize seeds. Normal atmospheric oxygen content: 20.9%

Time (months)	Moisture content (%)			
	11.5	12.5	13.5	14.5
3	20.0 ±0.2 Ac	19.4 ±0.4 Ab	1.3±1.1 Aa	0.0±0.1 Aa
6	20.1 ±0.2 Ac	18.3±0.4 Ab	2.6±1.1 Aa	0.5±0.1 Ba

Within each column, means followed by different upper case letters are significantly different; within each row means followed by different lower case letters are significantly different ($P<0.05$)

Tab. **3** shows the results of the microbiological analyses before and after 6 months of hermetic storage. The initial percentages of infected seeds were similar for the different m.c.s. After the storage period, the percentages of infected seeds were similar at 11.5% and 12.5% m.c.s., and remained on average below 40%. The final percentage of infected seeds increased abruptly at 13.5% m.c., where practically all the seeds were infected by molds. The percentage of infected seeds at 14.5% was near 60%, and was lower than at 13.5% m.c. despite the higher e.r.h. However, due to variability in the data, the differences were not significant.), which became limiting for further fungal development. The results of this experiment agree with the findings of various authors who reported reduction of microbial activity in low-oxygen environments (Hocking 1990; Weinberg et al. 2008; Taniwaki et al. 2009; Marcos Valle 2015). The high percentage of infected grains and CFU counts at the end of storage at 13.5% m.c. could be, in effect, a picture of what happened before oxygen became limiting for the microflora. Intermediate measurements of oxygen and microbial indicators are needed to characterize the fungal development more precisely. The low-oxygen atmosphere might have also contributed to limit other intrinsic degenerative reactions in the seed, what remains to be evaluated. Despite final germination was similar at 13.5% and 12.5% m.c., the former is a more risky condition from the microbiological point of view. Any change in the storage conditions could rapidly impact germination.

14.5% m.c. resulted a high-risk condition for hermetic storage at 25ºC (Tab.4). Final germination dropped markedly to 50%. The high e.r.h. (79% on average through the whole storage period) enabled an intense respiration that generated an anaerobic atmosphere by 3 months of storage. Microbial indicators, however, were lower at 14.5% than at 13.5% m.c., probably because oxygen was consumed earlier at 14.5% m.c. and rapidly became limiting for microbial growth. At 14.5% m.c., indeed, the oxygen concentration reached 0%. Some authors report that fungal growth is completely inhibited only below 0.5-1% of oxygen (Taniwaki et al., 2009; Marcos Valle, 2015). The anaerobic atmosphere, nevertheless, was not effective to protect germination. Open-air versus hermetic storage experiments are needed at 13.5 and 14.5% m.c.s. to explore the benefits of storing the seeds in a low-oxygen atmosphere.

Finally, this research was carried out using a single corn hybrid. Since there is evidence that seed storability is affected by genotype (Friday et al., 1989; Marks and Stroshine, 1995), additional research including different hybrids should be conducted before drawing general conclusions.

Tab.4. Summary of safe storage moisture contents for maize seeds under hermetic storage.

	Moisture content (%)			
	11.5	12.5	13.5	14.5
Final germination >90%	Yes	No	No	No
Absence of microbial activity	Yes	Yes	No	No
Safe storage moisture content	Yes	No	No	No

Conclusions

The results of this work show that 11.5% m.c. is a safe m.c. for storing maize seeds hermetically for 6 months at 25ºC, because germination remains above 90% (official standard for maize seed) and the seed microflora is inactive. At 12.5% m.c. the microflora is still inactive but germination falls below the official standard. Hence, seeds should only be stored at 12.5% m.c. if they are not intended for marketing. Moisture contents of 13.5% m.c. and above are not compatible with safe seed storage because of microbiological activity and damage to germination.

At 11.5 and 12.5% m.c.s., the hermetic storage was not useful to generate low-oxygen self-modified atmospheres. In contrast, self-modified atmospheres were observed for m.c.s. of 13.5% and above (e.r.h. higher than 75%). The self-modified atmospheres, however, were not effective to protect germination.

Further research is needed on the potential of low-oxygen modified atmospheres for seed storage. In the future it would be important to study the effect of anaerobic environments on germination and microbial activity from the beginning of storage, by means of controlled atmospheres. It would also be important to expand the temperature range, to include seed aging indicators for a better understanding of the mechanisms of germination loss, and to extend the studies to other maize genotypes.

Acknowledgement

The authors are thankful to the National Institute of Agricultural Technology (INTA) through the projects PNAlyAV-1130023 and PNCyO-1123023, and also to the Innovation and Transfer Project of the Buenos Aires Province Research Council (CIC) (PIT AP BA) for the financial support for this research.

References

ABREU, L, CARVALHO, M, PINTO, C, KATAOKA, V, AND SILVA, T, 2013: Deterioration of Sunflower Seeds during Storage. Journal of Seed Science **35**:2.240–47. https://doi.org/10.1590/S2317-15372013000200015.

ASAE, 2003: Moisture Measurement — Unground Grain and Seeds **1988**:2–4.

BARZALI, M., LOHWASSER, U., NIEDZIELSKI, M., AND BÖRNER, A., 2005: Effects of Different Temperatures and Atmospheres on Seed and Seedling Traits in a Long-Term Storage Experiment on Rye (Secale Cereale L.). Seed Science and Technology **33**:3.713–21.

BERARDO, N, PISACANE, V, BATTILANI, P, SCANDOLARA, A, PIETRI, A, AND MAROCCO, A, 2005: Rapid Detection of Kernel Rots and Mycotoxins in Maize by near-Infrared Reflectance Spectroscopy. Journal of Agricultural and Food Chemistry **53**:21.8128–34. https://doi.org/10.1021/jf0512297.

BEWLEY, J D, BRADFORD, KJ, HILHORST, HWM, AND NONOGAKI, H, 2013: Seeds. Physiology of Development, Germination, and Dormancy. 3rd ed. New York: Springer Science & Business Media.

CARDOSO, L, BARTOSIK, R, CASTELLARI, C, ABADÍA, B, LA TORRE, D DE, AND TAHER, H, 2016: Hermetic Storage of Wet Corn in Liners with and without EVOH Barrier. In Proceedings of the 10th International Conference of Controlled Atmospheres and Fumigation of Stored Products, 117–28. New Delhi.

CASTRO, M, BRAGAGNOLO, N, AND VALENTINI, S, 2002: The Relationship between Fungi Growth and Aflatoxin Production with Ergosterol Content of Corn Grains. Brazilian Journal of Microbiology **33**:1. SBM22–26. https://doi.org/10.1590/S1517-83822002000100004.

CHIU, K.Y, CHEN, C.L, AND SUNG, J.M, 2003: Partial Vacuum Storage Improves the Longevity of Primed Sh-2 Sweet Corn Seeds. Scientia Horticulturae **98**:2.99–111. https://doi.org/10.1016/S0304-4238(02)00206-6.

FRIDAY, D, TUITE, J, AND STROSHINE, R, 1989: Effect of Hybrid and Physical Damage on Mold Development and Carbon Dioxide Production during Storage of High-Moisture Shelled Corn. Cereal Chem, 422–26. http://www.aaccnet.org/publications/cc/backissues/1989/Documents/66_422.pdf.

GINER, S, AND GELY, M, 2005: Sorptional Parameters of Sunflower Seeds of Use in Drying and Storage Stability Studies. Biosystems Engineering **92**:2.217–27. https://doi.org/10.1016/j.biosystemseng.2005.06.002.

GROOT, S, GROOT, L DE, KODDE, J, AND TREUREN, R VAN, 2015: Prolonging the Longevity of Ex Situ Conserved Seeds by Storage under Anoxia. Plant Genetic Resources **13**:01.18–26.

HOCKING, A, 1990: Responses of Fungi to Modified Atmospheres. Fumigation and Controlled Atmosphere Storage of Grain: Proceedings of an International Conference, Singapore, 14-18 February 1989. ACIAR Proceedings No. 25., 70–82.

ISTA, 2015: International Rules for Seed Testing.

KONG, L, HUO, H, AND MAO, P, 2015: Antioxidant Response and Related Gene Expression in Aged Oat Seed. Frontiers in Plant Science **6**:March.158–66. https://doi.org/10.3389/fpls.2015.00158.

KRISHNAN, P., NAGARAJAN, SHANTHA, AND MOHARIR, A. V., 2004: Thermodynamic Characterisation of Seed Deterioration during Storage under Accelerated Ageing Conditions. Biosystems Engineering **89**:4.425–33. https://doi.org/10.1016/j.biosystemseng.2004.09.004.

LENTH, R, 2018: Emmeans: Estimated Marginal Means, Aka Least-Squares Means. https://cran.r-project.org/package=emmeans.

MANSOURI-FAR, C, GOODARZIAN-GHAHFAROKHI, M, SAEIDI, M, AND ABDOLI, M, 2015: Antioxidant Enzyme Activity and Germination Characteristics of Different Maize Hybrid Seeds during Ageing, 177–82.

MARCOS VALLE, FJ, 2015: Tasa Respiratoria de Granos de Maíz (Zea Mays) Y Su Microbiota Asociada En Almacenamiento Hermético. Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata.

MARKS, B. P., AND STROSHINE, R. L., 1995: Effects of Previous Storage History, Hybrid, and Drying Method on the Storability of Maize Grain (corn). Journal of Stored Products Research **31**:4.343–54. https://doi.org/10.1016/0022-474X(95)00020-8.

MORENO, E, BENAVIDES, C, AND RAMÍREZ, J, 1988: The Influence of Hermetic Storage on the Behaviour of Maize Seed Germination. Seed Science & Tecnology **16**:427–34.

NAVARRO, S, AND DONAHAYE, J, 2005: Innovative Environmentally Friendly Technologies to Maintain Quality of Durable Agricultural Produce. In Environmentally Friendly Technologies for Agricultural Produce Quality, edited by S B Yeoshua, 203–60. CRC Press LLC, Boca Raton, Fla, USA.

PINHEIRO, J, BATES, D, DEBROY, S, SARKAR, D, AND TEAM, R CORE, 2017: Nlme: Linear and Nonlinear Mixed Effects Models. <URL: https://CRAN.R-project.org/package=nlme>.

PIXTON, S.W., 1967: Moisture content—Its Significance and Measurement in Stored Products. Journal of Stored Products Research **3**:1.35–47. https://doi.org/10.1016/0022-474X(67)90085-9.

ROBERTSON, J A, CHAPMAN, G W, WILSON, R L, AND RUSSELL, R B, 1984: Effect of Moisture Content of Oil Type Sunflower Seed on Fungal Growth and Seed Quality During Storage **61**:4.768–71.

SAGYP, 1993: Resolución 2270/93. http://www.inase.gov.ar/index.php?option=com_remository&Itemid=102&func=startdown&id=562.

SARKAR, D, 2008: Lattice: Multivariate Data Visualization with R. New York: Springer. http://lmdvr.r-forge.r-project.org.

SUN, Q, WANG, J, AND SUN, B, 2007: Advances on Seed Vigor Physiological and Genetic Mechanisms. Agricultural Sciences in China **6**:9.1060–66. https://doi.org/10.1016/S1671-2927(07)60147-3.

TANIWAKI, M. H., HOCKING, A. D., PITT, J. I., AND FLEET, G. H., 2009: Growth and Mycotoxin Production by Food Spoilage Fungi under High Carbon Dioxide and Low Oxygen Atmospheres. International Journal of Food Microbiology **132**:2-3. Elsevier B.V.100–108. https://doi.org/10.1016/j.ijfoodmicro.2009.04.005.

WEINBERG, Z. G., YAN, Y., CHEN, Y., FINKELMAN, S., ASHBELL, G., AND NAVARRO, S., 2008: The Effect of Moisture Level on High-Moisture Maize (Zea Mays L.) under Hermetic Storage Conditions-in Vitro Studies. Journal of Stored Products Research **44**:2.136–44. https://doi.org/10.1016/j.jspr.2007.08.006.

YEH, Y.M., CHIU, K.Y., CHEN, C.L., AND SUNG, J.M., 2005: Partial Vacuum Extends the Longevity of Primed Bitter Gourd Seeds by Enhancing Their Anti-Oxidative Activities during Storage. Scientia Horticulturae **104**:1.101–12. https://doi.org/10.1016/j.scienta.2004.08.006.

Application of $ECO_2FUME^®$ Phosphine Fumigant for the Complete Control of Major Stored Product Insect Pests in Milled Rice in Thailand

Rungsima Kengkanpanich*, Duangsamorn Suthisut, Saruta Sitthichaiyakul

Post-harvest and Processing Research and Development Office, DOA, 50 Phaholyothin Road, Chatuchak, Bangkok, Thailand 10900
*Corresponding author, Email: koong_12@yahoo.com
DOI 10.5073/jka.2018.463.133

Abstract

$ECO_2FUME^®$ phosphine fumigant was used to fumigate milled rice in a commercial plastic bag (5 kg) and milled rice in a jumbo bag (1,000 kg) under gas-proof sheets to assess its performance against a mixed-age culture of *Sitophilus zeamais, Tribolium castaneum* and *Oryzaephilus surinamensis*. The trials were divided into 2 groups: 1) milled rice in a commercial plastic bag (packed rice) treated with a 50 g/m^3 $ECO_2FUME^®$ application rate (700 ppm phosphine) for 2 days with two bag stacks of 46 m^3 and 55 m^3 and for 3 days with two bag stacks of 50 m^3 each; and 2) milled rice in a jumbo bag (raw material rice) with stack size of 314 m^3 treated with a 35 g/m^3 $ECO_2FUME^®$ application rate (500 ppm phosphine) for 3 days and a stack size of 435 m^3 treated with 50 g/m^3 $ECO_2FUME^®$ application rate for 2 days. Gas sampling lines were installed in the stack to measure the phosphine concentrations during the fumigation period. The results of the fumigation trials showed that mixed-age cultures of the three insect species in packed rice stacks were completely controlled at 2 and 3 days when applied with an $ECO_2FUME^®$ application rate of 50 g/m^3, whereas most insects in untreated control cages remained alive. $ECO_2FUME^®$ was also 100% effective in raw material rice stacks with complete control of mixed-age cultures of the three insect species using 35 g/m^3 of $ECO_2FUME^®$ for 3 days and 50 g/m^3 of $ECO_2FUME^®$ for 2 days. Commercial tarp fumigation of milled rice with $ECO_2FUME^®$ can be fumigated successfully without "top up" with good sealing procedures. Gas monitoring at regular intervals throughout the whole fumigation period is part of best fumigation practice to ensure that the minimum recommended phosphine concentration is maintained for complete control of all stages of target insect pests.

Keywords: $ECO_2FUME^®$ phosphine fumigant, fumigation, stored-product insect pests, milled rice, commercial plastic bag (5 kg), jumbo bag (1,000 kg).

1. Introduction

One of the largest rice-producing countries in the world, Thailand has an estimated 1.06 million hectares of cultivated land. In 2016, Thailand exported 9.88 million tons of rice with a total value of 154,434 million Baht (Office of Agricultural Economics, 2016).

Milled rice is prone to damage due to insect infestation. When the grain has no protection, the insect population will build up rapidly. Therefore, the losses and damage to stored milled rice due to insect