Effect of oxygen reducing atmospheres on the quality and safety of stored shelled Brazil nut packs

Scussel, V.M.*#, Tanello, A.C., Giordano, B.N., Manfio, D., Galvão, S., Rodrigues, M.N.F.
Laboratory of Mycotoxicology and Food Contaminants, Department of Food Science and Technology, Center of Agricultural Sciences, PO. Box 476, Federal University of Santa Catarina, Florianopolis, SC, Brazil.
Email: vildes@cca.ufsc.br

* Corresponding author
# Presenting author

DOI: 10.5073/jka.2010.425.167.229

Abstract

High moisture content, relative humidity, temperature and environment rich in oxygen (O_2) are the main factors for tree nuts to get infected by fungi and so aflatoxins (AFLs) contaminated. During storage and commercialization dry Brazil nuts packs need to maintain their safety and quality. Modified atmospheres in storage (macro-environment) and packaging (micro-environment) have been used to prolong food shelf life by reducing O_2 concentration with inhibitory gases or, more recently, by adding O_2 absorber pads. This work reports the application of O_2 atmosphere reducing methods on stored shelled Brazil nut packs aiming fungi and AFL degradation as well as hygienic conditions improvements. The methods applied were: (a) ozone - O_3, (b) carbon dioxide - CO_2 and (c) O_2 absorber pads with and without vacuum. Nuts were submitted to microbiological tests (fungi, aflatoxigenic strains, yeast and bacteria), moisture content and AFLs analysis. From all O_2 reducing atmosphere evaluated, the best performance was obtained with O_3. A reduction on fungi growth (1.8×10^4 cfu.g^{-1} to 2.6×10 cfu.g^{-1}) and yeast destruction after the first month of storage were registered. Also O_3 was the only nut treatment that was able to degrade AFLs. None of the spiked (AFLs: 15 ppb) nut samples O_3 treated had AFLs detected up to the LOQ of the method (0.36 μg.kg^{-1} for AFB_1+AFB_2+AFG_1+AFG_2) i.e., much lower than the allowed by the European Union regulation (MRL: 4 and 2 ppb for total and AFB_1, respectively), thus producing safer nuts. All other treatments stabilized and/or inhibited microorganisms growth. Add CO_2 and O_2 pads played an important role on nut quality. Further study will be carried out in order to adjust O_3 concentration and application conditions for longer period of storage.

1. Introduction

In the natural environment, Brazil nuts (*Bertholletia excelsa* Humb. and Bonpl) that grow in the Amazon forest may get contaminated by fungi and aflatoxins (AFLs) (Steiner et al., 1992, Pacheco and Scussel, 2009), as do other tree nuts. The aflatoxigenic *Aspergillus* species that have been isolated from Brazil nuts are *A. flavus, A. parasiticus* and *A. nomius* (Cartaxo et al., 2003; Castrillon et al., 2003; Arrus et al., 2005; Scussel, 2004; Olsen et al., 2008). Their growth is directly related to the climate conditions of that region and to the conditions during their storage, transport and commercialization, if there is no control of moisture content (m.c.) and temperature. That can also occur if nuts are packaged in a microclimate rich in oxygen (O_2) and m.c. enough to allow microorganisms to grow (McKenzie et al., 1998; Pacheco and Scussel, 2006).

Studies have reported the use of modified atmospheres in food storage, extensive to packaging, to reduce O_2 concentration by adding gases such as nitrogen, carbon dioxide (CO_2) and ozone (O_3) which lead to microorganisms (fungi, yeast and bacteria) inhibition, maintenance of lipid stability and reduction of grains/nuts/vegetable respiration (Zhao and Cranston, 1995; Kim and Yousef, 2000; Achen and Yousef, 2001; Sharma et al., 2002; Yelsincemin and Murat, 2006; Olmez, 2009). Vacuum also is an alternative for O_2 reduction and in recent years the addition of O_2 absorber pads have been the newest alternative in packaged food (Mexis et al., 2010; Freshpax, 2009; Ageless, 2009). Studies has been reported the effect O_3 and CO_2 on controlling microorganism growth in agricultural commodities (Mason et al., 1997; Maskan et al., 1999; Mazza et al., 2001; Yelsincemin and Murat, 2006). CO_2 is a promising and efficient inactivating microorganisms' gas for application on non-thermal sterilization process (Kaliyan et al., 2007; Van der Steen et al., 2009). Maeba et al. (1988) reported the destruction and disinfection of AFB_1 e AFG_1 in agricultural products treated with 1.1 ppm of O_3 during 5 min. An advantage of O_3, apart from being a powerful disinfectant, oxidant and AFLs degrader, is that it decomposes quite fast into O_2 and

does not have toxic effects (Samarajeeva et al., 1990; Mckenzie et al., 1998). This work reports the application of O_2 atmosphere reducing methods (vacuum, CO_2, O_3, and O_2 absorber) and their influence on fungal growth and AFL degradation on stored packaged shelled Brazil nuts.

2. Materials and methods

2.1. Samples

Shelled dry (processed) Brazil nuts (15 kg) were provided by Renmero Factory, Cameta city, in the State of Para, northern Brazil. The nut type and condition were as follows: medium size, 40-50 mm of length according to standard nut size by De Melo and Scussel (2007); initial m.c. and total fungi load of 6.5% and 1.83 log cfu.g^{-1}. No AFLs contamination was detected up to the method LOQ applied, respectively.

2.2. Chemicals, reagents and culture media

For analytical purposes, the following reagents and chemicals were used: potassium iodine, sulphuric acid, sodium thiosulphate (J.T. Baker); Solvents: methanol, ethanol, acetonitrile, benzene and toluene (Carlo Erba); Starch indicator (Synth). Ultrapure water (MilliQ system, Millipore); AFL standards: AFB_1, AFB_2, AFG_1, AFG_2 (Sigma); malt extract agar-MEA (Himedia), *A. flavus* and *parasiticus* agar-AFPA (Fluka), peptone agar (Himedia) and Tween 80 (CRQ); Violet red bile agar, Baird Parker agar tellurite potassium, serenity cysteine broth, tetrathionate broth, brilliant-green and phenol-red lactose sucrose agar (Merck).

2.3. Equipment and apparatus

The materials that were used: homogenizer (IKA T 25-Ultra Turrax); water bath (Quimis-Dubnoff Q226D); autoclave (Phoenix); microscope (100-400x PZO); incubator set at 20-25°C (ZET); microscope stereoscope (Carl Zeiss); colonies counter (Phoenix); microbiological oven (OLM); analytical (Mettler) and semi-analytical (CAB) scales; thermometer and hygrometer (CE); Altima C_{18} column (150 x 3.2 mm, 5 µm) (Alltech) at 30ºC; liquid chromatograph (LC) system (Waters Alliance 2695 separation module) with a 20 µl injection loop (Waters Corp.) coupled to a Quatro Ultima triple quadrupole mass spectrometer (Micromass) equipped with APCI as ionization source.

2.4. Application of O_2 reducing atmospheres

Shelled Brazil nuts were divided into two groups. (a) Group I - as Controls: nuts packed (a.1) loose - only air inside and (a.2) under vacuum. (b) Group II - AFL 15 ppb spiked nuts with O_2 reducing atmosphere: nuts were divided into the following sub-groups: packed (b.1) loose - only air inside; (b.2) vacuum; (b.3) O_3 treated* (packed with and without vacuum); (b.4) CO_2 gas added into packs; and (b.5) O_2 absorber pads (packed with and without vacuum). The series *O_3 (11.14 mg.L^{-1} - 90 min) was applied on the spiked nuts separately and then aseptically packaged. O_3 concentration checking was performed by the iodine metric test (APHA, 1980).

2.5. Packaging and storage conditions

Packs dimensions for length and width were of 20x25 cm, respectively, made with polypropylene film (with O_2 and UV barrier). For storage, the packs (260 g nut portions each) were heat sealed and stored in an incubator at 27ºC during two months.

2.6. Sample collection for analysis

Individual packs of shelled Brazil nuts were collected at Day one and every 30 days. Samples collected for analysis were in duplicate (n = *2*).

2.7. Shelled Brazil nut analysis

The analyses carried out were microbiological, m.c., temperature and AFLs. The methods applied for total fungi count was of Pit and Hocking (1997). The aflatoxigenicity of fungal strains was checked utilizing the AFPA by Pitt et al. (1983) and the identification of fungi in genus and species was carried out according to the keys of Samsom et al. (2004). *Salmonella* spp.*, Staphilococcus* spp. and coliforms (45ºC) were checked by APHA (1997). Moisture content was determined by gravimetry (AOAC, 2005) and AFLs content by LC tandem mass spectrometry (Xavier and Scussel, 2008) (limit of quantification - LOQ: 0.358 $\mu g.kg^{-1}$ for AFB_1+AFB_2+AFG_1+AFG_2, respectively).

2.8. Statistical analysis

The results were expressed as the mean values and standard errors. Statistical analysis was performed by analysis of variance (ANOVA) and included the Tukey´s test to evaluate significant differences among the means ($p < 0.05$). Figure 1 shows the flowchart on the whole study.

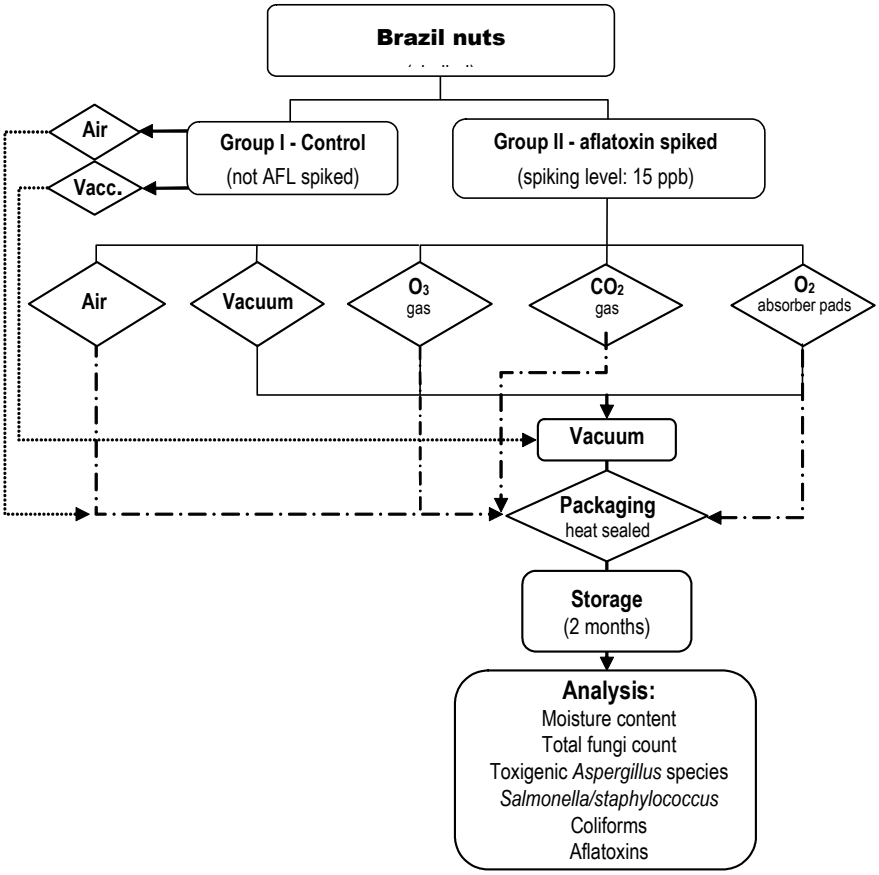

Figure 1 Flow chart of the oxygen reducing atmospheres application on shelled Brazil nuts stored in packages.

3. Results and discussion

All modified atmosphere treatments applied presented better nut quality after the period of study, when compared to the Control Group of nut packed loose i.e., with air inside (high total fungi and yeast count) (Table 1).

Table 1 Effect of O_2 reducing atmosphere on packs of shelled Brazil nuts microorganisms and aflatoxins

Storage	**Microorganisms (log cfu/g)**				**m.c. (%)**	**Aflatoxin total (ppb)**
Atmosphere Day	**Fungi/toxigenic strain**	**Coliform**	***Salmonella***	***Staphylococcus***		
Group I – Control[a]						
Air						
Initial	1.83/*A.f.; A.p.*[c]	ND	ND	ND	6.5	15.00
Final	2.69/*A.f.;A.p.*	ND	ND	ND	7.1	15.00
Vacuum						
Initial	1.83/*A.f.;A.p.*	ND	ND	ND	4.2	15.00
Final	0.70/*A.f.;A.p.*	ND	ND	ND	4.2	15.00
Group II[b]						
Air						
1	1.83/*A.f.;A.p.*	ND	ND	ND	6.5	15.00
30	2.96/*A.f.;A.p.*	ND	ND	ND	7.1	15.00
60	6.30/*A.f.;A.p.*	ND	ND	ND	7.1	14:89
Vaccum						
1	1.83/*A.f.;A.p.*	ND	ND	ND	4.2	15.00
30	0.56	ND	ND	ND	4.2	15.00
60	0.10	ND	ND	ND	4.2	14:99
Ozone						
1	1.83/*A.f.;A.p.*	ND	ND	ND	5.0	ND
30	NG	ND	ND	ND	4.9	ND
60	NG	ND	ND	ND	4.7	ND
Ozone + vacuum						
1	1.83/*A.f.;A.p.*	ND	ND	ND	3.1	ND
30	NG	ND	ND	ND	33.1	ND
60	NG	ND	ND	ND	3.0	ND
Carbon dioxide						
1	1.83/*A.f.;A.p.*	ND	ND	ND	6.5	15.00
30	NG	ND	ND	ND	7.0	15.00
60	NG	ND	ND	ND	7.0	14:99
Oxygene absorber pad						
1	1.83/*A.f.;A.p.*	ND	ND	ND	6.5	15.00
30	NG	ND	ND	ND	6.5	14.90
60	NG	ND	ND	ND	6.5	15:00
Oxygene absorber pad + vacuum						
1	1.83/*A.f.;A.p.*	ND	ND	ND	4.0	15.00
30	NG	ND	ND	ND	3.9	15.01
60	NG	ND	ND	ND	4.0	1498

ND: not detected [a] not aflatoxin spiked (AFL total < lower than the method LOQ: 0.350 µg/kg), [b] AFLs-15 ppb spiked *A. parasiticus A. flavus NG no grow*

3.1. Total fungi and aflatoxigenic strains

A substantial fungi reduction was observed after the study both with O_2 absorber and O_3 packaged under vacuum as well as nuts O_3 loose packed. CO_2 also plays an important role on the microorganism reduction in the current experiment ranging from 1.8 x 10^4 cfu.g^{-1} to 2.6 x 10 cfu.g^{-1}. Applying vacuum improved quality further. As far as mycoflora and contamination the main genera and species isolated from the untreated shelled nuts received from the factory were *Acremonium* sp., *A. ochraceus*, *Cladosporium* sp., *P. corylophium and Rhizopus* sp. followed by *A. niger; A. parasiticus, A. versicolor and P. crustosum*. However, infection was reduced when atmospheres were applied.

3.2. Hygienic bacterial indicators

Regarding to what was observed for fungi and yeast O_3, all gases and O_2 absorbers as well as vacuum did not allow neither *Salmonella*, *Staphylococcus* or coliform to grow showing the safe power of the treatments for microbial population control.

3.3. Moisture content and AFLs

Nuts presented m.c. reduction during the after vacuum application specially the O_3 treated throughout the whole storage period which kept nuts cruncher. That was probably due to the fact that during O_3 application occurs an exposure of nuts to 90 minutes with O_3 stream that can take moist from nut surface. The lower total fungi count was detected in the packs that were submitted to O_3 (reduction of 5.01 %) suggesting that apart from the fungi destruction by the O_3/vaccuum application, the reduction of m.c. powered fungi reduction.

3.4. Aflatoxins

It was possible to observe in the AFLs spiked samples, that O_3 was able to degrade them because none when analysed had AFLs detected up to the method LOQ used when compared to the Control Groups. That was different of the other O2 reducing atmospheres. They were able, only to stabilize/reduce the microorganisms growth keeping nuts safe but AFLs. In that sense the pack with O_3 and vacuum applied brings an alternative for AFL degradation and also m.c. reduction, a factor that is directly related to fungi proliferation and development of possible aflatoxigenic strains. Nuts treated with O_3 in the study showed to be good for consumption, as no AFLs were detected in them.

References

Achen, M., Yousef, A.E., 2001. Efficacy of ozone against *Escherichia coli* O157:H7 on apples. Journal of Food Science 66, 1380-1384.

Ageless, 2009. AGELESS® Oxygen Absorbers - Longer Shelf-Life for Refrigerated Food, Meat, Nuts and Dietary Supplements http://www.idspackaging.com/packaging/us.

AOAC - Official Methods of Analysis International Nuts and Nuts Products, 2005. 18th ed, Vol, II, chapter 40.

APHA - American Public Health Association, 1997. Compedium of methods for the microbiological examinatium of foods, 3rd ed.

Arrus, K., Blank, G., Clear, R., Holley, R. A., 2005. Aflatoxin production by *Aspergillus flavus* in Brazil nuts. Journal of Stored Products Research 41, 513-527.

Cartaxo, C., Souza, J., Correa, T., Costa, P., Freitas-Costa, O., 2003. Occurrence of aflatoxins and filamentous fungi contamination in Brazil nuts in the forest. In: IVth Congresso Latino-Americano de Micotoxicologia, Havana, Cuba, Sep. 24-26.

CONFORCAST, 2008. Plano de amostragem para castanha-*do*-Brasil. Access: 02/06/2008, //www.nutfruit.org.

De-Mello, F.R., Scussel, V.M., 2007. Characteristics of in-shell Brazil nuts and aflatoxin contamination: criteria for sorting. Journal of Agriculture and Food Chemistry 55, 9305-9310.

European Union – EU, 2006. Commission Regulation 1881/2006 of 19 Dec 2006, setting maximum levels for certain contaminants in foodstuff. Official Journal of European Communities, L.364/5-364/24.

Freshpax, 2009. http://www.multisorb.com/products/freshpax.html

Inan, F., Pala, M., Doymaz, I., 2007. Use of ozone in detoxification of aflatoxin B1 in red pepper. Journal of Stored Products Research 43, 425-429.

Kaliyan, N., Morey, R.V., Wilcke, W. F., Alagusundaram K., Gayathri P., 2007. Applications of carbon dioxide in food and processing industries: Current status and future thrusts. American Society of Agricultural Engineers, Annual Meeting 076113, www.asabe.org.

Kim, J.G., Yousef, A.E., 2000. Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. Journal of Food Science 65, 521-528.

Maeba, H., Takamoto, Y., Kamimura, M., Miura, T., 1988. Destruction and detoxification of aflatoxins with ozone. Journal of Food Science 53, 667–668.

Maskan, M., Karatas, S., 1999. Storage stability of whole-split pistachio nuts (*Pistachia vera* L.) at various conditions. Food Chemistry 66, 227 - 233.

Mason, L.J., Woloshuk, C.P., Maier, D.E., 1997. Efficacy of ozone to control insects, mould and mycotoxins. In: Donahaye, E.J., Navarro, S., Varnava, A. (Eds), Proceedings of International Conference on Controlled Atmosphere and Fumigation in Stored Products, Cyprus Ltd., Nicosia, pp. 665–670.

Mazza, G., Jayas, D.S., 2001. Controlled and Modified Atmosphere Storage. In: Eskin, N. A. M., Robinson, D.S. (Eds), Food Shelf Life Stability. Chemical, Biochemical and Microbiological Changes. CRC Press, Boca Raton.

McKenzie, K.S., Sarr, A.B., Maymura, K., Bailey, R.H., Miller, D.R., Rogers, T.D., Norred, W.P., Voss, K.A., Plattner, R.D., Kubena, L.F., Phillips, T.D., 1997. Oxidative degradation and detoxification of mycotoxins using a novel source of ozone. Food and Chemical Toxicology 35, 807-820.

Mexis, S.F., Badeka, A.V., Riganakos K.A., Kontominas M.G., 2010. Effect of active and modified atmosphere packaging on quality retention of dark chocolate with hazelnuts. Innovative Food Science and Emerging Technologies 11, 177-186.

Olsen, M., Johsson, P., Moller, T., Paladino, R., Lindblat, M., 2008. *Aspergillus nomius*, an important aflatoxin producer in Brazil nuts? World Mycotoxin Journal 1, 123-126.

Pacheco, A.M. Scussel, V.M., 2006. Castanha do Brasil – da Floresta ao Consumidor. Ed. Editograff, Brazil.

Pacheco, A.M., Scussel, V.M., 2007. Selenium and aflatoxin levels in raw Brazil nuts from the Amazon basin. Journal of Agriculture and Food Chemistry 55, 11087-11092.

Pacheco, A.M., Scussel, V.M., 2009. Aflatoxins evaluation on in-shell and shelled Brazil nuts for export by LC-MS/MS - 2006 & 2007 harvests. World Mycotoxin Journal 2, 295-304.

Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage. 2nd edition, Blackie Academic and Professional, London.

Pitt, J.I., Hocking, A.D., Glenn, D.R., 1983. An improved medium for the detection of *Aspergillus flavus* and *A.parasiticus*. Journal of Applied Bacteriology 54, 109-114.

Prado-Filho, L.G., 1994. Umidade relativa de equilíbrio e oxidação de lipídios em farinhas de castanha-do-Pará, de macadâmia e de soja. Scientia Agricola 51, 357-362.

Samarajeewa, U., Sen, A.C., Cohen, M.D., Wei, C.I., 1990. Detoxification of aflatoxins in foods and feeds by physical, chemical methods. Journal of Food Protection 53, 489-501.

Samsom, R.A., Hoesktra, E.S., Frisvad, J.C., 2004. Introduction to Food and Airborne Fungi. 7th edition, CBS, The Netherlands.

Scussel, V.M., 2004. Aflatoxin and food safety: Recent South American perspectives. Journal of Toxicology, Section Toxin Review, 179-216.

Sharma, R.R., Demirci, A., Beuchat, L.R., Fett, W.F., 2002. Inactivation of *Escherichia coli* on alfalfa seeds with ozonated water and heat treatment. Journal of Food Protection 65, 447-451.

Van der Steen, C., Devlieghere, F., Debevere, J., 2009. High oxygen concentration in combination with elevated carbon dioxide to affect growth of fresh-cut produce micro organisms. International Society for Horticultural Science, Acta Horticulturae 1, 599.

Xavier, J.J.M., Scussel, V.M., 2008. Development of methodology by LC-MS/MS for aflatoxins B_1, B_2, G_1 and G_2 in Brazil nuts for export. International Journal of Environmental Chemistry 88, 425-433.

Yesilcimen, A.M., Murat, O., 2006. Effect of treatments on AFLs degradation and physicochemical properties of pistachios. Journal of the Science of Food and Agriculture 86, 2099-2104.

Zhao, J., Cranston, P.M., 1995. Microbial descontamination of black pepper by ozone and effects on volatile oil of spices. Journal of the Science of Food and Agriculture 68, 11-18.