Improvement of stored Brazil nuts (*Bertholletia excelsa* H.B.K.) quality by ozone gas

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Abstract

This study reports an investigation on *in-shell* Brazil nuts (*Bertholletia excelsa* H.B.K.) quality improvement by ozone gas treatment (at different concentrations and time of exposure) during 6 month storage. Nuts were treated with O_3 gas at 10, 14, 31.5 $mg.l^{-1}$ (Group I, II and III, respectively) keeping one as Control (no O_3 treated: Group C) and stored in silos for 180 days. Samples were collected each 30 days of storage and submitted to 18 trained panelists for nuts sensory evaluation and also analyzed for their lipid stability and moisture content. The nuts (O_3 treated) sensory parameters evaluated received from the panelists, high scores: either for flavor (odour/taste), texture and slicing firmness, which indicated maintenance of nuts quality after gas treatment despite of the storage period (up to 180 days) studied. In addition, the nuts length of exposure time to the gas reduced their mc (-1.4/-1.7/-2.9%) for the 3 treated Groups, leading to cruncher nuts (an important sensorial quality attribute for the consumers acceptance and for nut safety by reducing one of the factors for fungi proliferation). Therefore, apart from safety (regarding fungi and aflatoxin degradation), O_3 treatments, under the storage conditions applied, were able to improve Brazil nuts quality, not interfering to their lipid content stability. The fatty acid oxidation indicator, malonaldialdehyde acid, was detected in low amounts throughout the 180 days period, which were not high enough to be detected by the panelists' sensory evaluation indicating the low effect of O_3 treatment on nuts lipids under the experimental conditions.

Keywords: in-shell Brazil nut, quality improvement, ozone, storage, sensory evaluation

1. Introduction

Brazil nut (*Bertholletia excelsa* H.B.K.), which is a major non-timber Amazon forest product, has important nutritional (high quality proteins & lipids) and antioxidant (high selenium content) properties (Arrus et al., 2005; Wadt et al., 2005; Pacheco and Scussel, 2006, 2007a,b; 2011; FAO, 2010; Scussel et al., 2011; Manfio et al., 2012).

Despite those positive qualities, Brazil nuts can have problems for export regarding fungi (*Aspergillus* genera - *A. flavus*, *A. parasiticus* and *A. nomius*) proliferation and toxins formation, leading to shell & nuts edible part spoilage (EU, 2006; De-Mello and Scussel, 2007; Brazil, 2008; FAO, 2010; Scussel et al., 2014a,b). Several methods have been developed to reduce fungi and toxin contamination, either by (a) controlling conditions for their proliferation such as temperature, moisture content –mc- and water activity or decontamination via chemical degradation such as solvents, chemical compounds (sodium and ammonium hydroxide) and gas (carbon dioxide, oxygen pads and ozone - O_3)] and some of them recommended to reduced the problem (Scussel et al., 2011).

Among them, O_3 gas have been suggested to be used as an effective alternative to destroy those microorganisms and degrade AFLs in several foods including Brazil Nuts (McKenzie et al., 1997; Akbas and Ozdemir, 2006; Giordano et al., 2013; Savi et al., 2014a,b,c; Beber et al,

2015). O_3 is known as a powerful disinfectant, deodorizer (McKenzie et al., 1997). As a disinfectant, it is one and a half time stronger than chlorine being effective over a wider spectrum of microorganisms (Xu, 1999; Kim et al., 1999; Savi et al., 2014a,b,c). Its application efficacy has been reported in several foods and their different production stages (Akbas and Ozdemir, 2006; Song et al., 2006; Olmez and Akbas, 2009; Giordano et al., 2013;), mainly at post-harvest with the purpose of bacterial growth inactivation (Xu, 1999; Sharma et al., 2002), fungal decay prevention (Perez et al., 1999; Palou et al., 2002; Scussel et al., 2011, Savi et al., 2014a,b), pesticides & chemical residues degradation (Hwang et al., 2001; Ong et al., 1996; Savi et al., 2014d; de Freitas et al., 2014) and storage pests control (Kells et al., 2001; Mendez et al., 2002; Faroni et al., 2007).

Given the fact that the Brazil nuts are important for the Amazon region economy and rainforest maintenance; the need of green & effective methods for fungi control and toxin reduction as well as the O_3 gas has been reported as a promising anti-fungi and anti-toxin treatment in nuts; and the lack of sensory information on O_3 effect especially on the Brazil nut: the present study investigates the O_3 gas effect (at different concentrations & time of exposure) on improvement at long term storage in-shell Brazil nuts sensory attributes (odor / color / stain / texture / taste / slicing / firmness).

2. Materials and Methods

2.1. Materials

2.1.1. Sample

Dry *in-shell* Brazil nuts (14 kg) for export, Medium size Type (40-50 mm length). Moisture content (mc) and lipids of 6.5% and 68±1.9 %, respectively. Malonaldialdehyde (MDA) of 7.16 $mg.kg^{-1}$ and no AFLs contamination (method LOQ = 1.34 $\mu g.kg^{-1}$ - Sobolev, 2007).

2.1.2. Reagents, solvents, equipment and others

(a) Reagents and solvents. Potassium iodine, sulphuric acid, sodium thiosulfate, 2-thiobarbituric acid (TBA), trichloroacetic, butylated hydroxytoluene, ethanol (all Analar, Vetec) and starch indicator (Synth), *(b) Equipment.* vertical silos (2 kg capacity), ozone generator (Megazon), industrial nut cracker (CIEX), spectrophotometer (Hitachi), homogenizer Ultra Turrax (IKA), thermometer and hygrometer (CE), analytical scale (Mettler) and water bath (Quimis-Dubnoff), *(c) Others:* polyethylene plates (∅150 mm) and cups (vol. 50 ml), spring water and a group of 18 trained panelists.

2.2. Methods

2.2.1. O_3 gas treatment and storage

The in-shell nuts (2 kg portions) were divided into 4 Groups [*C*: as Control (no O_3 application) and *I, II* & *III* (for O_3 treatment at concentrations of 10, 14 and 31.5 $mg.l^{-1}$, respectively)], placed in silos and upper lid closed (Figure 1). *(a) O_3 Treatment:* it was carried out with O_3 gas applied through the lower lateral silo aperture by means of an O_3 generator for one, three and five hours to reach the above O_3 concentrations for nuts *Groups I, II* and *III*, respectively (n=2). The O_3 concentrations were checked by iodinemetric analysis (APHA , 1980). *(b) Storage:* The silos top aperture was closed and were nuts kept stored for 180 days (6 months). *(c) Sample Collection:* The nuts were collected for analysis from each silo in the first day and every 30 days, up to the end of storage period. They were kept (a) in-shell and (b) shelled for analysis (sensory evaluation and fatty acid oxidation & mc analysis - *whole* and *ground* edible part, respectively). The samples collected were in triplicate. Figure 2 shows details of the whole experiment.

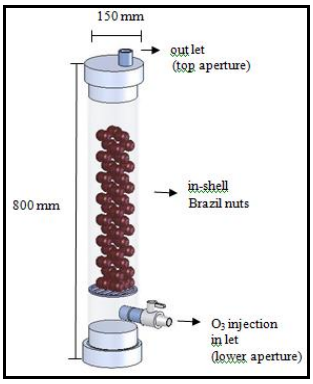

Figure 1 Dimensions and silos building details utilized for the Brazil nut (*Bertholletia excelsa* H.B.K.) ozone gas application experiment (Giordano et al., 2013).

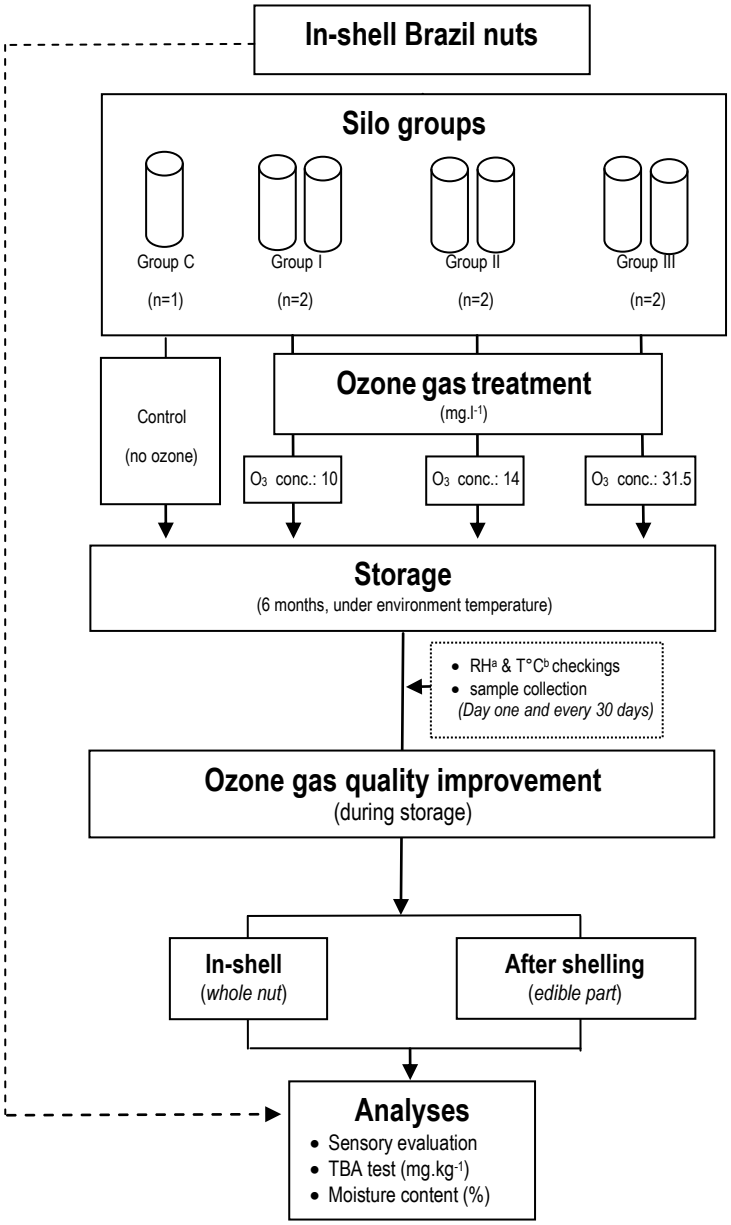

Figure 2 Flowchart of the whole experiment for sensory evaluation of ozone gas treated and stored Brazil nut. [a]relative humidity [b]temperature (Giordano et al., 2013 modified)

2.2.2. Sensory evaluation

It was carried out by descriptive quantitative analysis (Stone & Sidel, 1993) and conducted with a team of 20 trained panelists during four sessions (n=4). *(a) Sample types tested:* in-shell and shelled (edible part) Brazil nuts from the different storage periods (days one, 30, 60, 90, 120, 150, 180) and O_3 treatment groups (I, II, III and C) were served at room temperature, in polyethylene cups that received a three-digits code number, in randomized order of presentation. *(b) Panelist sensorial attributes and scores:* the panelists were encouraged, in each session, to use associative and cognitive terms to describe impressions perceived using a hedonic scale of 5 points (scores: *5 for like very much, 4 for like, 3 for neither like nor dislike, 2 for dislike and 1 for dislike very much*). The sensory attributes of Brazil nuts evaluated were: shell & nut edible part *appearance* (color, stains), *strange odor, texture* (*crunchiness*), *rancidity flavor and slicing firmness* (Figure 3).

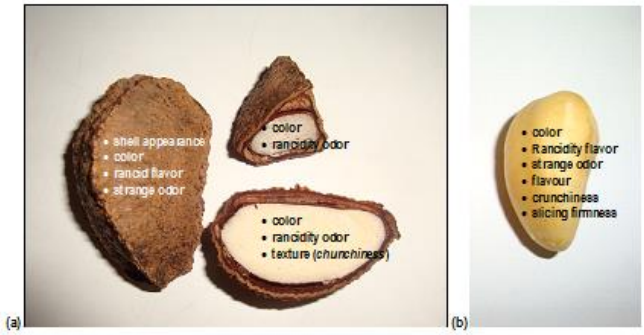

Figure 3 Parts of the in-shell Brazil nuts (*Bertholletia excelsa* H.B.K.) O_3 gas treated and their specific atributes applied for the sensory chracteristics evaluation: (a) whole and (b) shelled.

2.2.3. Thiobarbituric acid test

That analysis was performed by the TBA test ((Yaacoub et al., 2008). Briefly, ground and homogenized portions of edible Brazil nuts were added of trichloroacetic aqueous solution, filtered and mixed with TBA solution (20 $mmol.l^{-1}$) in stopper test tubes in a water bath for MDA formation. After cooling, MDA was measured at 532 nm and results were expressed as mg of MDA equivalents per kg of nut sample using a molar extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1} for MDA. The method LOD was 0.37 $mg.kg^{-1}$.

2.2.4. Moisture content

It was determined by gravimetry (Mendez et al., 2002).

2.2.5. External silo environment conditions

Relative humidity - RH (%) and temperature (°C) data were monthly collected from the Santa Catarina State Agriculture Research Company (Epagri) website.

2.2.6. Statistical analysis

Statistics was performed by analysis of variance (ANOVA) and included the Turkey´s test to evaluate significant differences among the means ($p< 0.05$). The results were expressed as the mean values and standard errors.

3. Results and Discussion

From the sensory & analytical data obtained on the O_3 treated Brazil nuts (stored for 180 days), it was possible to observe that the gas showed quite slight changes i.e, it was not able

to highly interfere on the odor/color/texture/firmness/lipids stability under the experiment conditions applied. In fact, for some sensorial attributes, the gas inclusive improved the sensory nuts acceptance (for: crunchiness and odor). Data on sensory evaluation, fatty acid oxidation stability, m.c. and storage environment conditions (R.H. and temperature) are shown in Table 1 and Figure 4.

3.1. O_3 treatment improvements on stored nuts quality sensory attributes

Data on the sensory attributes of shell & nut edible part for *appearance* (color / stains), *strange odor* (aroma), *texture* (crunchiness), *rancidity flavor* & *slicing firmness* showed no significant changes between the shell and edible nut scores for *appearances / strange odor / texture / rancidity flavor / firmness* of the ozonated and stored (6 months) Brazil nuts evaluated (Figure 4). All sensory evaluation scores for the O_3 treated nut Groups, despite of the O_3 concentrations, did not differ. They were between 4 (like) and 3 (indifferent) throughout the storage period. They were different of the Control Group exposed to air/no O_3 treated that, as expected, reduced acceptance by panelist as the storage time increased and received score 2 for most of the attributes, except for strange odor and shell color (score 3). The current data are corroborated by studies carried out and published on pistachio nuts and red peppers. When Yesilcimen and Murat (2006) studied pistachio, they observed no significant changes between sweetness, rancid flavor, appearance and overall palatability of ozonated nuts. Also Inan et al., (2007) observed that the color values in red peppers did not present any significant changes after O_3 treatment and the appearance was quite acceptable. According to the present work carried out with Brazil nuts O_3 treated and the sensory evaluation scores plus TBA test (Section 3.2), one can conclude that the gas treatment did not interfere greatly. As mentioned previously, nuts were with shell on being only shelled at the sensory panelists session (tissue protected), thus the only factor that mainly could interfere directly to sensorial characteristics in the current experiment would be fungi proliferation along the nut locule/channel (Scussel et al, 2014a,b). However if that condition is controlled by O_3, the nut characteristics for consumer acceptance would be kept/protect.

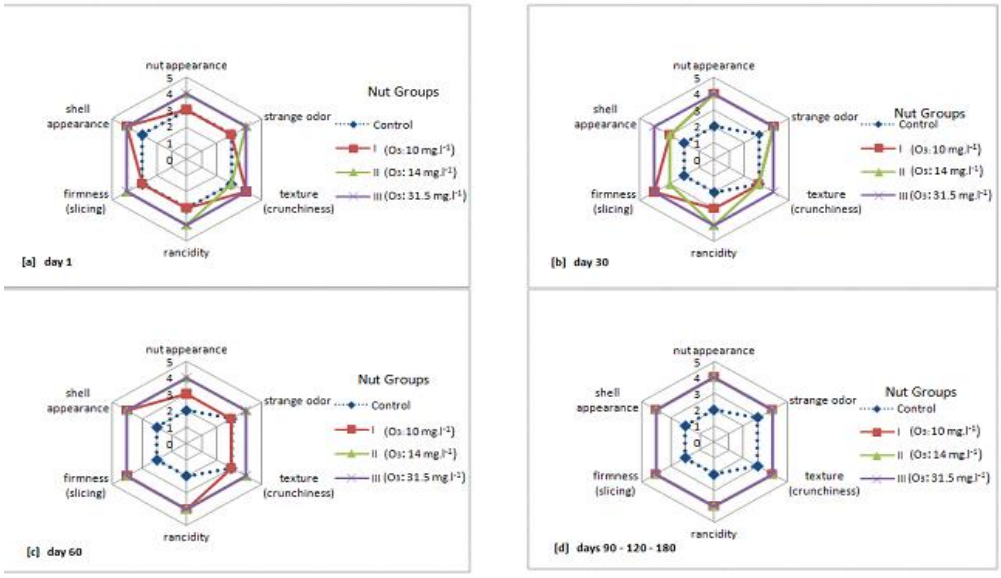

Figure 4 Stored in-shell Brazil nuts O_3 treated (at different O_3 concentrations Groups & sample collection Days) panelist scores: Groups C = Control (no O_3 treated) and Groups I, II & III O_3 treated (10.0, 14.0 & 31.5 mg.l^{-1}). Sampling collection Day:[a] 1; [b] 30; [c] 60 and [d] 90 to 180. (values obtained as mean scores of 20 individual trained panelists, based on a 5-points hedonic scale *[5 for like very much, 4 for like, 3 for neither like nor dislike, 2 for dislike, 1 dislike very much]).*

It is known, apart from other sensory attributes, that Brazil nuts high lipid content can be affected by air exposure leading to rancidity when *shelled* (the most straight forward detectable odor by consumers). Methodologies for lipid oxidation detection and quantification vary and none of them can soley define the reaction extent/stage (initiation, propagation and paralization steps). It is necessary to use a combination of methods, i.e., *sensory* evaluation together with, *chemical* determinations (we utilized here the detection of MDA).

3.2. O_3 treatments maintenance of lipid stability

Regarding the fatty acid stability, the Table 1 shows the results of TBA tests on the nut extracts from all Groups and time of storage. The levels of MDA formed by the TBA reactants in the *in-shell* Brazil nut O_3 treated did not change significantly, despite of the concentration applied and time of storage of the Brazil nut (up to 6 months) which was quite good. That occurred also in the Group III (MDA: 7.13 to 7.20 $mg.kg^{-1}$), which had the highest O_3 concentration applied with the longest time of nuts under the gas stream exposure (up to the end of storage period: Day 180). Therefore, the 3 Groups' MDA levels slightly lowered and/or kept constant throughout the whole period of storage (from 7.15-7.18; 7.13-7.17 & 7.13-7.20 $mg.kg^{-1}$, respectively). These results can be attributed to low oxidation speed conditions present in the storage silos, indicating no interference of O_3 in the process, including other factors such as (a) lower environment temperature throughout the storage period (19.6°C, min 17.9; max 20.5°C), (b) darkness (lack of light &UV), (c) edible part was nut shell protected and (d) the hermetic quality of the silos, leading to unfavorable conditions either for inner/surface nut chemical reactions and possible fungi growth. The same effect was reported in aloe powders submitted to O_3 treatment (18 $mg.l^{-1}$), where the TBA test did not detect significant changes in the lipid oxidation (Byun et al., 1997). Similarly, in other studies (Inan et al., 2007) when working with red pepper, Zhao & Cranston (1995) with black pepper and Yesilcimen and Murat (2006) with pistachio, showed no significant changes after the O_3 treatment. Nevertheless, the Control (not O_3) has been detecting some lipid oxidation during the last part of the storage period. Moreover, in a study carried out for Scussel et al (2011) with *shelled* Brazil nuts packs, re-enforce the lipid low oxidation speed when O_3 treated. In the current experiment, the O_3 treated and stored Brazil nuts were *in-shelled*, thus with no edible tissue mechanic damage (by shelling processes) and tissue enzymes released, apart from being protected by the shell from environment O_2 and UV light (if no or low fungi proliferation) thus no free radicals (either alkyl or peroxyl) formed. The only possible factors for developing lipid and/or other sensory alterations would be fungi growth, high mc, high temperature and shell cracks which were not present. Our data experiments achieved with TBA test were better understood and corroborated by the sensory evaluation (Section 3).

Table 1 Evaluation of lipid oxidative stability by the thiobarbituric acid[a] method of in-shell Brazil nuts stored under different ozone gas concentrations.

In-shell Brazil nut[b]		TBA test ($mg.kg^{-1}$)[c] /day of storage						Mc[d]	
Groups	O_3 treatment[e] ($mg.l^{-1}$)	Day 1	Day 30	Day 60	Day 90	Day 120	Day 180	Day 1 (%)	Day 180 (diff. %)
Control									
C[f]	no O_3	7.16±0.3	7.25±1.2	7.38±1.1	7.59±2.2	7.85±4.0	8.26±3.4	6.6	+0.1
Ozone treated									
I	10	7.15±0.2	7.14±0.1	7.14±0.2	7.17±0.9	7.25±1.2	7.18±1.6	5.1	-1.4
II	14	7.17±0.8	7.18±0.4	7.18±0.3	7.19±1.7	7.20±3.8	7.13±3.1	4.8	-1.7
III	31.5	7.13±1.2	7.17±0.4	7.11±0.9	7.19±1.2	7.20±2.1	7.20±0.1	3.0	-2.9
External storage environment[g]									
Temperature (°C)	A[f]	19.3	17.5	18.0	18.4	17.6	20.5	NA	NA
RH[g] (%)	NA	76.2	79.6	84.2	82.5	78.4	85.4	NA	NA

[a] 2-thiobarbituric acid; [b] lipid content: 68% (edible part) or 51% (shell+edible part); initial m.c.: 6.5%; [c] data as malondialdehyde (MDA) level; [d] final moisture content, average (n=3); [e] ozone concentration ($mg.l^{-1}$); [f] control (no O_3 treatment); [g] stored at room temperature in hermetic silo [f]not applicable [g] relative humidity

3.3. O_3 treatment improvement on nut crunchiness

It was observed that nuts presented reduction of m.c. after the O_3 applications, reaching lower levels by the time (60 to 300 min.) of gas stream (passing through nuts – 2 kg silos) to reach the target concentration (mc difference: -1.4; -1.7 & -2.9% for Groups I, II & III, respectively). They were low throughout the whole storage period and improved one of the most appreciated sensory nuts attribute: crunchiness. The O_3 gas stream nuts exposure was able to take moist from nut surface, apart from its known reaction with atmospheric water, reducing the micro-environment air relative humidity (Xu, 1999; Scussel et al., 2011). Therefore, the mc reduction varied, depending on the time that O_3 was applied (reaching a reduction of 2.9% for Group III). Apart from keeping nuts crunchier, the gas stream mc reduction produces an inappropriate environment for fungi spore growth (if any would survive the O_3 treatment) or spores reaching the nuts (by post-storage environment contamination). Furthermore the hermetic type of silo construction and external RH also played a role on keeping mc low. It is important to emphasize also the improvement of the flavor, mainly, odor, as the gas stream carries out of the silos/nuts micro-environment, any strange odor that can remain, improving also that sensory parameter of acceptance. In a work carried out by Scussel et al (2011), authors also reported an improvement of crunchiness and reduction of strange odor in shelled Brazil nut packs due to O_3 (10 $mg.kg^{-1}$) application which corroborates to the present findings.

4. Conclusions

This study showed that, under the conditions of O_3 gas treatment (at 10,14 and 31.5 $mg.l^{-1}$) applied on in-shell Brazil nuts, that gas was able to *keep* their sensory characteristics of consumer acceptance throughout the storage period, inclusive *improving* some of them such as aroma & crunchiness (as the gas stream eliminated strange odors & humidity, respectively).

Currently, *in-shell* Brazil nuts are stored in bulk (loose) – in silos or raffia bags (50 kg) at the warehouses for national industries processing. Similar occurs for shipping nuts abroad, however held in bags & big-bags (1000 Kg) piled up inside containers. By applying O_3 in (a) closed/ hermetic silos instead of warehouses, nuts could be kept away from fungi and last, safer, and longer. In addition, during shipping abroad, O_3 could be applied into the (b) containers (especially built for that purpose) with cocoon sheet/bag, and sealed up, until the importers' harbor reach, which could be, then, safely opened.

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