Silva JR, Soares CE, Kreibich HH, Pereira MN, Maria GS, Runtzel C, Scussel VM (2016) Effect of ozone gas on water activity conditions during extruded food storage and fungi spores inactivation. Pp. 235–240 In: Navarro S, Jayas DS, Alagusundaram K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Effect of ozone gas on water activity conditions during extruded food storage and fungi spores inactivation

J R SILVA*, C E SOARES, H H KREIBICH, M N PEREIRA, G S MARIA, C RUNTZEL, V M SCUSSEL

Santa Catarina Federal University, 476, Florianopolis – SC, Brazil

ABSTRACT

The antifungal properties of ozone (O_3) gas exposure, as well as its use to control/reduce water activity (a_w) were evaluated in stored-extruded food. The O_3 gas was applied at two concentrations (40 and 60 μmol/mol) and exposure times (60 and120 min) then stored for three months. Samples were collected at day zero, and each 30 days of storage. It efficiently inhibited the fungi colonies development (to NG: no growth), led to slight sample a_w reduction (0.62 to 0.58) as well as m. c. (8.85 to 7.99%). Regarding the food extrusion characteristics and moist release during gas application and through the storage, it was observed that factors such as (*a*) the extrusion process pores/bubbles formed (number/size/distribution) and the (*b*) gas flow stream applied, behaved as moist (*a*) channels release and (*b*) transport mean respectively. In contrary, the lipid content (a final product fat coating applied) had an opposite moist effect by blocking some of those pore channels. The current experiment showed that O_3 gas atmosphere was effective in controlling fungi growth (antifungal propriety) which is one of the main problems associated to food deterioration, as well as moist reduction (m.c. and a_w) parameters that are used by the extruded food industry to extend the shelf life and reduce deterioration of the final products.

Key words: Extrusion, Fungi, Ozone, Storage, Water activity

There has been a constant search in the food industry to apply processes that prolong shelf life. The techniques that prevents and/or slows down spoilage are a combination of parameters (mainly reduction of water activity – a_w and moisture content –m.c.), which may act synergistically to inhibit or retard the growth of microorganisms (fungi, yeast, bacteria). The main processes known are heating, pressure (both inactivate enzymes and destroy living organisms) and/or modified atmospheres (gases application), either separately or in combination (Leistner, 1992).

The development of fungi is one of the major problems of stored vegetable based dry foods, despite whether they are raw or processed. It is known that fungal spores are much more resistant to adverse processes and storage conditions (moisture and temperature) than its vegetative (mycelia) form. However, when exposed to adequate conditions, they can develop (even the field ones – *Fusarium* genera) leading to deterioration of stored-food (Lorini, 2008).

Starchy vegetables, such as cereals (rice, maize, wheat, barley and oats) have been utilized as ingredients (energy source) in mixed/extruded food and reported susceptible to fungi deterioration during storage. That condition lead to quality and safety reduction, including possible toxins production if toxigenic strains are present (Scussel et al., 2011).

The extrusion process is a technique applied by the food industries (both for human and animal) in order to alter texture, facilitate nutrients absorption and improve flavour. The extrusion also keeps and improves food safety regarding m.c. and a_w reduction (< 9%).Therefore it prolongs the storage period by slowing down growth of microorganisms and food deterioration (Krabbe, 2009). Extrusion is considered a high-temperature and short-time (HTST) process, combined with pressure, moisture and mechanical work. The food standing time in the extruder is from 1 to 2 min. When high pressure is applied to the food in a short time, it can influence the characteristics of final product such as size pores (empty spaces) and

*Corresponding author e-mail: *vildescussel_2000@yahoo.co.in*

nutritional profile (Bhandari et al., 2001).

Ozone (O_3) gas is a known powerful oxidizing agent and disinfectant. It has been studied to improve food quality and avoid quantitative losses by microorganisms spoilage (Palou et al., 2002; Savi and Scussel, 2014; Christ et al., 2016; Kreibich et al., 2016). Some studies have reported O_3 m.c. reduction, however the most important humidity factor (microorganisms growth wise) is the a_w (inherent to food compounds water bound).

Considering that there are no reports in the literature to our knowledge on the O_3 gas application in extruded pet foods (a high pressure -34–37 atm and temperature - 120°C cooked vegetable and animal ingredients mix) as a decontamination method, the aim of this study was to investigate that effect of gas on extruded dogs food a_w in controlling development of fungi spores at the day of application (day zero) and throughout the storage period.

MATERIALS AND METHODS

Sample

Extruded dry food (kibbles) for dogs, sold in bulk (5 kg) with m.c., a_w and fungi load of 8.85%, 0.62 and 2×10 CFU/g respectively. Table 1 shows the ingredient types, their decreasing quantity of inclusion order and the proximate composition.

Pilot silos

A total of 7 silos built with polyvinyl chloride material, dimensions of 250 mm × 100 mm (height × diameter respectively) and two openings: one for O_3 gas inlet at the bottom of the silo and the other to exit at the top of the silo (Fig. 1).

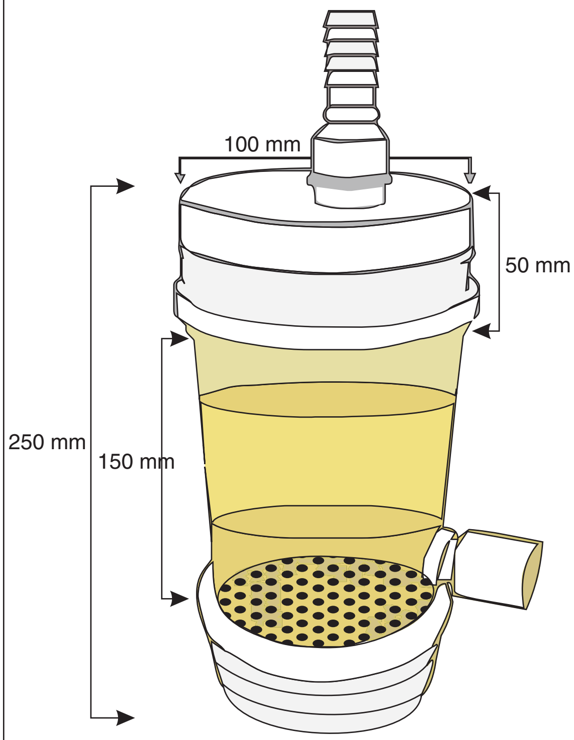


Fig. 1. Ozone treatment silo scheme, showing the lower (gas inlet) and upper (gas outlet) openings with dimensions

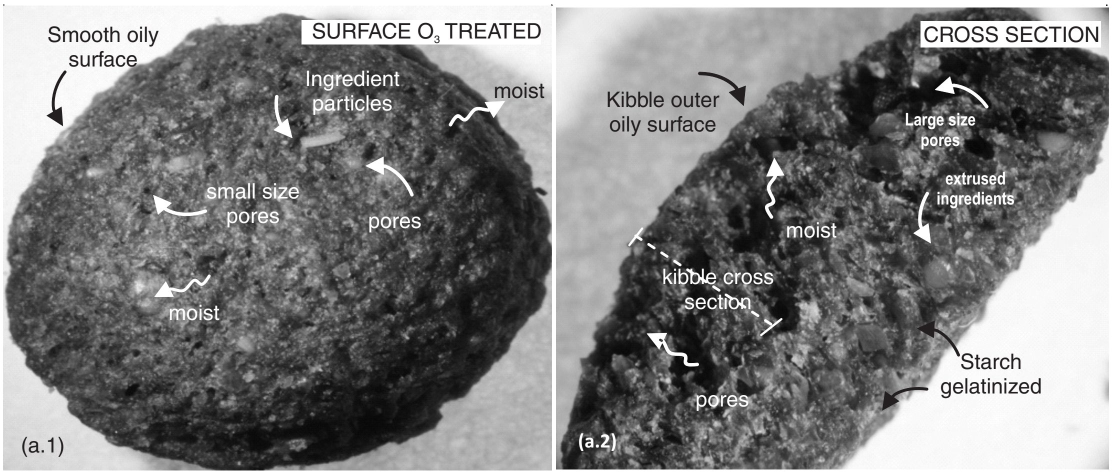
Sample preparation for O_3 application

Different portions (25g) of dry extruded food were separated for the O_3 gas application and further storage. They were divided into three Groups as: I and II Group (O_3 treated, at concentrations of 40 and 60

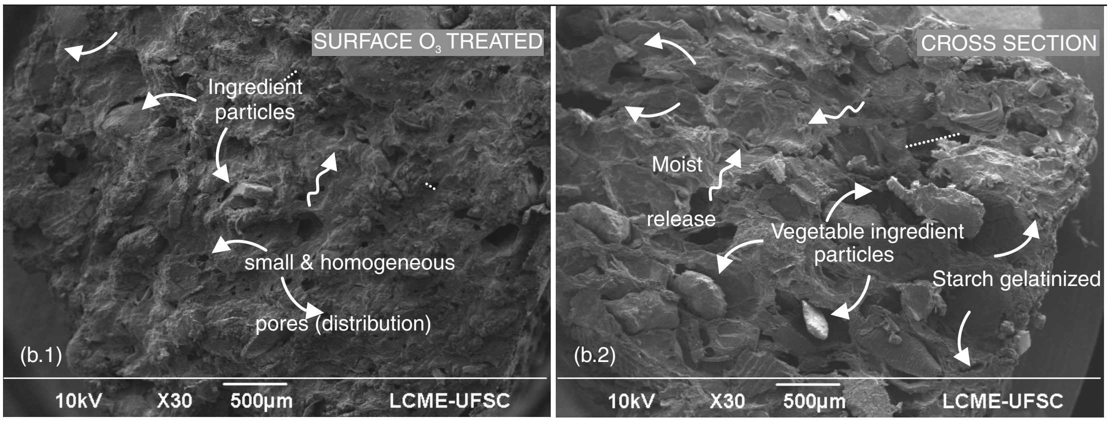
Table 1 Animal and vegetables ingredients of extruded food and its proximate composition

Food group	Extruded food ingredients				Proximate composition	
	Type	Product	Group	Order[a]	Parameter	(%)
			Animal			
	Meat	Flour	Protein	1	CP[c]	17.82
	Viscera	Meal	Protein	2	EEHA[d]	7.16
	Chicken	Oil	Lipid	6	CF[e]	2.42
			Vegetable		MM[f]	11.71
	Corn[b]	Ground	Carbohydrate	5	MC[g]	8.33
	Rice	Broken	Carbohydrate	4		
		Bran	Fibre	8		
	Wheat	Bran	Fibre	7		
		Ground*	Carbohydrate	3		
	Beans	Ground	Carbohydrate	9		

[a]Decreasing quantity of inclusion order (1-12); [b]whole; [c]crude protein; [d]ether extract in acid hydrolysis; [e]crude fiber; [f]mineral matter; [g]moist content; *pre-cooked (biscuit)



(a) Stereo microscopy



(b) Scanning electron microscopy

Fig 2. Extruded kibbles ozone treated micrographs (a) stereo (a.1) surface and (a.2) cross section [5/7 ’×’] and (b) scanning electron (b.1) surface and (b.2) cross section [30 ‘×’].

ppm, respectively, n=2) for two gas exposure times (60 and 120 min, each) and C Group (Control, no O_3 treated, n=1).

O_3 treatment

The gas was applied according to Giordano et al. (2012). Briefly, by connecting the ozonator at the lower silo opening (gas inlet) O_3 was allowed to pass through the extruded food samples inside (with its exit at the top gas outlet), until reaching the pre-established O_3 concentrations. After application, silos were completely closed and stored for 90 days (at room temperature). [The O_3 gas concentrations measurement was carried out by titration (iodine test) according to APHA (1999) in the O_3 generator output.]

Sample collection

Samples were collected just after the gas application (day zero), and after 30, 60 and 90 days of storage for analysis.

Water activity and moisture content determination

Water activity (a_w) of sample portions was measured utilizing an a_w Meter (at 25ºC) according to Aqualab (2011) and the moisture content (*m.c.*) was measured by the gravimetric method as described by AOAC (2005).

Mycological tests

The colonies enumeration technique was applied to evaluate fungal total load according to Silva et al. (2010). The results were expressed as colony forming units per gram (CFU/g).

Stereo microscopy (SM) and scanning electron microscopy (SEM)

Sample of whole kibbles were cut in cross and length sections with a thin saw for SM observation (pores characterization and distribution) and after 40 nm layer gold coated, they were SEM evaluated (pores dimensions and ingredients identification) (Scussel et al., 2012).

Table 2 Ozone gas effect on water activity, moisture content and fungi inhibition in extruded food for dogs

O_3 treatment[a]			Humidity a_w^b/mc[c]					Total fungi load (× 10 CFU/g)				
Group	Conc. (µmol/mol)	Time (min)	Prior gas treatment	Day Zero	Storage (Day) 30th	60th	90th	Prior gas treatment	Day Zero	Storage (Day) 30th	60th	90th
I	40	60	0.62/8.85	0.60/8.01	0.61/8.76	0.61/8.77	0.63/8.82		NG[d]	NG	NG	NG
		120		0.58/7.99	0.58/8.74	0.59/8.74	0.60/8.75		NG	NG	NG	NG
II	60	60		0.58/8.00	0.58/8.66	0.60/8.68	0.64/8.80	20	NG	NG	NG	NG
		120		0.57/7.98	0.58/8.53	0.62/8.56	0.62/8.78		NG	NG	NG	NG
Control	No O_3	60[e]		NA[f]	0.62/8.84	0.62/8.85	0.63/8.88		2.0	2.0	2.0	2.0
		120[e]		NA	0.62/8.84	0.62/8.84	0.63/8.86		2.0	2.0	2.0	2.0

[a]Ozone gas; [b]water activity; [c]moisture content; [d]no grow; [e]air; [f]not applicable

RESULTS AND DISCUSSION

Data obtained from the O_3 gas applications effect on extruded food a_w and antifungal proprieties, showed some moist release and effective fungi inactivation. Tables 1 and 2 present the gas effect on humidity (a_w and m.c.) with fungal load control and Fig. 2 the SM and SEM dry extruded food characteristics that can induce moist release.

O_3 effect on extruded food humidity and antifungal properties

Extruded food fungal growth is inhibited at a_w lower than 0.60, which can be controlled with drying. The same for m.c. (≤ 10%) in order to prevent undesirable microorganisms growth, such as fungi and yeasts (Andersson and Hedlund, 1990). However, excessive drying can result in ingredients loss such as antioxidants and other thermolabile components thus reducing the palatability and needs to be controlled (Krabbe, 2009; Rokey et al., 2012).

Food humidity: In the present study, a_w was of 0.62, slightly higher prior O_3 application; however, it reached 0.58 after that. The same happened for m.c. (8.85 to 7.99). By using O_3, the a_w and m.c. parameters remained below the recommended, thus safe fungi wise. The application of O_3 gas in controlling those moist parameters was reported by Rodrigues et al. (2015) and reported the effects of modified atmosphere (O_3) application on rice (*Oryza sativa*) mycobiota and the distribution of moist (a_w and m.c.) to enhance the safety during storage. From applications of three different O_3 concentrations (10, 20 and 40 mg/l) for 1.6 minutes of ozonized gas stream over 30 min rest, the m.c. and a_w showed slight reductions from 0.67 to 0.63 and 12.03 to 11.61%, respectively.

Antifungal properties

The O_3 gas also efficiently inhibited the development of fungal colonies from 2.0 CFU/g to NG (no growth) throughout the storage period (Table 2). It is important to note that there are no reports on O_3 gas application in extruded food as a decontamination method. However, O_3 gas has been used in food and grains for that purpose. Giordano et al. (2012) observed the destruction of fungi 4.83 CFU/g to NG, in Brazil nuts, corroborating our results. In another study, O_3 gas was effective against the growth of fungi, especially after 120 min at concentration of 60 µmol/mol in wheat grains (Savi and Scussel, 2014). Several studies have shown that the O_3 gas reduces fungal population, corresponding to an effective method for grains disinfestations and disinfection (Guzel-Seydim et al., 2004; White et al., 2010). Due to powerful oxidizing capacity, the O_3 gas is very effective in destroying microorganisms and, target organisms has reduced chance to develop resistance to O_3 across (Khadre et al., 2011).

Effect of O_3 on extruded food characteristics and humidity alterations

Extruded food composition versus lipid content and coating : As for humans, the main nutrients to sustain life and optimize the performance of pets include proteins, fats, carbohydrates, fibre, vitamins and minerals (França et al., 2011). Table 1 shows the extruded details of food ingredients and the proximate composition. Regarding proteins, these nutrients come from meat (bovine/pork/fish/poultry – flour) and eggs (powder). The samples utilized in the current study had 17.8% protein, accomplishing the recommended level, which is easily extrusion cooked. The carbohydrate content (energy source) that suffers gelatinization during extrusion (aggregates/encapsulates other ingredients) is present in high percentages (40 to 55). They come from cereals such as corn (plain and extruded grain, starch and germ meal), rice (whole, broken grain), sorghum [*Sorghum bicolor* (L.) Moench], wheat (*Triticum aestivum* L.) (bran) and barley (*Hordeum vulgare* L.) (whole grain) (Twomey et al., 2003). Despite that, the current study samples had in its composition only corn (ground), wheat (bran/ground) and rice (broken/bran) – highly susceptible fungi deterioration/contamination.

Regarding lipids (fat/oil), they play important functions on dogs extruded food, as they provide energy, essential fatty acids and taste. According to Willard (2003), animal lipids are more palatable than the vegetables ones, being most of them from chicken (saturated fatty acids) and fish (poli-unsaturated fatty acids). As far as lipid content and humidity release are concerned, samples utilized present 7% lipid, which are added as a fluid fat coating (called chicken oil) at the extrusion process end (Carciofi, 2008).High lipids levels (≥ 6 to 7%) control expansion of extruded food (the reason of adding them at the final step) (AAFCO, 2003; Cheftel, 1986). The fat coating behaves as a protection layer for humidity loss. It was observed that when the kibbles surface was applied with lipid led to some pore/bubbles blockage (reducing moist release/fungi spores development).

Extruded food morphology and humidity release: Depending on the ingredients composition, extruded foods may have different texture properties, such as airiness, crispiness and hardness. Fig. 2 shows the ingredients (vegetables and animals) alterations post-extrusion (morphology) and the pores/bubbles (crunchy/crispness characteristics) formation. *Carbohydrates × humidity* – The expanded starchy material is inversely proportional to the extruded humidity. The water has an opposition effect on expansion, acting as a starch material plasticizer (reduces viscosity and dissipates mechanical energy) in the extruder (Ding et al., 2005).The product becomes denser and bubbles formation is reduced. In a study, increasing water level in the cooking dough resulted in more fluid, compromising the starch gelatinization (decreased the dough bubbles, lead to more compact and dense kibble, thus harming the texture and crispness (Murakami, 2010). It is important that the kibbles oil bath is carried out while they are still hot (Riaz, 2003; Rokey et al., 2012). The Fig. 2 shows the difference among the pores/bubbles produced on the surface (oil coated) and inside kibbles (low lipid content). They were of smaller size (mean: 232 µm ×120 µm, length × width respectively) than the ones extrusion produced inside the kibbles (mean: 435 µm × 250 µm).

CONCLUSION

The effect of concentration and time of O_3 gas treatment were found effective on the extruded food on the main inner moist condition (food substrate wise) to control fungi growth start up (spores development). These findings can contribute to the prevention of microorganisms, thus improvement of extruded food safety. O_3 gas may be a promising green method to be applied in the storage and packaging of such a food type. This is the first work regarding usage of O_3 gas application in extruded dogs food as decontamination method.

ACKNOWLEDGMENT

The authors thank staff members of the Electron Microscopy Laboratory Center for the SEM analyses; the National Council of Technological and Scientific Development (CNPq) for JRdaS and Nutricol for providing the extruded samples.

REFERENCES

AAFCO (2003) Official Publications. Association of American Feed Control Officials, USA.

AOAC (2005) AOAC Official Methods of Analysis,Thiex, NJW (Ed), Association of Official Analytical Chemists, 18 edn, Maryland, USA.

Anderson Y, Hedlund B (1990) Extruded wheat flour: correlation between processing and product quality parameters. Food Quality and Preference **2**: 201–216.

Carciofi AC (2008) Sources of protein and carbohydrates for dogs and cats. Revista Brasileira deZootecnia **37**: 28–41.

Bhandari B, d'arcy B, Young G (2001) Flavour retention during high temperature short time extrusion cooking process: a review. International Journal of Food Science and Technology **36**: 453–461.

Cheftel JC (1986) Nutritional effects of extrusion cooking. Food Chemistry **20**: 263–283.

Christ D, Savi GD, Scussel VM (2016) Effectiveness of ozone gas in raw and processed food for fungi and mycotoxin decontamination – a review. Journal of Chemical Biology and Physical Sciences **6**(2): 326–348.

Ding Q, Ainsworth P, Tucker G, Marsom H (2005) The effect of extrusion conditions on the physicochemical properties and sensory characteristics of 23 rice-based expanded snacks. Journal of Food Engineering **66**: 283-289.

Giordano BNE, Nones J, Scussel VM (2012) Susceptibility of the in-shell Brazil nut mycoflora and aflatoxin contamination to ozone gas treatment during storage. Journal of Agricultural Science **4**: 1–10.

Guzel-Seydim ZB, Greene AK, Seydim AC (2004) Use of ozone in the food industry. Lebensmittel-Wissenschaft und Technologie **37**: 453–460.

Hardin JA (2010) Ozone fumigation of stored grain; closed-loop recirculation and the rate of ozone consumption. Journal of Stored Products Research **41**: 149–154.

Khadre MA, Yousef AE, Kim JG (2001) Microbial aspects of ozone applications in food: a review. Journal of Food Science **66**: 1242–1252.

Krabbe EL (2009) Controle da atividade de água e produção de alimentos secos e semi-úmidos. In: I Cong. Internacional e sobre nutrição de animais de estimação - CBNA. Campinas – SP.

Kreibich HH, Christ D, Savi GD, Silva JR, Scussel VM (2016) Decontamination of cocoa beans (*Theobroma cocoa* L.) inoculated *Aspergillus flavus* by ozone gas. Journal of Chemical Biology and Physical Sciences **4**: 567–580.

Leistner L (1992) Food preservation by combined methods. Applied Technology **5**: 151–158.

Lorini I (2008) Manejo Integrado de Pragas de Grãos de Cereais Armazenados. Passo Fundo, Embrapa Trigo, Empresa Brasileira de Pesquisa Agropecuária, 72 p.

Murakami FY (2010) Water impact in the extrusion digestibiliy and physico-chemical properties of dogs food. Curitiba, Federal University of Parana (Veterinary Ciences MSc).

Palou L (2002) Effects of continuous 0.3 ppm ozone exposure on decay development and physical responses of peaches and table grapes in cold storage. Postharvest Biology and Technology **24**: 39–48.

Riaz MN (2003) Extrusion basics. (In) Kvamme JL, Phillips TD Pet Food Technology. Illinois Mt Morris, pp 347–360.

Rokey G (2010) Feed extrusion process description. Revista Brasiliera Zootecnia **39**: 510–518.

Savi GD, Scussel VM (2014) Effects of ozone gas exposure on toxigenic fungi species from *Fusarium*, *Aspergillus*, and *Penicillium* genera. Ozone-Science and Engineering **36**: 144–152.

Silvan N (2010) Food and Water Microbiology Methods of Analysis Guide, Varela 4th Ed, p 624. São Paulo, Brazil

Tran QD (2008) Effects of extrusion processing on nutrients in dry pet food. Journal of the Science of Food and Agriculture **88**: 1487–1493.

Twomey LN, Pluske JR, Rowe JB (2003) The replacement value of sorghum and maize with or without supplemental enzymes for rice in extruded dog food. Animal Feed Science and Technology **18**: 61–69.

White SD, Murphy PT, Bern CJ, Leeuwen J (2010) Controlling deterioration of high-moisture maize with ozone treatment. Journal of Stored Products Research **36**: 7–12.

Willard T (2003) Choosing and sourcing the best ingredients. (In) Kvamme, JL, Phillips, TD (Eds.) Petfood Technology. Watt Mt Morris, pp 76–81.