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

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ABSTRACT

The antifungal properties of ozone (O₃) gas exposure, as well as its use to control/reduce water activity (a_w) were evaluated in stored-extruded food. The O₃ gas was applied at two concentrations (40 and 60 μmol/mol) and exposure times (60 and 120 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 O₃ 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
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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).

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

<table>
<thead>
<tr>
<th>Food group</th>
<th>Extruded food ingredients</th>
<th>Proximate composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Product</td>
</tr>
<tr>
<td>Animal</td>
<td>Meat</td>
<td>Flour</td>
</tr>
<tr>
<td></td>
<td>Viscera</td>
<td>Meal</td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>Oil</td>
</tr>
<tr>
<td></td>
<td>Corn$^b$</td>
<td>Broken</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Bran</td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>Bran</td>
</tr>
<tr>
<td></td>
<td>Beans</td>
<td>Ground*</td>
</tr>
</tbody>
</table>

$^a$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)
ppm, respectively, n=2) for two gas exposure times (60 and 120 min, each) and C Group (Control, no O₃ treated, n=1).

O₃ treatment

The gas was applied according to Giordano et al. (2012). Briefly, by connecting the ozonator at the lower silo opening (gas inlet) O₃ was allowed to pass through the extruded food samples inside (with its exit at the top gas outlet), until reaching the pre-established O₃ concentrations. After application, silos were completely closed and stored for 90 days (at room temperature). [The O₃ gas concentrations measurement was carried out by titration (iodine test) according to APHA (1999) in the O₃ 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.

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Water activity and moisture content determination

Water activity (a_w) of sample portions was measured utilizing an a_w Meter (at 25°C) 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).
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Table 2 Ozone gas effect on water activity, moisture content and fungi inhibition in extruded food for dogs

<table>
<thead>
<tr>
<th>O₃ treatment²</th>
<th>Humidity aₑ/mᶜ³</th>
<th>Total fungi load (× 10 CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30th</td>
<td>60th</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>No O₃</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

²Ozone gas; ³water activity; ⁴moisture content; ⁵no grow; ⁶air; ⁷not applicable

RESULTS AND DISCUSSION

Data obtained from the O₃ gas applications effect on extruded food aₑ and antifungal properties, showed some moist release and effective fungi inactivation. Tables 1 and 2 present the gas effect on humidity (aₑ and m.c.) with fungal load control and Fig. 2 the SM and SEM dry extruded food characteristics that can induce moist release.

O₃ effect on extruded food humidity and antifungal properties

Extruded food fungal growth is inhibited at aₑ 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, aₑ was of 0.62, slightly higher prior O₃ application; however, it reached 0.58 after that. The same happened for m.c. (8.85 to 7.99). By using O₃, the aₑ and m.c. parameters remained below the recommended, thus safe fungal wise. The application of O₃ gas in controlling those moist parameters was reported by Rodrigues et al. (2015) and reported the effects of modified atmosphere (O₂) application on rice (Oryza sativa) mycobiota and the distribution of moist (aₑ and m.c.) to enhance the safety during storage. From applications of three different O₃ concentrations (10, 20 and 40 mg/l) for 1.6 minutes of ozonized gas stream over 30 min rest, the m.c. and aₑ showed slight reductions from 0.67 to 0.63 and 12.03 to 11.61%, respectively.

Antifungal properties

The O₃ 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 O₃ gas application in extruded food as a decontamination method. However, O₃ 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, O₃ 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 O₃ gas reduces fungal population, corresponding to an effective method for grains disinfections and disinfection (Guzel-Seydim et al., 2004; White et al., 2010). Due to powerful oxidizing capacity, the O₃ gas is very effective in destroying microorganisms and, target organisms has reduced chance to develop resistance to O₃ across (Khadre et al., 2011).

Effect of O₃ 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 (polyunsaturated 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 moisture 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 O3 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. O3 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 O3 gas application in extruded dogs food as decontamination method.

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