Effects of Antimicrobial Addition on Shelf Life of Rendered Chicken Fat

Janak Dhakal[1], Dalton Holt[1], Charles Aldrich[1], and Carl Knueven[2]

[1]Kansas State University
[2]Jones-Hamilton Co

April 28, 2020

Abstract

This study evaluated the effects of antimicrobial addition on the shelf-life of rendered chicken fat. Sodium bisulfate (SBS) and lactic acid (LA), were each added at 0.5% to chicken fat and incubated for 6 weeks at 40°C. Peroxide value (PV), p-anisidine value (AV) and free fatty acid (FFA) levels were measured at day 0 (D0), 1(D1), 3 (D3), 5 (D5), and 7 (D7), and week 2 (W2), 3 (W3), 4 (W4), 5 (W5), and 6 (W6). The FFA level of untreated-control fat was ˜7% and remained consistent throughout the incubation until W6 (˜8.5%; (P<0.05). The FFA values in SBS treated fat were constant (range 7.25-8.30%) throughout the incubation; whereas, the LA treated fat peaked at W5 (9.3%, P<0.05). For the control fat, PVs were between 0.56-0.67 meqv/100gm until W1 then declined. For the SBS treated fat, the PVs remained low and like the control with the exception of a slight increase on W4 to 0.38 meqv/100gm, P<0.05). In the LA treated fat PV was greater than the control from W1 and increased to a peak on W5 (2.52 meqv/100gm). The AV of control fat averaged 2.12 at D0 and increased through W2. In control and LA treated fat, the AV values declined slightly thereafter; whereas, SBS treated fat increased (P<0.05) to 10.28 on W5.This study indicates that at antimicrobial effective doses the LA in rendered chicken fat may impact the shelf-life, but SBS had a minimal effect over a period of 6 weeks.

INTRODUCTION

It is common to add fat topically to extruded pet food. This provides a source of added calories, essential fatty acids, flavor and texture for the dog and cat. The fat also aids absorption of fat-soluble vitamins (Bauer, 2006).

Most fats added to pet food are derived from animal sources as a function of rendering. Rendering is an effective means for separating the fat from animal tissue and does so through application of heat (Romans et al., 2001). This thermal process is also effective in killing pathogenic organisms. However, recent indications suggest that following rendering the fat could be re-contaminated through handling, transport and storage. Since fat is surface applied following the established kill step (extrusion in pet food) this represents a potential vector for reintroduction of pathogens onto the food.

Commonly found in poultry products, *Salmonella* remains a significant economic and safety hazard in the food system (Batz et al., 2012). Although the application of heat during the rendering process is effective at inactivating most microorganisms, it does not provide protection for post-rendering re-contamination (Cochrane et al., 2016; Meeker, 2006). Recent work would suggest that acidulants such as lactic acid, phosphoric acid, or sodium bisulfate may provide residual preventive controls to pathogens introduced into fat (Dhakal et al., 2019). However, no data exists as to how the addition of these antimicrobial agents affect lipid oxidation or affect the shelf-life of chicken fat. Therefore, it was our objective to determine the effect of pathogen preventive acidulants at their previously established doses (minimum inhibitory concentration) on measures of oxidation and shelf life in treated poultry fat.

EXPERIMENTAL PROCEDURES

Chicken fat. Rendered chicken fat was procured from a regional poultry renderer (Simmons Foods®, Silom Springs, AR). The experiment was begun within one week of the fat rendering.

Treatment of fat with antimicrobials . An aliquot of chicken fat was transferred into 90 clean and grease-free plastic cups and treated with two of the acidulants, 0.5% SBS, 0.5% LA or left untreated (control). The control consisted of distilled water only. The treatments were added to attain a 3% added moisture level in fat. All the sample cups were incubated at 40°C for up to 6 weeks. All subsamples for oxidative measurements were from the middle of sample cups and were only chicken fat (not added water) at the various pre-determined time intervals.

Shelf life study of rendered chicken fat. For the determination of free fatty acids, (American Oil Chemists' Society (AOCS) official method Ca 51-40 was used) 75 ml of hot neutralized alcohol was added to 7.05 gm of well mixed sample in an Erlenmeyer flask followed by 2 ml of phenolphthalein indicator. Shaking vigorously, the content was titrated using 0.1% potassium hydroxide (KOH) until the appearance of first permanent pink color of the same intensity as that of the hot neutralized alcohol which persisted for at least 30 seconds.

The peroxide value of the fat was determined (AOAC official method 965.33) by adding 30 ml of acetic acid: chloroform (3:2, v/v) to 3 gm of sample in an Erlenmeyer flask with a glass stopper. After swirling to dissolve, 1 ml of saturated potassium iodide solution was added and mixed well. Then, after 1 min, 100 ml of distilled water was added to stop the reaction. After the addition of 1 ml of starch indicator, the mixture was titrated with 0.01N sodium thiosulfate under constant shaking until the blue color disappeared. A blank sample with 30mL of acetic acid: chloroform and 1.0mL of starch solution was also prepared.

The p-anisidine value (AV) was determined (AOCS official method Cd 18-90) with 0.75 gm of the fat sample transferred into a 25 mL volumetric flask and dissolved with 25 ml of isooctane. After dissolving for approximately 4-5 minutes, 5ml of the sample was transferred into an aluminum foil wrapped test tube followed by the addition of 1ml of p-anisidine. After 10 minutes reaction time, the sample was measured on a spectrophotometer at 350 nm. Both the sample (without p-anisidine) and reactions (with p-anisidine) were read separately.

Statistical analysis . The study was conducted as a completely randomized design with time interval as the block. A total of 3 replications for each treatment was performed. At each of the10-time points a duplicate sample was tested. Statistical software (SAS; version 9.2) was used to analyze the data for the effects of treatments and within the time interval. Means were separated with a pairwise comparison and considered different at a P of 0.05.

RESULTS AND DISCUSSION

A shelf-life study is an objective, methodical means to determine the amount of time a food product can reasonably be expected to remain fresh without appreciable changes to quality/safety (Galic, 2009). For oils/fats and the food products that contain them, shelf-life is contingent upon lipid oxidation and hydrolysis during the time of processing and storage (Hu and Jacobsen, 2016; Kilcast and Subramaniam, 2000). Lipid oxidation is a series of chemical reactions involving oxygen which can be described in terms of oxidative rancidity, or deterioration of oils/fat causing undesirable changes in color, taste, odor, and palatability. This also threatens the quality of nutrients and can lead to the formation of toxic compounds (Miller, 2010). Oxidation rates in lipids are largely a function of the structure of the fatty acid chains (Kilcast and Subramaniam, 2000; Osawa et al., 2008). Poultry fats contain high percentages of unsaturated fatty acids (ranging 57%-75%) with linoleic acid (C18:2) accounting for as much as 20% of the total fatty acid profile, much higher than that of beef tallow and pork lard (Hu and Jacobsen, 2016). Unsaturated fatty acids contain reactive double bonds between some of their carbon atoms, this results in higher susceptibility to oxidation. Lipid oxidation is complex and involves a multiple number of reactions (Osawa et al., 2008). Numerous analytical methods exist for the measurement of lipid oxidation, each with their own desired value and limitations (Hu and Jacobsen, 2016; Gray, 2015).

The FFA value measures the concentration of free fatty acids cleaved from the triacylglyceride molecules by hydrolytic breaking of the ester bonds between the fatty acids and the glycerol backbone (Miller, 2010). The percentage FFA in this study was expressed as a percentage (in weight) of oleic acid based on titration with a standard solution of KOH using phenolphthalein as the indicator (AOCS, 2009). The free fatty acid concentration of the rendered chicken fat used in this study was approximately 6.88% on initiation of the study and remained consistent through week 5; however, on week 6, FFA increased slightly to 8.53% ($P<0.05$) (Fig. 1). The hydrolysis of fat can occur due to the enzymatic (lipase) hydrolysis before rendering, or through acid or steam hydrolysis after rendering. The increase in the FFA over time, especially in the LA added chicken fat could be due to the acid hydrolysis of fat by lactic acids leading to more free fatty acid production. This finding is also related to the finding by Tadesse et al. (Tadesse et al., 2017) who also reported a constant increase in the FFA over the incubation time with no regular pattern of increase. Osawa et al. (2008) reported that FFA level in dry pet food samples increased during the storage period which is indicative of hydrolytic rancidity. They reported a range of 4.6 to 28.0% FFA (as oleic acid equivalents) in the pet food samples. The FFA percentage as well as the range in our study was smaller compared to their finding which could be dependent on the fat source and storage time before analysis. Both the main effects (time and treatments) were significant ($P < 0.05$) whereas, the interactions were not.

Peroxide values measure the primary oxidative products of fat. In the control fat PV was in the range of 0-0.67 meqv/100 gm fat throughout the storage period (Fig. 2). The values were in a range of 0.56-0.67 meqv/100 gm fat for the first week before it declined. This decline could be due to primary oxidation products breaking down to form secondary oxidation product as the storage time increased. The PV value of chicken fat stored for 7 days at 4°C was measured at 0.215 meqv/100 gm of fat in a study reported by Shantha and Decker (1994). This lower PV value compared to our study is likely due to the lower storage temperature compared to ours (40°C). The PV values in the SBS treated fat were also in a close range of 0.11-0.39 meqv/100 gm of fat, with a higher value (<0.05) on day 5 and week 4 of the sampling. The reason for these elevated values is not immediately obvious. The continuous increase in the PV value up to day 5 in control and SBS treated fat in our study may be similar to the findings of Tadesse et al. (2017), wherein the PV value in animal butter continuously increased during storage period up to 72 hours when stored at 25°C and 65°C. However, in the LA added chicken fat the PV values increased over the storage period until 5 weeks, and then declined on 6^{th}week. The highest PV value of 2.53 meqv/100 gm of fat was recorded on 5^{th} week. The increase in the PV values before it started falling on 6^{th} week may be the effect of the acidulant (lactic acid) causing more lipid oxidation to form peroxides before they start breaking down to secondary oxidation products. The main effects and interactions of treatments and days were higher ($P < 0.05$). The PV values in the LA treated and control fat increased linearly ($P < 0.05$) over time.

The second stage of oxidation occurs as hydroperoxides are cleaved to form carbonyl compounds such as aldehydes, which can be measured as non-volatile secondary oxidation products such as AV. These secondary compounds can impact the sensory quality of foods, producing off-tastes and off-smells (Hu and Jacobsen, 2016). It is important to note that initially the peroxide values increased during lipid oxidation but as the secondary compounds formed the primary values dropped, so it is essential to use these tests in tandem to achieve meaningful information about what is occurring at the time of measurement. The AV values for the control fat ranged between 2.11 to 6.68. The LA treated fat had AV values between 1.89 on day 0 to 8.18 on week 2; whereas, the SBS treated fat had AV values of 2.00 on day 0 to 10.28 on the week 5 (Fig. 3). For a good quality fat or oil, the AV values should be lower than 10 (List et al., 1974). In our study, both the acidulant treated fats, as well as control fat had AV values at or below this threshold of 10, with the single exception of 10.28 for SBS treated fat on week 5. The higher AV value indicates lower oxidative stability of the fats and oils. Tadesse et al. (2017) reported that the AV values of animal butter increased during storage at 65°C which was similar to our findings at 40°C storage. Christensen and Holmer (1996) reported that the increase in the AV value of fats and oils were a function of storage time and temperature. The time effect and the interaction of the main effects were significant ($P < 0.05$). Both the acidulant treated samples as well as the control showed a linear ($P < 0.05$ increase in the AV value over the time.

In the fats and oil industry, the TOTOX value has been proposed as a mean to combine the anisidine value

and the peroxide value (Shahidi et al., 2002; O' Keefe et al., 2010). The TOTOX value provides a broad accounting for the history of an oil or fat, but it "does not have any sound scientific basis because it combines variables with different dimensions" (Shahidi et al., 2002). The TOTOX value is calculated by adding the AV value with twice the PV value. There was a linear increase ($P<0.05$) in TOTOX values over time for all treatments. Individually, the TOTOX values of control fat after 1 week of storage remained constant over the storage period. Whereas, the TOTOX values for the acidulant treated chicken fats increased throughout the storage time with a maximum TOTOX of 10.67 on week 5 for SBS treated fat and 11.36 on week 5 for LA treated fat (Fig. 4) and differed ($P<0.05$) from the control (TOTOX of 6.0).

In conclusion, this study demonstrated that the FFA of fat rose slightly over the 6 weeks regardless of the acidulant treatments. The addition of LA increased the PV of the chicken fat and SBS led to a slight increase in AV at the last two weeks of the study. Taken in combination the acidulant treatment each led to a rise in TOTOX values for the fat by weeks 5 and 6. This would indicate that use of acidulants for pathogen control are stable to oxidation for at least 4 weeks and that LA may have greater impact on the primary oxidation product (PV), whereas SBS more impact on secondary oxidation products (AV) beyond 4 weeks of storage. There may be limitations to these findings because the experiment was conducted in a "bulk oil" model and the results might be amplified if applied in a thin layer to pet foods where exposure to air would be greater. Future research should evaluate the effects on shelf life on dry pet food kibbles coated with rendered chicken fat treated with acidulants. In conclusion, while changes were observed over time due to acidulant use for 6 weeks, the oxidation products measured remained within acceptable levels for fat used in the production of the pet foods.

ACKNOWLEDGMENTS

We acknowledge the Jones-Hamilton Co. for partial funding of this research. C. Knueven (Jones-Hamilton Co.) provided input regarding experimental design and description of the SBS activity.

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FIGURE LEGENDS

FIGURE 1. Effects of acidulants in the free fatty acid levels of rendered chicken fat evaluated over the period of 6 weeks at 40°C. SBS: sodium bisulfate, LA: lactic acid, Control: no acidulant. *- LA > SBS, Control ($P < 0.05$).

FIGURE 2. Effects of acidulants in the peroxide value of rendered chicken fat evaluated over the period of 6 weeks at 40°C. SBS: sodium bisulfate, LA: lactic acid, Control: no acidulant. *- LA > SBS, Control ($P < 0.05$) (but, on week 1 it is LA, SBS > Control).

FIGURE 3. Effects of acidulants in the anisidine values of rendered chicken fat evaluated over the period of 6 weeks at 40°C. SBS: sodium bisulfate, LA: lactic acid, Control: no acidulant. *- LA > SBS, Control ($P < 0.05$).

FIGURE 4. Effects of acidulants in the TOTOX values of rendered chicken fat evaluated over the period of 6 weeks at 40°C. SBS: sodium bisulfate, LA: lactic acid, Control: no acidulant. *- LA, Control > SBS ($P < 0.05$).