Effect of Addition of Ghee Residue on the Microbiological and Keeping Quality of Pet Food

Rani.C*, Prem Anand Govande, Palat Kuttinarayanan Sathu.T and Ahire Girish Sureshrao
Department of Livestock Products Technology,
College of veterinary & Animal Sciences, Mannuthy-680651, Kerala, India

Abstract

A study was conducted to assess the keeping quality of pet food due to addition of ghee residue which is obtained as byproduct in ghee manufacture. The pet food were prepared using meat cum bone meal (48%), plain flour (maida) (34%), blood serum (6.5%), yeast (1%) and sugar (0.5%) and various treatments were prepared by adding rendered fat and ghee residue at three different combinations viz., 10% RF, 5% RF +5% GR, 10% GR. The quality parameters like Thio-Barbituric Acid Reacting Substances (TBARS), Aerobic plate count and Yeast and mould count were determined on day of preparation and on day 7, 14, 21, 28, 45, and 60 at room temperature storage. The TBARS values varied significantly between treatments with lowest values in ghee residue added samples in all days of analysis whereas the aerobic plate count and yeast mould plate count varied non significantly between treatments throughout the storage. Storage had a significant effect in enhancing the microbial count.

Key words:

Pet food, Ghee residue, TBARS, Aerobic plate count, Yeast and mould count, Anti-oxidant activity

Introduction

The demand for ready to eat pet food in India grew strongly, with an increasing number of owners serving packaged food at least once a day. There has been a sudden shift from traditional practice of feeding homemade food to use of packaged pet food with people searching for convenience, due to busy lifestyles. The pet food market is in nascent stage in India but increasing pet ownership and humanization of pets has made the domestic market attractive with a major focus on key urban cities. The market in India is largely concentrated on dry food and the market is categorized with high influx of foreign brands which are costly; hence there is huge scope for indigenous pet food industries in India. Meat industry by products can be profitably used to produce pet foods. Animal protein meals, which form an important ingredient of pet foods, possess a better balance of amino acids in comparison to protein from vegetable source. In addition the rendered fat particularly of inedible grades widely used in animal feeds. But the quality of such rendered fat is usually low and use of such fat in pet foods makes it susceptible to oxidative rancidity. Ghee residue obtained as a byproduct in ghee manufacture can be used as alternative to rendered fat. Ghee residue, brownish solid mass obtained as a by-product in ghee manufacture, contains considerable amount of milk fat (30-50 %), protein (17 -25 %) and minerals (2-6%). Ghee residue has antioxidant properties (Pagote and Bhandari, 1988) and rich flavor potential. Considering the growth of pet food industry and it future development potential the inclusion of ghee residue as an ingredient in pet food is subject which needs consideration. Taking this aspect into consideration, the study was undertaken to assess the suitability of Ghee residue as an ingredient in low moisture pet food in improving the microbiological and physic chemical qualities.

* *Email: ranichacko84@gmail.com*
Ph: 973946233

Materials and Methods

Preparation of sample

The low moisture content (dry) pet food were prepared using the formulations T1, T2, T3 given in Table 1 as per the method standardized by Rani *et al.,* 2011.

Table1: Shelf stable Pet food formulation

Ingredients / formulations used in the manufacture of shelf stable pet foods

Ingredients	Treatment1-T1 (%)	Treatment1-T2 (%)	Treatment1-T3 (%)
Meat cum bone meal	48.0	48.0	48.0
Refined wheat flour	34.0	34.0	34.0
Blood serum	6.5	6.5	6.5
Yeast	0.5	0.5	0.5
Sugar	1.0	1.0	1.0
Rendered fat **(RF)**	10.0	5.0	-
Ghee residue **(GH)**	-	5.0	10.0
Total	**100**	**100**	**100**

The ingredients like Meat Cum Bone Meal (prepared by dry rendering), cattle blood serum, rendered fat (RF) were procured from Meat Technology Unit, College Of Veterinary and Animal Science, Thrissur, kerala, refined wheat flour, sugar and yeast were brought from local market and ghee residue (GR) procured from Dairy Technology Unit, KVASU, Thrissur, Kerala were used as ingredients. Various treatments were prepared by adding rendered fat (RF) and ghee residue (GR) at three different combinations viz., 10% RF, 5% RF +5% GR, 10% GR and were designated as T1, T2 and T3 respectively. The dough prepared was kept for one hour at ambient temperature, molded into rectangular shaped biscuits, the biscuits were then baked in a hot air oven at 150°C for 45 minutes and then cooled to room temperature and each biscuits weighed around 2 grams. The moisture content in pet food was restricted to 1.5 -2 percent by standardizing the drying time as per Rani *et al.,* 2011. Six batches of pet food were prepared, packaged in oxygen permeable high-density polyethylene pouches (HDPE, 200 µM), sealed by pulsed sealing machine (Sevana, Kochi) and stored at room temperature. Samples were analysed for physicochemical (Thio Barbituric Acid Reacting Substances) and microbiological qualities (APC and Yeast and mould count) on day of preparation and on day 7, 14, 21, 28, 45, and 60 at room temperature storage.

Lipid Oxidation

The TBARS were determined as per Witte *et al*. (1970) with modifications as an indicator of lipid oxidation. Accurately weighed 20 g sample was blended with 50 ml chilled extracting solution containing 20 per cent trichloroacetic acid in 2 M ortho-phosphoric acid for 1.5 to 2 min. The resultant slurry was transferred to a 100 ml volumetric flask and the sample was made up to 100 ml using deionised distilled water. This solution was filtered through Whatman No.1 filter paper. Five ml filtrate was transferred to a screw capped vial followed by the equal quantity of 2-thiobarbituric acid solution (0.005 M in distilled water; Merck, Germany). The solution was mixed by inverting the vial and kept for 15 h in darkness at room temperature. The absorbance was determined at 530 nm against blank containing 5 ml distilled water and 5 ml 2-thiobarbituric acid solution (0.005 M in distilled water) in UV Vis Spectrophotometer 119 (Systronics, India). The absorbance was converted to TBARS values and was expressed as mg of malonaldehyde per kg (mg mal / kg) of pet food.

Microbiological Analysis

For bacteriological analysis 25 g of the sample was placed in a stomacher bag with 225 ml of 0.01 per cent sterile peptone water (diluent) and was homogenized for 30 seconds at 230 rpm with in a stomacher (Seward Stomacher®

400 circulator) so as to form one in 10 dilution of the sample. Serial decimal dilutions were prepared in peptone water and 1 ml samples of appropriate dilutions were poured on to designated petri dishes. Aerobic plate count (APC) of each sample was estimated by pour plate technique, as described by Mortan (2001) using Standard Plate Count Agar (HiMedia, Mumbai) which was incubated at 37°C for 24 hours in inverted position. The yeast and mould count of the sample was performed using the potato dextrose agar (HiMedia, Mumbai) as per the method described by Beuchat and Cousin (2001) and were incubated at 25-27oC for 3 days.

At the end of the incubation period, the plates having colonies between 20 and 200 were selected and counts were taken with the help of a digital colony counter (Royal, India). The number of cfu per g of the sample was calculated by taking the average of duplicate plates and multiplied by the dilution factor and converted to log10 cfu/g of sample.

Experimental design and analysis

The experimental design was a completely randomized block design, where each treatment served as a block. The effects of addition of GR and RF on different qualities of pet food were analysed using analysis of variance using IBM SPSS package (version 19) as per Snedecor and Cochran (1994). Means were considered significant at $P < 0.05$.

Results

Lipid Oxidation

Estimation of TBARS is done to evaluate the extent of oxidative rancidity changes in a product. The TBARS values of the pet foods are shown in table 2 as mg of malonaldehyde/ Kg of pet food.

Table 2 TBARS values of different treatments of pet food (mg malonaldehyde/ Kg)

Treatment	Storage days					
	0	7	14	28	45	60
T1	3.90 ± 0.02 aA	4.37 ± 0.04 aB	4.98 ± 0.05 aC	5.7 ± 0.02 aEF	5.81 ± 0.04 aF	6.08 ± 0.03 aG
T2	3.56 ± 0.02 bA	4.00 ± 0.07 bB	4.57 ± 0.02 bC	5.50 ± 0.03 bE	5.66 ± 0.01 bF	5.79 ± 0.01 bG
T3	3.19 ± 0.0 cA	3.40 ± 0.07 cB	3.82 ± 0.04 cC	5.2 ± 0.01 cE	5.55 ± 0.01 cF	5.62 ± 0.02 cF

Mean ± SE column with a different letter (a–d) are significantly different ($P<0.05$). Mean± SE within a row with a different letter (A-G) are significantly different ($P<0.05$).

The TBARS values of ghee residue added samples both at 5 percent and 10 percent levels had the lowest values indicating addition of ghee residue had significantly influenced TBARS values. The values TBARS values varies from 3.19±0.0 to 6.08±0.03 mg of malonaldehyde/Kg of sample.

Microbiological Analysis

The aerobic plate count and yeast and mould content of pet food expressed in log10 cfu/g during the storage period are shown in table 3 and 4 respectively.

Table 3 Total aerobic plate count of various treatments of pet food (log cfu per g)

Treatment	**Storage days**					
	0	**7**	**14**	**28**	**45**	**60**
T1	0.65± 0.05 aA	0.94± 0.02 aB	1.41± 0.03 aC	1.69± 0.01 aD	1.85± 0.01 aE	1.94± 0.01 aF
T2	0.63± 0.04 aA	0.91± 0.03 aB	1.23± 0.04 bC	1.66± 0.02 aD	1.85± 0.01 aE	1.96± 0.01 aF
T3	0.64± 0.04 aA	0.97± 0.11 aB	1.33± 0.05 abC	1.67± 0.04 aD	1.86± 0.03 aE	1.97± 0.01 aF

Mean± SE column with a different letter (a–d) are significantly different ($P<0.05$)
Mean± SE within a row with a different letter (A-G) are significantly different ($P<0.05$)

Table 4.Yeast and mould count of various treatments of pet food, log cfu per g

Treatment	Storage days					
	0	7	14	28	45	60
T1	0.10± 0.06 aA	0.16± 0.10 aA	0.36± 0.04 aB	0.66± 0.07 aC	0.93± 0.03 aD	1.07± 0.03 aD
T2	0.00± 0.00 aA	0.00± 0.00 aA	0.38± 0.09 aB	0.70± 0.03 aC	0.93± 0.03 aD	1.05± 0.01 aE
T3	0.05± 0.05 aA	0.13± 0.08 Aa	0.39± 0.04 aB	0.62± 0.07 aC	0.87± 0.02 aD	1.02± 0.02 aD

Mean± SE column with a different letter (a–d) are significantly different (P<0.05) and Mean± SE within a row with a different letter (A-G) are significantly different (P<0.05)

The aerobic plate count 0.65±0.05 was noticed in T1 samples and was non significant from other two treatments. Storage of the product under ambient temperature had a significant effect (P<0.05) in enhancing the aerobic plate count and yeast and mould count. The yeast and mould count of all treatments remained significantly low throughout the storage life in all treatments and the treatments differed non significantly in days of analysis maintaining almost equal counts in all days.

Discussion

Lipid Oxidation

There are various reports that ghee residue possess antioxidant activity. Ramamurthy *et al.* (1969) observed that the addition of ghee residue to ghee considerably increased the shelf life of ghee. It may be due to high content of phospholipids. Phospholipid acts synergistically with reducing substances in ghee residue and protects it from oxidative defect. Ghee residue particularly one obtained from creamery butter has higher content of phospholipid about 17.39 % of its total fat (Santha and Narayanan, 1978). Among various phospholipid fractions cephalin is having greatest antioxidant activity. Lal *et al*. (1984) reported that the residue from ghee manufacture large quantities of which are wasted contains 3.6 – 13. 2 per 100g of phospholipids and addition of 15- 20 g of ghee residue to 100g of flavored butter milk imparted a fairly good oxidative stability to flavored butter milk (Wadhwa *et al.,* 1991). The Storage had a significant effect on increasing the TBARS values in all treatments.

Microbiological Analysis

The aerobic plate counts in three treatments were in the range of 101 to 102 and the yeast and mould count were less than 101 in the entire storage study period. The result of the study is in agreement with Wodjat *et al.* (2005) and according to them average level of TPC in samples of food for pets was lower and ranged between 10 2 - 10 3 cfu/g. The same tendency was observed for the total number of yeast and moulds in dry pet food samples examined and were between 101 – 103 cfu/g. The addition of GR had no significant effect in microbiological quality of the product in all three treatments and it may be due to the low moisture content of the pet food.

Conclusion

From the observations recorded in the study it can be concluded that shelf stable low moisture pet food of acceptable quality with GR and relatively lower in cost could be prepared. Usage of meat and dairy industries byproduct in pet foods, not only lower the cost of pet food, it will also help to overcome the major problem of disposal of byproducts faced by these industries and it will be a remedy for the environmental contamination caused by such byproducts.

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