

## **Microbiology, Sensory Evaluation and Shelf Life of Irradiated Chicken Breast Fillets Stored in Air or Vacuum**

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### **ABSTRACT**

*This work investigated the effects of different packaging methods (air and vacuum) combined with irradiation (0.0, 2.0 and 3.0 kGy) on the preservation of chicken breast fillets stored at 1°C for up to 18 days by sensorial test, determination of pH and bacterial growth. The findings indicated that the post-irradiation lag phase increased with the dose, leading to an extension in shelf-life. Vacuum-packed samples irradiated at 3.0 kGy exhibited the longest shelf life. Among the analyzed bacteria, coliforms and *Listeria* spp. were most sensitive to gamma radiation. All the fillets acquired more attractive coloration and better overall impression with irradiation. The combined use of vacuum packaging and irradiation (3.0 kGy) reduced the microbial populations without causing change in pH and yielded a significant shelf-life extension of refrigerated fillets, besides improving its appearance.*

**Key words:** Irradiation, chicken fillets, modified atmosphere, microbiology, sensory tests

### **INTRODUCTION**

Between 2000 and 2008, the most rapid expansion, with an annual growth rate of more than six per cent a year, occurred in South America. Over three-quarters of this expansion was attributed to the growth in the industries in Brazil, where poultry meat output escalated by almost 75 %, from 6.1mt to 10.7mt between 2000 and 2008 (The Poultry Site, 2010).

Poultry meat is a nutritious food and it is consumed all over the world because of its relatively low cost and low fat content. However, it is highly perishable with a relatively short shelf life even when it is kept under refrigeration. Thus, developing more appropriate technologies for its

preservation could be highly useful. In order to increase the shelf life of meat products, vacuum-packaging has been used although it has not been able to extend the shelf life of the packaged product for a long time. The gamma irradiation has been used in combination with packaging in modified atmosphere in order to improve the safety and further enhance the shelf-life extension of meat.

The safety and efficiency of irradiation in food preservation has been thoroughly demonstrated worldwide (Pelczar et. al., 1997). The doses of 1.5 and 3.0 kGy for the treatment of refrigerated and frozen chicken meat, respectively, were authorized in the USA in 1992 (Lee, 1995). The Brazilian legislation for food irradiation has been regarded

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as one of the most advanced ones in the world, allowing the treatment of any kind of food (Vital and Freire Jr., 2008). However, the use of such technology in Brazil is still mostly limited to the treatment of spices, animal feed and other food products to be exported. However, there has been an increasing joint effort geared at informing the population on the principles, safety and benefits of treating foods with ionizing radiation (Hernandez et al., 2003).

Bacteria tend to be more resistant to radiation during the latency (lag phase), becoming more sensitive as they enter the logarithmic growth phase and reach the lowest resistance at its end (Jay, 2005). Gram-negative (pathogenic and spoilage) bacteria are generally more sensitive than the Gram-positive vegetative cells (Franco and Landgraf, 1996).

The combination of different methods of food preservation should be seen as an alternative in the food industry, for example, the use of vacuum-packed, gamma radiation and refrigeration. However, in order to ensure that the combined use of those techniques does not produce changes in the original characteristics of the products, testing of sensory acceptance must be performed.

The objective of this study was to determine the effectiveness of the combined treatment of gamma irradiation (0, 2.0 and 3.0 kGy) and air or vacuum packaging for the preservation of refrigerated chicken fillets by monitoring the microbial growth parameters in order to determine the shelf-life extension, variation of pH and sensory acceptance.

## MATERIALS AND METHODS

The experiments were performed in two phases. During the first day of phase one (day zero), 2 kg of refrigerated chicken breast fillets were purchased from a market in Niterói, RJ and transported in boxes of expanded polystyrene filled with ice to the Laboratory, where all the bacteriological analyses and determination of pH were performed starting from day zero. Following analysis were made: counting of heterotrophic aerobic mesophilic (HAM), counting of bacteria of the genera *Listeria*, *Yersinia* and *Aeromonas*, and enumeration of total and thermotolerant coliforms. Each fillet was aseptically divided in 14 pieces with 18 g and each of those samples was packed in a plastic bag having a multilayered structure with

low permeability to gases. Two different packing atmospheres were tested in two sets, including 10 samples each: 1) air-packed (control) and 2) vacuum-packed. A sample from each treatment was analyzed individually after 1, 3, 5, 7, 9, 12 or 18 days of storage at  $1^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

The following growth media were used: plate count agar (PCA; Merck) for HAMB and PAHB, Oxford *Listeria* Base (Himedia) with *Listeria* Selective Supplement (Oxford Formulation; Oxoid) (SR0140) for *Listeria* spp., Mac Conkey Agar with *Yersinia* Selective Supplement (SR109; Oxoid) for *Yersinia* spp., Starch-ampicillin Agar (SA; Himedia) enriched with 1% ampicillin for *Aeromonas* spp., and Fluorocult broth for enumeration of total and thermotolerant coliforms. Merck's miniaturized methodology (2000), as modified by Franco and Mantilla (2004), was used for coliform enumeration. It consisted of employing automatic pipetors connected to sterilized pointers for preparation and inoculation of 0.1 mL (100 $\mu$ L) from different dilutions into 1 mL (1000 $\mu$ L) of Fluorocult selective broth.

In the second phase of experiments, fillets were obtained in the same conditions as in phase 1, out of 4 kg of fresh chicken breast; however, different doses of radiation and packing atmospheres were tested yielding four sets of samples: 1) air-packed, irradiated with 2.0 kGy; 2) vacuum-packed, irradiated with 2.0 kGy; 3) air-packed, irradiated with 3.0 kGy and 4) vacuum-packed, irradiated with 3.0 kGy. The analyses performed were the same as those in phase 1.

Since the bacterial populations in the beginning of the two phases were different, normalization of the initial reading of each phase was applied to all data of the corresponding phase so that the bacterial growth during both the phases could be compared and described according to the modified Gompertz's equation (Gibson et al., 1987) by using a special computer program (Baranyi and Roberts, 1994).

The sensory evaluation was performed in the form of an acceptance test, where samples from different treatments were randomly subjected to judgment by 33 untrained panelists. Color and overall impression were judged according to a hedonic nine-point scale (Stone and Sidel, 1998), where, 9 corresponded to "disliked extremely" and 1 to "liked extremely". Scores from 1-5 were considered acceptable. Differences between the variables were tested for significance by one-way ANOVA with Tukey's post test using SAS

program. Differences at  $p < 0.05$  were considered to be significant.

## RESULTS AND DISCUSSION

The findings indicated that the post-irradiation lag phase increased with dose, leading to an extension in shelf life. Similar results were also found by Spoto et al. (2000), who concluded that irradiation could efficiently be used in the preservation of chicken meat.

### Shelf-life extension

The samples found to exhibit the longest shelf life were those vacuum-packed and irradiated with 3.0 kGy, followed by the air-packed ones also treated with 3.0 kGy (Table 1). Then came the samples exposed to 2.0 kGy (air- and vacuum-packed), followed by the unirradiated samples, first the vacuum-packed and finally the air-packed ones. As expected, a larger decrease in the population of bacteria was found in the samples irradiated with 3.0 kGy, consequently leading to a larger extension in the shelf life. Vacuum packaging combined with irradiation at 3.0 kGy resulted in

extending the shelf life to 12 days, which was more than double in comparison with the unirradiated air-packed samples that remained good for five days only. These findings were consistent with those obtained by Abu-Tarboush et al. (1997) who reported that irradiation of refrigerated chicken meat with 2.5 kGy led to a 12-day shelf life. Similar results were observed by Grandison and Jennings (1993), who reported that air-packed unirradiated samples of ground chicken meat deteriorated in two days of storage, while the treatment with 3.1 kGy significantly increased the shelf life of the samples. Chouliara et al. (2008) also found an extension in the shelf life of fresh poultry meat treated with 2.0 and 4.0 Gy. They reported that the total counts of bacteria in the unirradiated air-packed samples reached the microbiological limit on the 5-6<sup>th</sup> day of storage at 4° C, whereas those irradiated with 2.0 kGy reached such level on the 15<sup>th</sup> day, and those irradiated with 4.0 kGy only after 22-23 days of storage. Miyagusku et al. (2003) observed that the samples of refrigerated chicken breast vacuum-packed and irradiated at 3.0 kGy did not reach 7.0 log CFU /g until the 30<sup>th</sup> day of storage, remaining with scores of up to 5.0 Log CFU /g.

**Table 1** - Shelf-life and growth parameters of bacteria found in vacuum- and air-packed chicken breast fillets treated with 0, 2 and 3.0 kGy and stored for 18 days at 1°C.

Treatment (Packing/(Dose))	Shelf Life (days) Based on Limit: 10 <sup>7</sup> CFU/g	Bacterial Growth Parameters	Initial count normalized to 1.0 log cycle				
			Me	CT	CTer	Lis	Aero
Air / 0 kGy	5	g (days)	0.6	0.4	1.1	0.5	-
		Lag (days)	1.9	2.4	8.9	1.4	-
		CF (log CFU/g)	4	2.2	1.7	5	nd
Vacuum / 0 kGy	7	g (days)	0.7	0.7	-	0.2	-
		Lag (days)	3.8	2.8	-	4.2	-
		CF (log CFU/g)	3.8	3	nd	4.5	nd
Air / 2.0 kGy	9	g (days)	0.7	18.7	-	-	-
		Lag (days)	5	-	-	-	7.7
		CF (log CFU/g)	3.3	0.6	nd	nd	0.8
Vacuum / 2.0 kGy	9	g (days)	1	1.7	-	-	0.8
		Lag (days)	4.4	6.9	-	-	7.7
		CF (log CFU/g)	2.7	0.5	nd	nd	0.7
Air / 3.0 kGy	10.5	g (days)	0.4	-	-	-	0.3
		Lag (days)	5.8	-	-	-	7.7
		CF (log CFU/g)	1.7	nd	nd	nd	3.3
Vacuum / 3.0 kGy	12	g (days)	0.7	-	-	-	1.7
		Lag (days)	5.9	-	-	-	12
		CF (log CFU/g)	1.6	nd	nd	nd	0.7

g – Breeding time; Lag- Adaptation phase; CF – Final Relative Count; nd- No detected count; Me- mesophilics; CT – totals coliforms; CTer – Thermotolerant Coliforms; Lis- *Listeria* spp.; Aero- *Aeromonas* spp.

### Heterotrophic aerobic mesophilic bacteria

Irradiation with 3.0 kGy reduced the number of aerobic mesophilic bacteria in the air-packed samples by approximately 2 log cycles (Fig. 1 A), while a much smaller drop (0.81 log cycle) was found for the vacuum-packed fillets treated with the same dose. Lescano (1991) also found that the irradiation of chicken breast with 2.5 kGy reduced the total bacterial count by approximately 2 log cycles. Thayer et al. (1995) found that the total bacterial count of chicken wings was reduced by about 2 log cycles with irradiation at 1.4 kGy.

In the present experiment, the doubling time was higher in vacuum-packed than in air-packaged samples, hinting that the mesophilic bacteria were not able to start the growth in vacuum as they grew more easily in air. This was in agreement with the results of bacterial count at the end of storage, when the vacuum-packaged samples had lower bacterial count. The highest bacterial growth in chicken breast wrapped in air when compared with the vacuum-packed ones was also found by Jiménez et al. (1997). They reported a rapid growth of viable bacteria in air-packed samples, reaching a population of 8 log CFU/g after four to five days of storage at 4° C; the use of vacuum packaging extended the time required for the total count of bacteria to reach 8 log CFU/g to two or three days of storage.

In this work, shelf-life extension was mostly due to the irradiation-induced prolongation of the lag phase, found to be higher for the samples treated with 3.0 kGy.

### Coliforms

The thermotolerant coliforms were unable to grow in the samples packed in vacuum and in irradiated ones, and were detected only in the unirradiated fillets packed in air. Miyagusku et al. (2003) detected *E. coli* in the control samples only and no significant counts were found during the storage in the irradiated samples.

The total coliform group did not show detectable growth in the samples irradiated at 3.0 kGy (Fig. 1 C) and showed a longer lag phase in the vacuum-packaged samples treated with 2.0 kGy. According to the data that group was eliminated from the chicken fillets irradiated to 3.0 kGy, and had difficulty to start growth after irradiation with 2.0 kGy. Abu-Tarboush et al. (1997) also found that

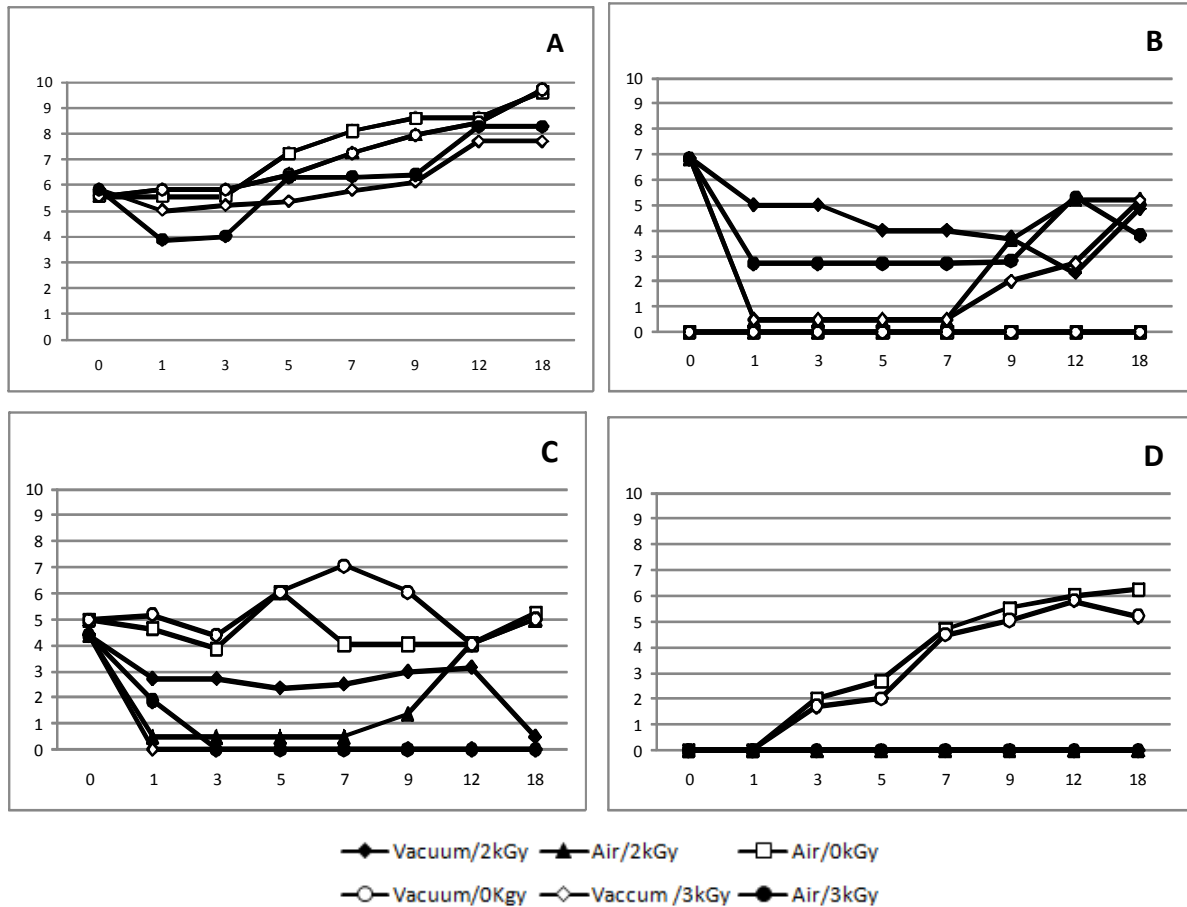
irradiation with 2.5 kGy and storage at 4°C for 21 days was sufficient to eliminate total coliforms in chicken meat. In another experiment, gamma irradiation of chicken with 1 and 1.8 kGy was sufficient to eliminate total coliforms (Lewis et al., 2002).

### Other bacteria

*Listeria* spp. was only detected in the control samples packed in air and vacuum, being eliminated with irradiation at 2.0 and 3.0 kGy (Fig. 1 D). Roberts and Weese (1995) reported that irradiation of meat with a dose up to 3.0 kGy effectively eliminated over 99% *L. monocytogenes*. Samelis et al. (2005) reported that 4.0 kGy completely eliminated *Listeria* spp. frozen meat. Trivedi et al. (2007), also observed that irradiation with 3.0 kGy was effective in eliminating 10<sup>2</sup> CFU/g of *L. monocytogenes* inoculated on diced chicken meat and turkey frankfurters.

*Aeromonas* spp. was not detected in control samples. Kumar et al. (2000) isolated *Aeromonas* spp. in 33 (13.41%) of 246 food samples of animal origin. In the present study, this genus was only detected in air- and vacuum-packed samples irradiated with 2.0 and 3.0 kGy (Fig. 1 B). A possible explanation for this could be that such bacterium hardly grew in media where it had to face competition with other microorganisms, as it was the case at the beginning of the experiment. Thus, when the microbiota was reduced by irradiation, *Aeromonas* spp. was able to develop. However, Thayer (1995) observed that doses below 3.0 kGy were sufficient to eliminate *Aeromonas hydrophila* in chicken meat.

*Yersinia* spp. was not detected in any sample from the beginning. According to Jay (2005), the pig is the most common source of this pathogen. In a pilot investigation, performed in Novi Sad, Trajković-Pavlović et al. (2007) did not find *Yersinia enterocolitica* in any of the tested samples (90 of fresh meat and 167 of ready-to-eat food). Bucher et al. (2008) examined the prevalence of pathogenic *Y. enterocolitica* in the animals and foodstuffs in Bavaria and reported it only from raw pork, especially from the edible offal. Some raw pork sausages and only one poultry sample were PCR positive.



**Figure 1** - Growth bacterial curves (Log CFU/g X Days of Storage) in samples of refrigerated chicken breast fillets subjected to six different treatments. (A)- Heterotrophic aerobic mesophilic bacteria; (B)- *Aeromonas* spp.; (C)- Total coliforms and (D)- *Listeria* spp.

### pH values

There were no changes in pH at the beginning of the experiment. As expected, the vacuum-packed samples consistently showed a lower pH value, due to the predominance of acid-producing bacteria. Samples packaged in the air showed an increase in pH during the storage in the same way as reported by Miyagasku (2008), which could be attributed to the faster growth of spoilage bacteria. The pH of air-packed irradiated and unirradiated samples ranged from 5.6 to 7.0 and 5.4 to 6.0, respectively (Fig. 2), confirming the data obtained by Pinto et al. (2005) who found that the samples of unirradiated chicken breast wrapped in air had a change in pH from 5.9 to 6.5 and the irradiated samples had pH ranging between 5.9 and 6.8, also informing that irradiation provided no changes in pH values compared with the control samples.

### Sensory acceptance

Table 2 showed that the judges preferred the air-packed samples irradiated with 2.0, although no significant difference was obtained relatively to the vacuum-packed samples irradiated with 3.0 kGy. All the irradiated fillets showed a color more attractive than non-irradiated. Lewis et al. (2002) concluded that irradiation at 1.0 kGy improved the color of chicken breast fillets. Nanke et al. (1998) also observed changes in the color of pork and turkey irradiated at doses of 1.5 kGy, 3.0 kGy, 4.5 kGy, 7.5 kGy and 10.5 kGy, which showed up red due to irradiation. Nam and Ahn (2002) found that Comioglobina was responsible for the pink color of chicken meat caused by the interaction with carbon monoxide during irradiation. Kim et al. (2002) also found that the development of red color in irradiated meat was

due to the production of gas, especially CO<sub>2</sub>. Similar results were found by Millar et al. (1995), who reported higher redness in the chicken breast irradiated at 5.0 kGy compared with unirradiated controls. Those authors concluded that ionizing radiation was able to change the coloration of poultry meat depending on the kind of muscle type and properties of the skin.

However, Kanatt et al. (1997) did not find any change in sensory attributes such as appearance,

color and odor after irradiating the chicken with 2.5 kGy. Similar reports were discussed by Souza et al. (2007) who investigated the influence of radiation on the levels of iron and color of pigments of thighs and chicken breast meat irradiated at doses 0, 1 and 2.0 kGy and found that the color was not influenced by those doses. The lack of changes in sensory properties of the chicken meat was probably due to the low doses (below 3.0 kGy) used by these researchers.

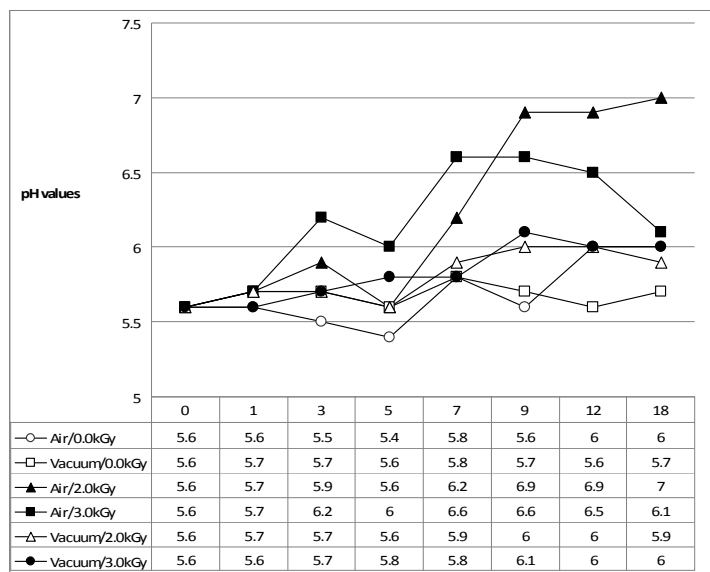


Figure 2- pH values for all treatments during the experiment.

Table 2 - Results from Tukey's Test for the color of chicken fillets subjected to different treatments.

Treatment	Mean
Vacuum/0.0 kGy	5.0 <sup>a</sup>
Air/0.0 kGy	4.7 <sup>ab</sup>
Air/ 3.0 kGy	3.97 <sup>b</sup>
Vacuum/2.0 kGy	3.67 <sup>bc</sup>
Vacuum/3.0 kGy	3.42 <sup>c</sup>
Air/2.0 kGy	3.36 <sup>c</sup>

\*Means followed by at least one letter the same, in the same column do not differ ( $p > 0.05$ ).

In relation to sensory acceptability of the chicken fillets to the overall impression (Table 3), it was found that only the vacuum-packed ones were rejected by the judges, probably due to the change from the original meat format. The treatment that received the highest scores in that attribute was air packaging combined with irradiation with 2.0 kGy. It was followed by vacuum packaging treated with 3.0 kGy, then with 2.0 kGy, followed by air packaging irradiated with 3.0 kGy and finally the

unirradiated sample packed in air. In this work, irradiation with 2.0 and 3.0 kGy improved the overall appearance of the samples. Hashim et al. (1995) also reported that irradiation did not affect the appearance (humidity and brightness) of breast and thigh chicken refrigerated raw chicken. Abu-Tarboush et al. (1997) observed that irradiation of chicken meat (2.5, 5.0, 5.7 and 10.0 kGy) caused minor changes in product acceptance in relation to appearance, aroma, texture and flavor.

**Table 3** - Results from Tukey's Test for the overall impression of chicken fillets subjected to different treatments.

Treatment	Mean
Vaccum/0.0 kGy	5.76 <sup>a</sup>
Air/0.0 kGy	4.81 <sup>ab</sup>
Air/3.0 kGy	4.63 <sup>abc</sup>
Vacuum/2.0 kGy	4.57 <sup>abc</sup>
Vacuum/3.0 kGy	4.51 <sup>abc</sup>
Air/2.0 kGy	3.67 <sup>c</sup>

\*Means followed by at least one letter the same, in the same column do not differ (p> 0.05).

## CONCLUSION

Based on the results obtained in this work, it could be concluded that irradiation at doses of 2.0 and 3.0 kGy significantly increased the shelf life of refrigerated chicken meat in comparison with the unirradiated air-packed samples. Irradiation of the samples under vacuum was more efficient in extending the shelf life and gave chicken fillets with color and overall more desirable to the consumers. Thermotolerant coliforms and *Listeria* spp. were eliminated with irradiation, whereas *Aeromonas* spp. was able to develop in the irradiated samples. The pH of the samples did not show large variations during the storage period. Irradiation improved the color of the fillets and maintained a overall good impression of the product. Results also demonstrated that the combined effect of vacuum-packed and irradiation (3.0 kGy) was able to extend the shelf life of refrigerated chicken fillets and control deteriorating and pathogenic microbiota, making them safer and more attractive to the consumers.

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