

EFFECT OF SORBIC ACID AND GAMMA IRRADIATION ON THE MICROBIAL FLORA OF SOY-BUFFALO PATTIES

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INTRODUCTION:

It is known that microbes play a major role in meat spoilage, and the type of spoilage obtained might be caused by very different organisms according to particular conditions of treatment and storage (Ingram and Dainty, 1971). Marriot et al. (1980) pointed out that the bacterial groups of Micrococci, Pseudomonas, Streptococci, Staphylococci, Flavobacterium, Lactobacilli and Coliforms were considered to be responsible for deterioration of appearance and taste attributes of ground beef.

Significant levels of soy proteins (25-30%) were found by many research workers to successfully replace meat or fat in different meat products (Sofos and Allen, 1977; Foster et al., 1978 and Kraft et al., 1979). Moreover, many investigators indicated that the presence of soy proteins (textured flours, concentrate and isolates) did not affect the microbial flora in meat patties (Judge et al., 1974; Sofos et al., 1979 and Emswiler et al., 1979). Stansbury (1975) reported a highly significant increase in the logarithmic aerobic and psychrophilic-mesophilic counts accompanied by every increase in the level of texturized vegetable proteins in ground beef patties. However, recently, Ray et al. (1981) indicated that total bacterial numbers in ground beef patties increased as the level of hydrated soy protein (up to 26%) increased.

Sorbic acid and potassium sorbate are widely used in the food industry for inhibiting molds and yeast. Both compounds are generally considered as safe by the code of Federal Regulations (section 121.101 of Food additives regulations) at the level used and does not affect the taste or flavor of the foods (Huhtanen and Feinberg, 1980). Pierson et al. (1979) found that potassium sorbate at the concentration of 0.13% delayed the growth of *Cl. botulinum* and the formation of toxin in bacon. Moreover, Buchway et al. (1982) concluded that potassium sorbate, up to 0.26%, reduced the number of aerobic microorganisms significantly in chickens white and patties.

The primary objective of treating flesh food with ionizing radiation is to extend their shelf-life throughout the reduction or total elimination of food spoilage microorganisms (Josephson et al., 1973). Bacteriological studies of Keskin (1977) indicated that irradiation, at 0.25 Mrad, permitted refrigerated storage of ground beef for 3 weeks and at 0.5 Mrad for 6 weeks, while completely inhibited coliforms. Wolin et al. (1975) found that *Pseudomonas geniculata* was among the most sensitive organisms to gamma irradiation. However, Sawickawrzošek (1978) concluded that a dose rate of 0.75 Mrad to 1.5 Mrad completely destroyed *E. coli* and markedly reduced the other counts.

This investigation was undertaken to study the effect of sorbic acid as well as gamma irradiation of buffalo meat patties, extended with 30% soy flour and contained two fat levels namely 10 and 20% on three of the most important groups of meat spoilage microorganisms i.e. psychrophilic, coliforms and Staphylococci during frozen storage. The effect of precooking treatment of patties on the microflora was also studied.

MATERIALS AND METHODS:

The source of meat and soy flour as well as methods of formulations, fabrication and storage have been given in a previous publication (El-Wakeil et al., 1982).

Buffalo meat patties, having two different compositions, were produced: (A) Buffalo meat with 30% soy flour and 10% fat. (B) Buffalo meat with 30% soy flour and 20% fat. Six different treatments were applied: (1) Raw frozen (control) (2) Raw frozen with 0.2% sorbic acid. (3) Raw frozen treated with irradiation (375 Krad). (4) Pre-cooked frozen. (5) Pre-cooked frozen with 0.2% sorbic acid. (6) Pre-cooked frozen treated with irradiation at 375 Krad. After freezing all samples were held frozen at $-10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ up to 6 months.

Examinations were carried out at 0, 30, 60, 90, 120, 150 and 180 days of storage for Psychrophilic, Coliforms and Staphylococci. Patties were thawed before examination in a refrigerator at about 5°C . Bacteriological procedures used are, hereafter, shown in Table I.

Table 1: Bacteriological procedures employed

Quantitative determinations (a)	Growth media	Plating technique	Incubation
Psychrophilic Coliforms	Trypticase soy agar.	Pour plates	50°C , 7 days
	Violet red bile agar (Dam et al., 1970)	Pour plates	37°C , 24 hr.
Staphylococci	Staph. 110 medium with egg yolk (Herman and Morelli, 1960)	Surface (b)	37°C , 48 hr.

(a) Thirty-gram sample.

(b) A swab technique was used for sampling about 50 cm^2 of surface

RESULTS AND DISCUSSION:

A. Total Psychrophilic count:

Figure (1) illustrates the changes which occurred in the total psychrophilic count as influenced by different treatments, i.e. addition of sorbic acid, gamma irradiation and fat content of soy-buffalo meat patties. The results clearly indicated that both sorbic acid and irradiation (even at the low dose of 375 Krad) clearly prohibited the total psychrophilic bacteria. However, irradiation had a more pronounced effect, as the percentage reduction reached as high as 80%, while with sorbic acid it reached only 68%. The results also indicated that the percentage fat added to the buffalo meat patties affected its microbial flora. With 10% fat the

percent reduction in the psychrophilic bacterial counts (in all treatments under investigation) was higher than with 20% fat. This might indicate that such fat had a protective effect on the microorganisms (Kraft *et al.*, 1979). Therefore, the fat content of meat patties should be considered during frozen storage in order to extend their shelf-life.

Precooking per se was found to lower the bacterial count as an effect to the heat treatment employed (Fig. 1). However, an addition of sorbic acid the effect was much more observable. On the other hand, irradiation with gamma rays combined with precooking of the prepared samples clearly eliminated the presence of psychrophilic bacteria. Moreover, irradiation even hampered the protective effect of the added fat as the patties with 20% fat did not show any growth for the psychrophilic bacteria.

As reported by Okafor (1973) an irradiation dose of 1.0 Mrad was required to eliminate most of the microorganisms present in meat. However, he found that at 1.0 Mrad many meat packs developed a "burnt-meat" odour, which might render them less acceptable for consumption. The present results revealed that low dose of irradiation (as low as 375 Krad) combined with precooking was capable for the elimination of the psychrophilic bacteria and maintained the acceptability of buffalo patties at the same time. (Table II).

During storage of buffalo meat patties, at $-10^{\circ}\text{C} \pm 2^{\circ}\text{C}$, up to 6 months, the numbers of total psychrophilic bacteria decreased gradually following the same trend. Irradiation still had the most effective influence and followed by the sorbic acid treated samples. Meanwhile, precooked treatments showed the best results as clearly indicated in (Fig. 1). At the end of storage period (6 months), irradiated precooked samples did not show any growth for the psychrophilic bacteria, while samples containing 0.2% sorbic acid revealed only 1.5×10^1 colonies, which could be considered of little significance in meat spoilage (Kraft *et al.*, 1979).

B. Total Coliform group:

The coliform group of bacteria is known as one of the major groups that affect the sanitary quality of ground meat, either raw or frozen (Okafor, 1973). Therefore, the coliform bacteria was determined in the soy-buffalo meat patties treated with different treatments and the results are graphically presented in Figure (2).

The total coliform count was highly affected by the addition of sorbic acid at the concentration of 0.2% and by the exposure to γ -rays at 375 Krad, than the total psychrophilic bacterial count in both raw frozen buffalo meat patties, i.e. with 10 and 20% fat. However, it seems that freezing alone had a slight effect on coliforms as the viable count was as high as 3.3×10^4 at zero time. Meanwhile, in the samples treated with sorbic acid and gamma irradiation, the viable counts were 4.5×10^3 and 3.0×10^2 respectively (at 10% fat level). On the other hand, at 20% fat level, the coliform count was almost as twice as much, as it reached 2.5×10^4 and 4.7×10^2 successively, which confirms the protective effect of fat on the microorganisms.

The data present in Fig. (2) revealed the high sensitivity of the coliform bacteria to heat. All precooked samples were completely free from the coliform groups in all treatments under investigation even the control ones. Therefore, it might be concluded that precooking per se (internal temperature 75°C) was capable of eliminating the health hazard of coliform bacteria.

During storage at $-10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 6 months, the viable counts of coliform bacteria in the raw frozen soy-buffalo-meat patties significantly decreased. However, irradiation completely destroyed the coliform groups in samples with 10% fat after 4 months of storage, while the same effect was obtained after 5 months of storage in the samples with 20% fat (Fig. 2 and Table III).

The addition of sorbic acid seems to have a lower effect on the coliform bacteria as compared with irradiation. Its effect was much more pronounced right after application as clearly shown in Fig. (2). This might be due to the possibility that during storage the coliform group well adapted to the low concentration of sorbic acid (0.2%), while at the beginning the lethal effect of the acid ion was more effective (Huhtanen and Feinberg, 1980).

The data concerning the precooked buffalo patties stored up to 6 months at $-10^{\circ}\text{C} \pm 2^{\circ}\text{C}$, confirm the fact that coliforms are sensitive to heat and, hence, might suggest that enteric pathogens (i.e. Salmonella) also might be destroyed (Kotula *et al.*, 1977).

C. Total staphylococci counts:

Fig. (3) shows the changes occurring in the total staphylococci counts as affected by sorbic acid (0.2%) and gamma irradiation (375 Krad.) in soy buffalo meat patties with two fat levels i.e. 10 and 20%.

The results clearly indicate the sensitivity of Staphylococci bacteria to gamma irradiation, even at the very low dose. The total staphylococci count was found to decrease immediately after irradiation by 99.43% and 99.24% in samples with 10 and 20% fat respectively. In the other words, the use of gamma rays prevented the protective effect of fat on the microorganisms, and, hence, allowed the use of higher fat percentage in meat patties. The same results were also reported by Okofor (1973) who studied the lethal effect of gamma irradiation at 0.3 Mrad on Staphylococci in ground beef.

On the other hand, the use of sorbic acid, at 0.2% level, clearly reduced the Staphylococci growth of the meat patties, but to a lower extent as the percentage reduction reached 94.86% and 94.39% in samples with 10% and 20% fat respectively.

During frozen storage of the different treatments of soy-buffalo meat patties, the staphylococci bacteria showed successive reduction, as shown in Fig. (3). The combined action of gamma irradiation and freezing reduced the samples completely free from Staphylococci group after one month. While, on the other hand the combined action of sorbic acid and frozen storage induced the same effect after 3 to 4 months depending on the fat level. According to Foster *et al.* (1978) the number of *Staph. aureus* allowable in ground beef in U.S.A. ranges from 0 to 2.5×10^2 per gram. The present results clearly indicated that in samples treated with 0.2% sorbic acid, the number of Staphylococci after only one month of storage was 4.8×10^1 , which is considered within such allowance.

One of the primary objectives of the cooking process is the thermal destruction of microorganisms. Pre-cooking per se of soy-buffalo meat patties, to an internal temperature of 75°C , decreased the total Staphylococci count by 97.8% and 97.98% in the two formulas under investigation (i.e. with 10 and 20% fat content respectively). The very low numbers of Staphylococci in the precooked patties were probably due to the combined treatments of pre-cooking followed immediately by freezing. Heat stressed Staphylococci that survived pre-cooking probably were susceptible to the additional stresses of freezing (Emswiler *et al.*, 1979).

Moreover, the obtained results indicated that in precooked treated samples the use of sorbic acid and gamma irradiation were of lower importance (Fig. 3). No growth of *Staphylococci* was encountered in any of the precooked treatment either in gamma irradiated treated samples nor, those treated with sorbic acid until the end of storage period (6 months at $-10^{\circ}\text{C} \pm 2^{\circ}\text{C}$).

REFERENCES:

- Bushway, A.A.; Ficker, N. and Jen, C.W. (1982). Effect of nitrite and sorbate on total number of aerobic microorganisms in chicken white and dark meat patties. *J. Food Sci.*, 47, 858.
- El-Wakeil, F.A.; El-Magoli, S.B.; Alian, N. and Abdel-Rahman, N. (1982). Lipid oxidation and quality characteristics of soy-buffalo meat-patties as affected by sorbic acid and gamma irradiation. *Ain-Shams Univ. Res. Bul.* No. 2050.
- Emswiler, B.S.; Pierson, C.J.; Kotula, A.W. and Cross, H.R. (1979). Microbiological evaluation of precooked beef patties containing soy protein. *J. Food Sci.*, 44, 154.
- Foster, J.F.; Guthertz, L.E.; Hunderfund, R.C. and Fowler, J.L. (1978). Comparison of bacterial species isolated from ground beef. Textured soy protein and ground beef extended with textured soy protein. *J. Food Sci. Protection*, 41, 961.
- Huhtanen, C.N. and Feinberg, J. (1980). Sorbic acid inhibition of *Clostridium botulinum* in nitrite-free poultry frankfurters. *J. Food Sci.*, 45, 453
- Ingram, M. and Dainty, R.H. (1971). Changes caused by microbes in spoilage of meat. *J. Appl. Bact.*, 34, 21
- Josephson, E.S.; Brynjolfsson, A.; Wierbicki, E.; Rowley, D.B.; Merritt, C.; Baker, R.W.; Killoran, J.J. and Thomas, M.H. (1973). Radiation preservation of food. Radappertization of meat, meat products and poultry. International Atomic Energy, Vienna, Austria, 471.
- Judge, M.D.; Haugh, C.G.; Zachariah, G.L.; Parmelec, C.E. and Pyle, R.L. (1974). Soy additives in beef patties. *J. Food Sci.*, 39, 137.
- Keskin, S. (1977). Extension of refrigerated storage of minced beef by irradiation. *Industries Alimentaires Et Agricoles*, 94, 1297.
- Kotula, A.W.; Chesnut, C.M.; Emswiler, B.S. and Young, E.P. (1977). Destruction of bacteria in beef patties by cooking. *J. Animal Sci.*, 45, 54.
- Kraft, A.A.; Reddy, K.V.; Sebranek, J.G.; Rust, R.E. and Hotchkiss, D.K. (1979). Effect of composition and methods of freezing on microbial flora of ground beef patties. *J. Food Sci.*, 44, 350.
- Marriott, N.G.; Garcios, R.A.; Pullan, J.; Handlee, D.R. (1980). Effect of thaw conditions on ground beef. *J. Food Protection*, 43, 180.

- Okafor, N. (1973). Microbial flora of gamma-irradiated vacuum-packed ground beef stored at different temperatures. *J. Food Sci. and Technol.*, 10, 95.
- Pierson, M.D.; Iveg, F.J.; Smoot, L.A. and Vantassel, K.R. (1979). Potassium sorbate inhibition of Clostridium botulinum in bacon. Annual Meeting of the American Society for Microbiology, 79, 217.
- Ray, F.K.; Parrett, N.A.; Vanstavern, B.D. and Ockerman, H.W. (1981). Effect of soy level and storage time on the quality characteristics of ground beef patties. *J. Food Sci.*, 46, 1662.
- Sawicka-Wrzosek, K. (1978). Study of sterilization of a spice mixture for frankfurters by γ -irradiation from a Co^{60} source. *Food Sci. and Technol. Abs.*, 11, 65, 1913.
- Sofos, J.N. and Allen, C.E. (1977). Effects of lean meat sources and levels of fat and soy protein on the properties of wiener-type products. *J. Food Sci.*, 42, 875.
- Sofos, J.N., Busta, F.F. and Allen, C.E. (1979). Effect of sodium nitrite on Clostridium botulinum toxin production in frankfurter emulsion formulated with meat and soy proteins. *J. Food Sci.*, 44, 1267.
- Stansburty, J.B. (1975). Quantitative determinations of selected bacteria in ground beef extended with varying levels of textured vegetable proteins. *Dissertation Abstracts International*, B36, 1127.
- Wolin, E.F.; Evans, J.B. and Niven, C.F. (1957). The microbiology of fresh and irradiated beef. *Food Research*, 22, 687.

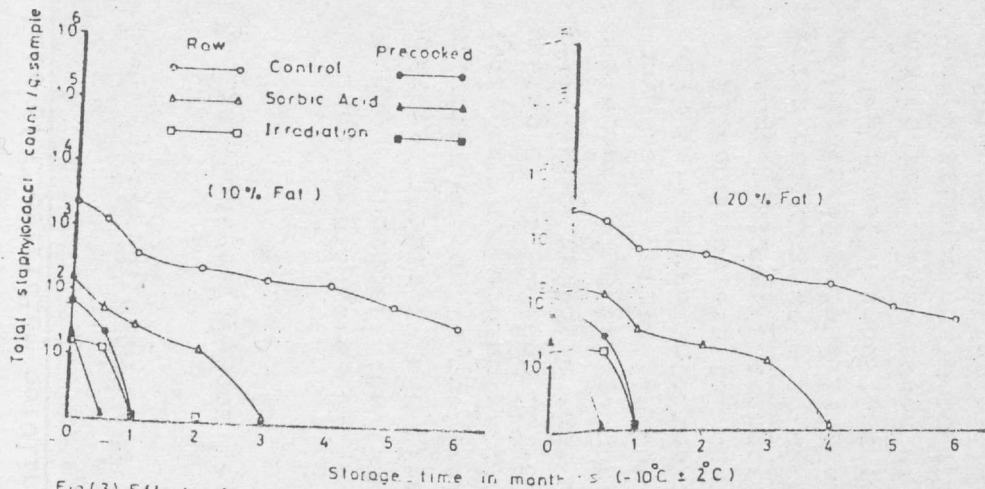


Fig.(3) Effect of sorbic acid and gamma irradiation on the total staphylococci bacteria count of soy-buffalo meat patties .

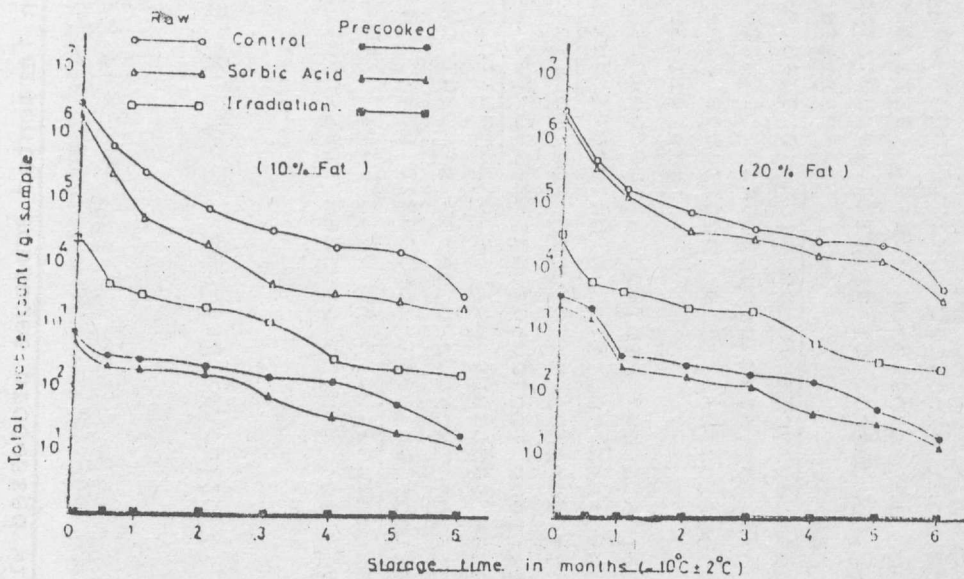


Fig.(1) Effect of sorbic acid and gamma irradiation on the total psychrophilic bacterial count of soy-buffalo meat patties

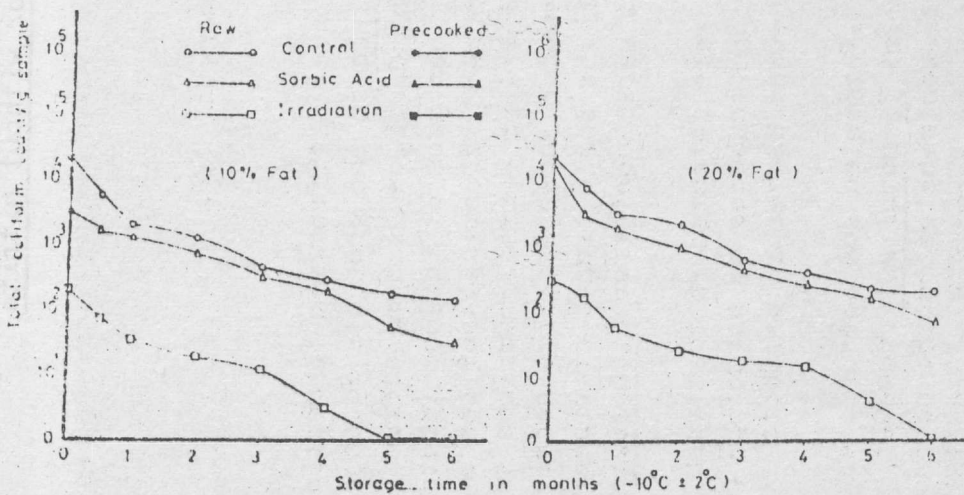


Fig.(2) Effect of sorbic acid and gamma irradiation on total coliform groups of soy-buffalo meat patties .