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# Use of the Ethanolic Extract of Bee Pollen (Bee Bread) and Gamma Irradiation for Keeping the Quality of Silver Carp (*Hypophthalmichthys Molitrix*) Fish Patties

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## ABSTRACT

**This investigation was carried out to extend the shelf-life of silver carp fish patties (*Hypophthalmichthys molitrix*) by the use of ethanolic extract of bee pollen (bee bread) at concentration of 4%, and gamma irradiation at doses of 1, 3 and 5 kGy as an individual treatment. The first group was control, the second group was silver carp fish patties samples treated with 4 % the ethanolic extract of bee pollen (bee bread) then irritated at doses of 1, 3 and 5 kGy. The effects of these treatments on the microbiological, chemical and sensory characteristics of silver carp fish patties samples have been observed. In addition, shelf-life periods were higher for silver carp fish patties samples treated by 4% the ethanolic extract of bee pollen (bee bread) and gamma radiation at dose of 5 kGy. This treatment was more effective as antimicrobial, consequently may be useful as natural food preservative.**

***Key Words: Ethanolic extract/ Bee pollen/ Silver carp/ Fish Patties/ Gamma irradiation***

## INTRODUCTION

In recent years, more concerns about food safety, together with the consumer's demand for safe and healthier products, have promoted studies of compounds with harmful effects on human health. Food processors and consumers have both expressed a desire to reduce the use of synthetic chemicals in food preservation. Common culinary herbs, spices and aromatic plants that exhibit antimicrobial activity could provide a source of accept Figure and natural alternatives. Antimicrobial agents, including food preservatives and organic acids, have been used to inhibit the growth of food borne bacteria and to extend the shelf-life of processed food. Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against food pathogens (**Castaldo, and Capasso 2002).**

Pollen is the reproductive cells of plants. Bees, other insects, wind and water pollinate plants help in transferring pollen from the stamen to the stigma of another plant. Honey bees collect pollen by adding sugars from nectar to hold the grains together and then transfer them back to the colony by packing them into hairs on the corbiculae (hind legs) of bees. Moreover, bee pollen, commonly referred to as the ''life-giving dust'', results from the agglutination of flower pollens with nectar and salivary substances of the honeybees and is used as food for all the developmental stages in the hive **(Almeida-Muradian,et al., 2005).** Bee pollen is considered a healthy food enjoying a wide range of therapeutic properties, among which: antimicrobial, antifungal, antioxidant, anti-radiation, hepatoprotective, chemoprotective and/or chemopreventive, anti-inflammatory activities, rich in nutrients and phytochemicals such as carotenoids, flavonoids and phytosterols (**Fatrcová-Šramková,et al., 2013).**

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Silver carp is a prevalent freshwater and extremely muddy flavor and many fish bones is a low market-value resource. The hydrolysis of protein with proteolytic enzymes can provide more marke Figure and value-added products of fish protein hydrolysate (**Suthasinee,et al., 2005).** In the United States, carp (including the silver carp) are generally considered to be using Fig. For direct human consumption due to the bony nature of their carcasses. However, various carp species have rapidly begun populating major bodies of fresh water to such an extent that commercial processing is now of interest. Carp have become a subject of research aiming to enable efficient utilization of their muscle protein and fat. (**Taskay, et al., 2009)** have shown that the materials recovered from the whole gutted silver carp using isoelectric solubilisation/precipitation have a high nutritional value and may be useful in the development of human food (or animal feeds).

Food irradiation was used for the safe production, storage and for the elimination of the harmful microorganisms that contaminate food. Commercial use of this technology has been actively utilized on an industrial scale in many countries (**Thayer, 1994**). However, several adverse effects (lipid oxidation, softening, etc.) caused by ionizing radiation have prevented this technology from being extended. Especially, lipid oxidation of meat products by irradiation is the most important factor for the decline of the quality. For preventing oxidation, the use of an antioxidant has been considered and practically applied in some products **(Kanatt, et al., 1998).** Natural antioxidants have been used to scavenge free radicals and to inhibit lipid oxidation owing to their safety and wholesomeness (**Lee, et al., 1999).**

Thus**,** the objectives of this study are to maintain the quality of silver carp (*Hypophthalmichthys molitrix*) fish patties by using the ethanolic extract of bee pollen (bee bread) and gamma irradiation.

## MATERIALS AND METHODS

### Preparation of the Ethanolic Extract of Bee Pollen (EEBP)

Dried bee pollen samples (300g) were purchased from local market, and then macerated in 500 ml ethanol (70%) by shaking for 7 days in the dark at room temperature. The ethanolic extract solution was then filtered and concentrated in a rotary evaporator under reduced pressure at 40 °C to a final volume of 5 ml. The extracts were stored in desiccators until use.

### Preparation of Minced Silver Carp (*Hypophthalmichthys Molitrix*) Fish Meat

Fresh silver carp (*Hypophthalmichthys molitrix*) fish, between 750 and 850 g in weight, were purchased from local market. Having been transferred to the laboratory, the fish were beheaded, gutted and washed. Then, they were filleted. The yield of flesh achieved by hand-filleting was 39.25% the fillets were minced in electrical minced.

### Preparation of Fish Patties

The silver carp patties mince included 93.5% silver carp mince, 1.5% salt, 1% sugar, 3% wheat flour, 0.243% cumin, 0.243% onion, 0.243% garlic powder, 0.243% pepper and 0.020% thyme (treated with 4% EEBP or untreated). The ingredients were homogenized with a kitchen blender to obtain fish patties which handily forming them into rounded flat patties. The raw patties' thickness and diameter were considered to be the inner height and diameter of the mould used (1.5 and 7.5 cm, respectively), then packaging in polyethylene bags. The untreated and treated samples of fish patties were divided into two groups; the first was used as a control group, the second was irradiated at dose levels of 1, 3 and 5 kGy for samples fish patties treated by 4% EEBP. Sampling for analysis was stopped with the rejection of samples if one or more of the following signs were observed: (1) visual observation of microbial growth on the surface of samples, (2) the deterioration of the odor, (3) and if the total plate count exceeded $10^7$ cfu/g.

**Irradiation Treatments**

For irradiation treatments, all fish patties groups were exposed to gamma irradiation at doses of 1, 3 and 5 kGy using an experimental $^{60}$Co Russian gamma chamber, (dose rate 3.2 kGy/hr), in the Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Abou Zaabal, Egypt. After irradiation, all samples were subjected to analysis.

**Microbiological Assay**

Colony forming units for total bacterial count were counted by plating on plate count agar medium and incubation at 30°C for 3-5 days **(APHA, 1992).** Lactic acid bacteria were counted by the pour plate over layer method on MRS medium **(Oxoid manual, 1982).** Enterobacteriaceae were counted on violet red bile glucose agar medium after incubation for 20–24 h at 37°C Roberts et al., (1995).Total count of molds and yeasts were counted on oxytetracycline glucose yeast extract agar medium according to **(10),** then the plates were incubated at 25°C for 3-5 days.

**Chemical Analysis**

Total volatile nitrogen (TVN) was determined as described by **(Mwanasyemela, 1992).** Trimethylamine (TMA) contents were determined for fish samples as described by **(EEC, 1995)**.

**Measurement of Lipid Peroxidation**

Thiobarbituric acid-reactive substances (TBARS) produced from lipid peroxidation were determined using the method of **(AMC, 1979).** A 4 g portion of each sample was blended with 16 ml of trichloroacetic acid solution (TCA 5%) and BHT (10 µg BHT/g of lipid ratio). Then it was filtered through Whatman filter paper (No. 4). Equal amounts of the filtrate and 0.02 M thiobarbituric acid was heated in boiling water bath for 20 min, cooled and the absorbance was measured at 532 nm.

**Sensory Evaluation**

Irradiated and non-irradiated prepared fish patties samples were periodically examined (every 3 days) for their appearance, texture and odor post treatments and during cold storage at 4±1ºC to determine the shelf-life of the samples. The panel consisted of ten members from our Department food irradiation laboratory and scores were obtained as described by **(Wierbicki, 1985)** by rating the above quality characteristics using the following rating scale: 9= Excellent, 8= Very good, 7= Good, 6= Below Good-above fair, 5= Fair, 4= Below fair-above poor, 3= Poor, 2= Very poor and 1= Extremely poor.

**Statistical Analysis**

The data were conducted to two-way analysis of variance to test the effect of ethanolic extract of bee pollen levels, gamma irradiation and time intervals. The differences among means were significant at significance level of $P < 0.05$ using Tukey test as a post-hoc test. All statistics were run on the computer using SAS program (SAS 2000, Version 6.12, SAS Institute Incorporation, and Cary, NC).

## RESULTS AND DISCUSSION

Figures (1) shows that the control batch of silver carp fish patties had initial counts of $7.6 \times 10^5$ and $6.2 \times 10^3$ cfu/g for total bacterial counts and lactic acid bacteria, respectively. The high level for the initial bacterial counts may be due to the presence of spore formers in the raw materials that could survive during cooking of silver carp fish patties and/or possible contamination during handling procedures. **Lopez-Cabaallero, et al., 2005)** have showed that initial total bacterial counts in the cod fillets were 4.2 log cfu/g. In addition **(Abedldaiem, 2009)** reported that bolti fish fillets possess initial counts of $6.7 \times 10^5$ and $3.2 \times 10^4$ cfu/g for total bacterial and total psychtrophilic bacteria, respectively.

Meanwhile, **(Tawfik, et al., 2007)** reported that control (non-irradiated) ready-to-eat cooked beef burger steaks had initial counts of $8.7xl0^4$ and $4.2xl0^5$ cfu/g for total bacterial and total psychtrophilic bacteria, respectively. Figure (1) Shows that the initial count of total moulds and yeast and *Enterobacteriacea* was $5.3xl0^3$ cfu/g in control samples. Irradiation of these samples at doses levels 3and 5 kGy caused a decrease in the initial count of total mould and yeast reached $7.0xl0^1$ and $2.0xl0^1$ cfu/g, respectively, and there was no colony growth of *Enterobacteriacea* neither post-irradiation nor during storage till 39 day (end of the experiment time).

In control samples when the total count exceeded the maximum accept Figure level of $lxl0^7$ cfu/g (**Alasnier, et al., 2000),** this leads to the rejection of the stored control samples. In the present study, the control samples were rejected at more than 3 days of storage and accept samples treated with 2% EEBP till 6 days of storage and 3 and 5 kGy irradiated samples till 15 and 21 days of storage (Fig. 1). The combined treatment of silver carp fish patties with 4% EEBP and 5 kGy increased the shelf-life till 45 days storage (Fig. 1). Presentation of outbreak of food borne diseases that are caused by pathogenic microorganisms and prevention of microbial spoilage of meat that leads to loss in the human health and economic society are very important (**Motamedee, et al., 2003).**

The treatment of foods with ionizing radiation in the form of gamma rays can produce beneficial effects such as inhibiting the growth of fungi pasteurizing fresh meat, poultry and seafood and sterilizing spices and food additives (**Cleland, et al., 2004).** In addition, irradiation is used for inactivate/eliminate the microbial load of meat products during storage period at refrigerators (**Tawfik, et al., 2007)**. The use of multiple antimicrobial treatments for decontaminating meat might provide a greater barrier to microbial survival and proliferation of beef by taking advantage of different weakness of differing microbial strains (**Pohman, et al., 2005)**. Bee pollen (bee bread) is increasingly recognized for their antibacterial and antifungal properties and the effects depend on the concentration of bee pollen (bee bread) extract and are influenced by the extraction method **(Koc, et al., 2011).**

**Basim, et al., 2006** reported that Turkish bee pollen extract have an inhibitory effect against 13 different bacterial species pathogens for plants (*Agrobacterium tumefaciens, A. vitis, Clavibacter michiganensis subsp. michiganensis, Erwinia amylovora, E. carotovora pv. carotovora, Pseudomonas corrugata, P. savastanoi v. savastanoi, P. syringae pv. phaseolicola, P. syringae pv. syringae, P. syringae v. tomato, Ralstonia solanacearum, Xanthomonas campestris v. Campestris* and *X. axonopodis v.vesicatoria*).

**Kacaniova, et al., 2012** have shown that extraction 99.9% and 70% methanol (aqueous, v/v) and 96% and 70% ethanol (aqueous, v/v) of bee pollen possess high antimicrobial activity against *Listeria monocytogenes* CC M 4699, *Pseudomonas aeruginosa* CC M 1960; *Staphylococcus aureus* CC M 3953; *Salmonella enterica* CC M 4420, *Escherichia coli* CC M 3988, three different strains of microscopic fungi, *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger,* and seven different strains of yeasts *Candida krusei, Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis, Geotrichum candidum* and *Rhodotorula mucilaginosa* in vitro. **(Koc, et al., 2011)** have shown that Pollen has also, antifungal activity against different pathogens.

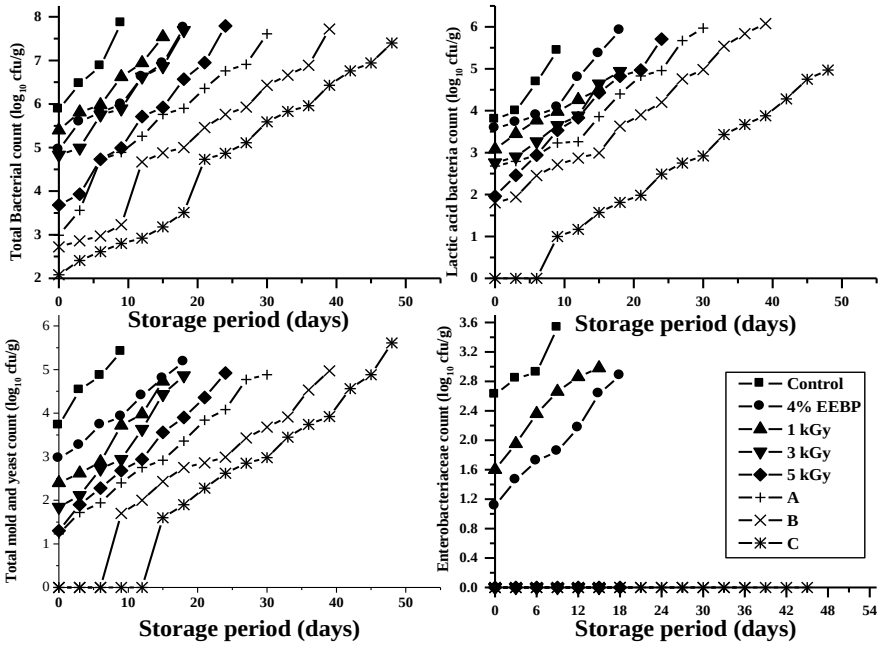


**Fig.(1)** The microbial load (Total bacterial count, lactic acid bacteria, enterobacteriaceae and the total mold and yeast $\log_{10}$ cfu/g) of silver carp fish patties as affected by different doses of gamma irradiation and 4% EEBP, whereas, A, B and C= Irradiated silver carp fish patties with 4% EEBP at dose level of 1, 3 and 5, respectively.

In the present study, the antimicrobial activity of the ethanolic extract of bee pollen (EEBP) against the pathogenic bacteria of silver carp fish patties was investigated (Fig.2). It is clear that the no growth of *Salmonella* and *Vibrio* was detected in the sample of 4% EEBP. The results showed that was active against *S. aureus*, *Entro Faecalis* and *B. cereus* (Fig. 2).

**Baltrusaityte, et al., 2007** have shown that the growth of *Streptococcus* was inhibited by the ethanol extract of bee pollen (bee bread) from various regions in Barazil and reported that bee pollen (bee bread) was active against gram-positive bacteria, yet it showed a limited activity against gram-negative bacteria. They found the bee pollen to possess an antibacterial activity against *Staph. aureus* and *S. epidermidis*. Meanwhile, **(Carpes, et al., 2007)** have showen that 80 % ethanol extracts of the Brazilian pollen antibacterial activity was exibited against *Staphylococcus aureus, Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella sp*. **(Tich, and Novak, 2000)** the antibacterial substances of pollen, active against *Streptococcus viridans* are similar to the ones found in propolis and honey combs **(Koc et al., 2011)** have shown that EEBP may have successfully inhibited the *E. coli* development in vitro at safe levels for human consumption and consequently, they could be useful as ground fresh beef natural preserver or as unspecific antibacterial food preserver. In the present study (Figure 2), the growth of *Salmonella* sp, *Vibrio* sp, *S. aureus* and *B. cereus* was completely inhibited at irradiation dose levels of 1, 3 and 5 kGy, respectively. From Figure (2), it is clear that the combined effect of treatment of silver carp fish patties with 4% EEBP and 3 kGy of gamma irradiation is accepted till 36 days of storage and the combined treatment of 4% EEBP and 5 kGy were accepted till 45 days of storage. Food spoilage is caused by the action of microorganisms among other factors. The food can be preserved when the basic causes of its spoilage is controlled. The sensitivity of pathologic microorganisms against gamma irradiation has been extensively studied (**Kanatt, et al., 2005 and Tawfik, et al., 2007).**

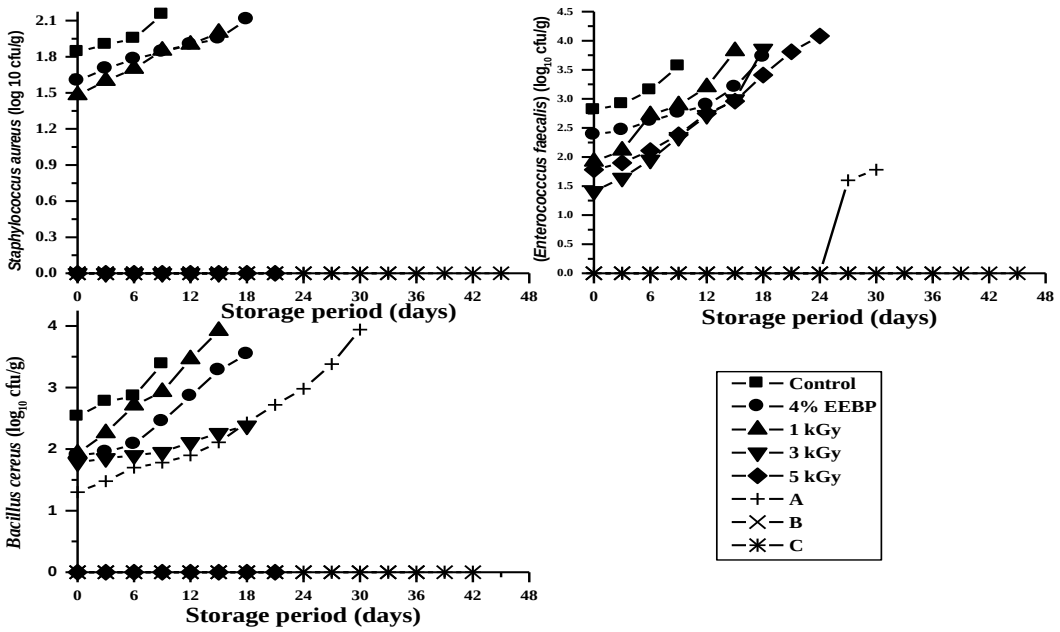


**Fig. (2)** The pathogenic bacteria (*Staphylococcus aureus*, *Enterococccus faecalis* and *Bacillus cereus* $\log_{10}$ cfu/g) of silver carp fish patties as affected by different doses of gamma irradiation and 4% EEBP, whereas, A, B and C= Irradiated silver carp fish patties with 4% EEBP at dose level of 1, 3 and 5, respectively.

## Chemical Analysis

Total volatile basic nitrogen (TVBN) is considered a storage stability test and an index to microbial decomposition of muscle proteins of meat products **(Hammad, et al., 2000**). The TVBN of silver carp fish patties with 2% EEBP and subjected to gamma irradiation reached 35.22 mg N/100g after 36 days of storage comp aired with control sample. From Figure (3), the thiobarbituric acid reactive substances (TBARS) of control, treated 2% EEBP and gamma irradiated chicken burgers were increased by increasing the storage period. The TVBN may be considered as a quality index for fish because its increase is related to the activity of spoilage bacteria and endogenous enzymes (**Ruiz-Capillas and Moral 2005**) and acceptability limit set by the EU (**EEC 1995**) for TVBN values of fish (35 mg N/100 g of fish fresh). Moreover, the TMA-N content is often used as a biochemical index to assess keeping quality and shelf-life of fish **(Connell, 1990)**. In marine fish, as is sea bream, TMA which is formed from trimethylamine oxide (TMAO) as a result of bacterial enzyme activity, is the main component responsible for an unpleasant ‘‘fishy’’ odor and TMA-N limit for fish is 10–15 mg N/100 g . In addition the TBARS value is an index of lipid oxidation measuring malondialdehyde (MDA) content. MDA formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (**Fernandez, et al., 1997).**

Figure (6) Shows the effect of ethanol extract of bee pollen (bee bread) (4% EEBP), subjected to gamma irradiation doses on TVBN, TMAN and TBARS of fish patties. The results showed that the concentrations of TVBN and TBARS were increased after irradiation. These concentrations were significantly increased by increasing the storage period for silver carp fish patties treated with 4% EEBP, gamma irradiation and combined treatment.

In a previous study, **(Tawfik, et al., 2007)** have shown that TVBN, TMA and AN values of beef burger steaks irradiated at dose levels 3 and 4 kGy were less than the accepted limits (25, 5.8 and 385

mg/l00g), respectively (AMI, 2001). Meanwhile, the acceptability limit of the TBARS value in this study was 1.0. Earlier workers reported that meat samples having TBARS value less than 1 possess no off odor (**Tarladgis, et al., 1960)** and said that for secondary oxidation products, such as, TBA, no legal threshold exists, but a limit of 1 mg malonaldehyde/kg meat has been suggested for sensory perceived rancidity.

In agreement with these results, **Gomes, 2002** reported that the irradiation process produced characteristic volatile compounds apparent as irradiation odor. These were lost from the meat during refrigerated storage, which is not the case for the un-refrigerated samples comparing with un-refrigerated samples. The major volatile compounds responsible for off-odor in irradiated meats were mainly sulphur compounds**.** The present data (Figure 2) are in agreement with that recorded by (**Tawfik, et al., 2007)** who revealed that the initial concentration of TEARS and -SH radicals were increased after irradiation. At the end of storage period, TEARS and -SH values of beef burger steaks were further increased. It was reported that -SH groups are known to be very sensitive to gamma irradiation, which is attributed to the reduction of the pH of beef burger samples resulting from the incorporation of this compound **(Lopez-Caballero, et al., 2005)** have shown that Mimosa pollen displayed the highest antioxidant activity. These may be due to its highest content of total polyphenolics, flavanols, flavones such as p-hydroxybenzoic, p-coumaric, vanillic, gallic and ferulic acid.

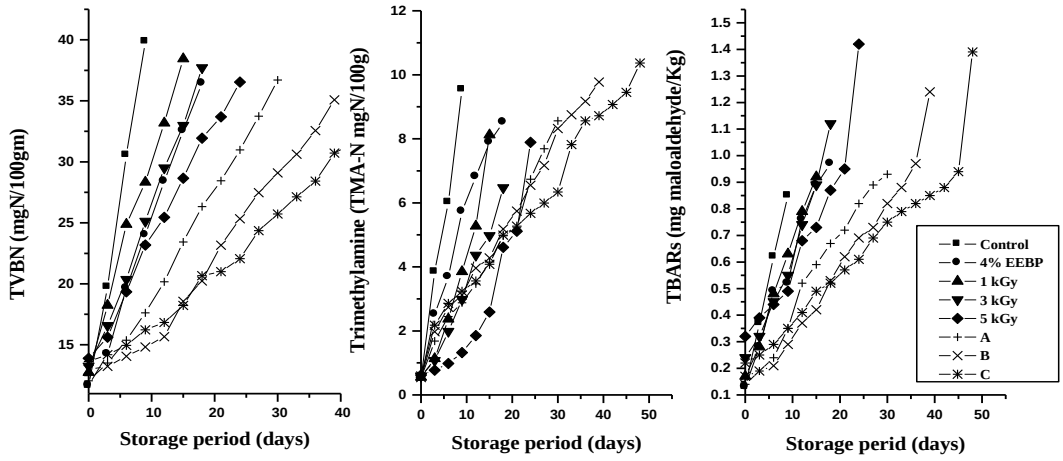


**Fig. (3)** Effect of ethanol extract of bee pollen (4% EEBP) and gamma irradiation doses on some chemical characteristics of chicken burger on some chemical quality index, (TVBN mg N/100g, TMA-N mg N/100g and TBARs mg maloaldehyde/Kg), whereas, A, B and C= Irradiated silver carp fish patties with 4% EEBP at dose level of 1, 3 and 5, respectively.

**Sensory Evaluation**

Sensory attributes for appearance, odor and texture of silver carp fish patties as affected by combined treatments 4% EEBP and gamma irradiation during cold storage at 4°C are shown in Figure (4). Sensory evaluation showed that gamma irradiation and treatment with the ethanol extract of bee pollen (bee bread) was better in appearance, odor and texture than non-treated control. The results indicated that irradiation at dose level of 5 kGy was effective to ensure safety of the tested silver carp fish patties up to 21 days of storage at 4°C, whereas the combined treatment between 5 kGy gamma irradiation and the 4% EEBP ethanol extract of bee pollen (bee bread) increased the accept Figure quality during the storage period (45 days). Sensory evaluation (appearance, odor and texture) was

better for the combined treatment than control. The samples were rejected at a concentration of A, B and C due to the emergence of fungal growths.

The variation of the antibacterial activities of the tested bee pollen (bee bread) extract may be due to their constituents and the probable presence of non-volatile compounds of extracts (**Gomes, 2002).** The major polyphenols were flavanoids, accompanied by phenolic acids and esters, phenolic aldhydes, ketones, etc. Bee pollen (bee bread) is considered safe in low doses and the use of standardized preparation of bee pollen (bee bread) is safe and less toxic than many synthetic medicines (**Basim, et al., 2006**). The present data are in agreement with that recorded with **(Hammad, et al., 2002) and (Tawfik, et al., 2007).** They have shown that panelists could not

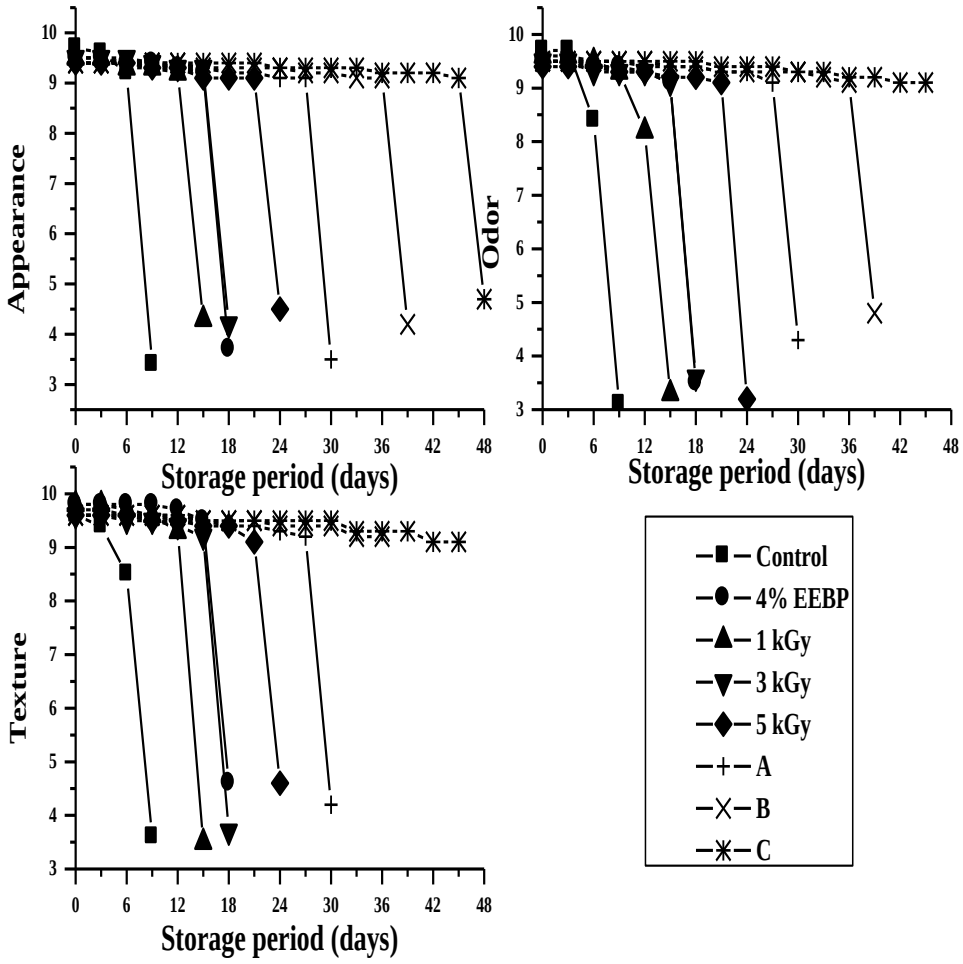


**Fig. (4)** Changes in the sensory characteristics for appearance, odor and texture of silver carp fish patties as affected by different doses of gamma irradiation and (4% EEBP) combined treatments during cold storage at (4±1ºC), whereas, A, B and C= Irradiated silver carp fish patties with 4% EEBP at dose level of 1, 3 and 5, respectively.

Differentiation between irradiated minced meat at low dose of radiation (3 kGy) and the quality was accepted during storage period of 30 days. Samples irradiated at dose of 5 kGy, samples showed high scores for their odor till the day 36 of cold storage, then lower scores were recorded for samples

when the panelists detected the odor of oxidation. However, scores indicated an accept Figure samples including those rejected when their total bacterial count exceeded $1x10^7$cfu/g.

## CONCLUSION

Generally, The results obtained from this study showed that combination treatments between gamma irradiation at doses of 1, 3 and 5 kGy and the ethanolic extract of bee pollen (bee bread) (4% concentration) led to improving the quality and safety of silver carp fish patties through its effectiveness in eliminating bacteria of public health and extending the refrigerated shelf-life to 27, 36 and 45 days, respectively, compared to 6 days for control samples without any adverse changes in their chemical and sensory properties. Thus, bee pollen, which is successfully introduced as a food supplement and can be easily up-taken through normal diet.

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