Extending the shelf life of refrigerated beef balls using methanol extracts of gamma-irradiated mushrooms

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Extracts of γ-irradiated (at dose levels of 0, 4, 8 and 12 kGy) mushrooms (*Agaricus ostreatus*) (EIM) were prepared to be used as natural antioxidant and antimicrobial agents for extending the shelf life of meat products. The properties of EIM-added minced beef balls (BB) were studied during refrigerated storage (4 °C). The antiradical potential of EIM against DPPH˙ radicals and the antioxidant trait of EIM in a β-carotene-linoleate model system were studied. The antimicrobial properties of EIM against bacterial strains including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli, Klebsilla penumoneae, Pseudomonas aeruginosa* and *Salmonella* Typhumurium as well as fungal strains including *Aspergillus niger* and *Penicillium expansum* were studied. The EIM from mushroom treated with 8 kGy had the highest antioxidant and antimicrobial properties. Thus, the 8-kGy-EIM treatment was added with BB at levels of 3, 6 and 9% (w/w). The BB treated with 3, 6 and 9% concentrations were evaluated as rejected by a trained panel after 9, 12 and 15 days of refrigerated storage, while the control sample was rejected after only 6 days. It is anticipated that EIM could be applied as natural antioxidant and antimicrobial agent to extend the shelf life of meat products.

Keywords

- Meat products
- Meat balls
- Natural antioxidants
- Antimicrobial agents
- γ-irradiation
- Food processing

The development and utilization of more effective natural antioxidants and antimicrobial agents to be applied in meat products is of major interest. Mushrooms held an important place in human diet which was shown by the statistical information regarding world production of mushrooms which reached 3 414 392 t in 2007 (USDA, 2009; Fernandes et al., 2012; Sławinska et al., 2016). The global economic value of mushrooms is staggering due to their value as food (Kalac, 2009) and their nutraceutical traits (Ferreira, Barros and Abreu, 2009; Ferreira, Vaz, Vasconcelos and Martins, 2010; Fernandes et al., 2012). Their fruiting bodies, contain about 39.9% carbohydrate, 17.5% protein and 2.9% fats, with the rest consisting of minerals. Cultivated mushrooms are a good source of vitamin B_2, niacin and folates (Wasser, 2002). Organic acids such as ascorbate, shikimate, malate and fumarate, also carbohydrates such as β-glucans, in addition monoterpenoid and diterpenoid lipids in combiantion with proteins such as hydrophobins and trace elements such as selenium were found in mushrooms (Dikeman et al., 2005). These substances have been found through several *in vitro* and *in vivo* studies to be responsible for the antimicrobial, antioxidant and antiaging potentials of edible mushrooms (Lin et al., 2015). The medicinal potential of mushrooms such as anticarcinogenic, anti-inflammatory and antibacterial were reported (Sabari et al. 2002; Lindequist et al., 2005).

Gamma-irradiation had been reported to be a potential tool in extending the shelf-life of mushrooms (Beaulieu et al., 2002; Fernandes et al., 2012). Substances like *p*-hydroxybenzoic, *p*-coumaric and cinnamic acids were reported in mushrooms' extracts (Taofiq et al., 2015). Lin et al. (2015) reported that the ethyl acetate fraction from the mushroom sclerotium of *Pleurotus tuber-regium* was rich in total phenolic content (41.4 mg/g extract) constituting of 58% chlorogenic acid and 21% syringic acid. An increase in phenolics and flavonoids was reported in mushrooms as affected by γ-irradiation (Jiang et al., 2010). Lower and similar phenolic levels were registered in *A. bisporus* also submitted to UV-C radiation (Guan et al., 2012; Fernandes et al., 2012). Kim et al. (2009) reported that γ-irradiation improved the antioxidant properties of *Inonotus obliquus* extract, through the increase in total phenolic content. Akram and Kwon (2010) mentioned that irradiation of mushrooms might be a safe and cost-effective tool to enhance the shelf-life and to the ensure sensory quality and hygienie. Different detection techniques for irradiated mushrooms, such as photostimulated luminescence, electron spin resonance, and thermoluminescence are available, effective and validated. In addition, the safety of irradiated mushrooms was well documented with some added values.

The goal of this work was to test the extracts of γ-irradiated (at dose levels of 0, 4, 8 and 12 kGy) mushrooms (*Agaricus ostreatus*) (EIM) as a natural antioxidant and antimicrobial agent in beef balls (BB) during cold storage.

Materials and methods

Mushrooms

Fresh samples of mushroom (*Agaricus ostreatus*) were obtained from local markets. The samples were dried at 45 °C for 72 h and then ground to a fine powder in a coffee grinder for 2 min. (particle size 2 µm). The ground mushrooms were defatted with *n*-hexane and then packaged in polyethylene pouches (each portion 20 g) to perform the irradiation.

For the irradiation treatments the defatted mushroom powder was exposed to γ-irradiation at dose levels of 0, 4, 8 and 12 kGy using 60Co from the Gamma Chamber 4000 unit at the National Center for Radiation Research and Technology (NCCRT, Atomic Energy Authority, Egypt). The dose rate at the time of experimentation was 2.8 kGy/h.

Preparation of EIM

For the preparation of EIM, γ-irradiated samples at dose levels of 0, 4, 8 and 12 kGy were immersed in aqueous methanol (methanol:water, 80:20, v/v) by shaking for 7 days in the dark at

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room temperature. The methanol extract was filtered and concentrated in a rotary evaporator under reduced pressure at 40 °C to a final volume of 10 ml.

Determination of total phenolics content (TPC)

The TPC was determined by the Folin-Ciocalteu method according to Arabshahi-Delouee and Urooj (2007). A volume of 200 µl of each extract was mixed with 1 ml of Folin–Ciocalteu's reagent (1 ml reagent with 9 ml distilled water). After 5 min 1.5 ml distilled water was added and then 1 ml of 75 g l^{-1} Na_2CO_3 solution was added. The mixture was incubated in a shaking incubator at ambient temperature for 60 min. and its absorbance at 760 nm was measured. Gallic acid was used as a standard for the calibration curve (correlation coefficient R^2= 0.9944).

DPPH˙ radical scavenging assay

The scavenging effect of EIM using the DPPH˙ radical was determined by the methods of Brand-Williams et al. (1995). The extracts of non-irradiated and irradiated samples at 4, 8 and 12 kGy of defatted mushroom samples were separately mixed with ethanol to prepare the test solution of 1 mg/ml sample. DPPH˙ was dissolved in ethanol and mixed with the extracts of the defatted mushroom samples. The solution was adjusted to a final DPPH˙ concentration of 100 µM. The mixture was shaken vigorously and left to stand for 5–60 min in the darkness at room temperature. The amount of DPPH˙ remaining in each period of stand was determined spectrophotometrically at 540 nm, using a microtiter plate reader (Biorad 680, USA). After vigorous shake, the mixtures were left to stand for 30 min. *Tert*-butylhydroquinone (TBHQ) was used to compare the scavenging activity. The radical scavenging activity was calculated as % inhibition from the following equation:

$$\% \text{ inhibition as OD} = \{(OD_{sample} - OD_{blank}) / OD_{blank}\} \times 100$$

β-Carotene/linoleic acid (βCB) bleaching assay

The ability of EIM and synthetic antioxidants to prevent the bleaching of *β*-carotene was assessed (Keyvan et al., 2007). In brief, 0.2 mg of *β*-carotene in 1 ml of chloroform, 20 mg of linoleic acid and 200 mg of Tween-20 were placed in a round-bottom flask. After removal of the chloroform, 50 ml of distilled water was added and the resulting mixture was stirred vigorously. Aliquots (3 ml) of the emulsion were transferred to test tubes containing EIM or the synthetic antioxidant. Immediately after mixing, 0.5 ml of EIM, an aliquot from each tube was transferred to a cuvette and the absorbance at 470 nm was recorded (Abs^0). The remaining samples were placed in a water bath at 50 °C for 2 h, then the absorbance at 470 nm was recorded (Abs^{120}). A control without added extracts was also analyzed. The antioxidant activity was calculated as follows:

$$\text{Antioxidant activity (\%)} = [1 - (Abs^0{}_s - Abs^{120}{}_s) / (Abs^0{}_c - Abs^{120}{}_c)] \times 100$$

Where:
$Abs^0{}_s$: is the absorbance of sample at zero time.
$Abs^{120}{}_s$: is the absorbance after 120 min.
$Abs^0{}_c$: is absorbance of control at zero time.
$Abs^{120}{}_c$: is the absorbance of control after 120 min.

Antibacterial activity

The antibacterial activity of non-irradiated mushroom extracts and EIM was determined according to Baydar et al. (2004). Nutrient broth cultures of *Bacillus cereus* and *Staphylococcus aureus* (Gram positive bacteria) as well as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella* Typhimurium (Gram negative strains) were grown at 35 °C for 22 h. Suspensions (250µl) of bacteria were added to flasks containing 25 mL sterile nutrient agar at 43–45 °C and poured into Petri-plates (10 cm diameter). The agar was allowed to solidify at 4 °C for 1 h. Wells (8 mm in diameter) were made in the media using a sterilized stainless steel borer. Each well was filled with 70 µl of each extract. The plates were left at room temperature for 30 min to allow diffusion of the materials in the media. The used solvents were examined as a control. the plates were incubated at 37 °C for 18–24 h. The inhibition zones in mm (including well diameter) around wells were measured. The antibacterial activity was expressed as the diameter of inhibition zones produced by the extracts against the tested bacteria.

Antifungal activity

Two plant pathogenic fungi strains namely *Aspergillus niger* and *Penicillium expansum* (obtained from Agriculture Research Center, Giza, Egypt) were cultured in yeast and mold extract broth (YM, Difco) for 48 h at 25 °C. The antifungal activity of non-irradiated mushroom extracts and EIM were determined according to Shin and Lim (2004) with some modifications. Fungal broth culture aliquots were added to potato dextrose agar medium (PDA) and uniformly distributed. Wells (8 mm in diameter) were made in the media using a sterilized stainless steel borer. Each well was filled with 70 µl of each extract. The used solvents were examined as a control. The inhibition zones in mm (including well diameter) around wells were measured. The antifungal activity was expressed as the diameter of the inhibition zones produced by the extracts against the test fungi after cultivation at 25 °C for 72 h.

Preparation of minced beef balls (BB)

Cuts of beef meat were minced in electrical mincer. A mixture of 1.7% salt, 1.5% spices (consisting of 15 g white pepper, 15 g black pepper, 10 g nutmeg, 10 g coriander, 10 g garlic powder, 10 g onion powder, 10 g cumin, 10 g fennel, 5 g ginger and 5 g clove) and 11.5% rusk flour was added to the minced beef meat (either treated with different concentrations of 0, 3, 6 and 9% of EIM at dose level of 8 kGy) and homogenized to obtain BB which shaped handily to ball-shape pieces of 35 ± 2 g and packed in polyethylene bags.

Microbiological analysis

The colony forming units for the aerobic mesophilic bacterial count were counted by plating on plate count agar medium and incubation at 30 °C for 3–5 days (APHA, 1992). The aerobic psychrophilic bacteria were enumerated on plate count agar medium after incubation at 5 °C for 7 days as recommended by APHA, 1992. Molds and yeasts were counted on oxytetracycline glucose yeast extract agar medium according to the Oxoid manual (1998). The plates were incubated at 25 °C for 3–5 days.

Chemical analysis and measurement of lipid peroxidation

The total volatile nitrogen (TVN) was determined as described by Mwansyemela (1992). Thiobarbituric acid-reactive substances (TBARS) produced from lipid oxidation were determined using the method of Alasnier et al. (2000). 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 a Whatman filter paper (No. 4). Equal amounts of the filtrate and 0.02 M thiobarbituric acid were heated in a boiling water bath for 20 min. and then cooled and the absorbance was measured at 532 nm.

Tab. 1: TPC (mg/100g as GAE/dry weight) of EIM

	Control	Gamma irradiation doss (kGy)		
		4	8	12
TPC (mg/100 g as GAE)	$2376.34^{A \pm 0.12}$	$2424.57^{B \pm 0.44}$	$2614.63^{C \pm 0.19}$	$2356.51^{B \pm 0.63}$

Capital letters were used for comparing between means in the rows.
Means with the same letters are not significantly different (p>0.05).

Source: Abdeldaiem, Ali and Hassanien FleischWirtschaft International 5/2016

Sensory evaluation

BB samples from the 8 kG γ-irradiated EIM groups and the non-irradiated mushroom extract sample were periodically examined (every 3 days) for their appearance, texture and odor post treatments during cold storage at 4 °C to determine the shelf-life of the samples. The panel consisted of ten trained panel from the laboratory wherein 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 a two-way analysis of variance. The differences among means were significant at significance level of $p<0.05$ using the Tukey test as a *post-hoc* test. All statistics were run on the computer using the SAS program (SAS 2000, version 6.12, SAS Institute Incorporation, and Cary, NC).

Results and discussion

Total phenolic content (TPC)

The results in Table 1 show the TPC (mg/100g as GAE/dry weight) of EIM. The data illustrate that EIM from samples irradiated at 4, 8 and 12 kGy had marked the TPC. Meanwhile, no significant differences of the TPC in EIM from sample irradiated at 12 kGy were noted. However, the results exhibite that EIM from samples irradiated at 4 and 8 kGy possess significant a higher TPC as compared with EIM from sample irradiated at 12 kGy. Furthermore, the EIM from samples irradiated at 8 kGy possess a significant TPC in comparison with methanol extracts from non-irradiated samples and γ-irradiated mushrooms at dose levels of 4 and 12 kGy. Harrison and Were (2007) suggested that the increased TPC in γ-irradiated almond skin extract could be attributed to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones. Otherwise, the increase in TPC was associated with the degradation of tannins as a result of irradiation. Also, plants with appreciable amounts of hydrolysable tannins may be more susceptible to irradiation compared with condensed tannins present in other spices (Khattak et al., 2008). Kumari et al. (2009) mentioned that water extracts of irradiated Triphala, a mixture of *Emblica officinalis*, *Terminalia chebula* and *Terminalia bellirica* showed linearly increasing concentrations of gallic acid (from 3.3 to 4.5 times), TPC (from 2.16 to 2.87 times), and antioxidant properties with an increasing radiation dose up to 25 kGy. The increase could be attributed to an easy release of active ingredients from their radiation degraded complex forms. Khattak (2012) found that medicinal plants (*Eruca sativa*, *Tagetes patula*, *Ocimum sanctum*, *Zizyphus nummularia* and *Morus nigra*) clearly exhibited an increase in the alkaloidal contents after radiation. The tannin content of radiated *Zizyphus nummularia*, *Tagetes patula*, *Morus nigra* and *Bryophyllum pinnatum* remained unaffected up to the dose level of 12 kGy, while tannins increased in irradiated samples of *Ocimum sanctum*, *Eruca sativa* and *Carum copticum*. The difference in the effects of γ-irradiation on the phytochemicals may be due to differences in the structure of the compounds and types of the plants.

Radical scavenging activity (DPPH˙ assay)

The antioxidant activity of phenolic compounds may result from the neutralization of free radicals initiating oxidation processes or from the termination of radical chain reactions. Also, the antioxidant activity of phenolics is due to their high tendency to chelate metals. Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups (Heim et al., 2002). The scavenging activity (%) of the DPPH˙ radical upon the treatment with methanol extracts from non-irradiated mushrooms and different EIM are tabulated in Table 2. The results showe that EIM from samples irradiated at 4, 8 and 12 kGy had a marked antioxidant activity on DPPH˙. Moreover, EIM from samples irradiated at 8 kGy possess high radical scavenging activity against DPPH˙ compared with non-irradiated and EIM from samples irradiated at 4 and 12 kGy. Breitfellner et al. (2002) have reported that γ-irradiation (1–10 kGy) of strawberries lead to the degradation of phenolic acids like cinnamic, *p*-coumaric, gallic and hydroxybenzoic acids. The hydroxylation (decomposition) of these phenolic acids has been attributed to the formation of free hydroxyl (OH^-) radicals during the treatment. Radiation treatments have been shown to either increase or decrease the antioxidant content of fresh plant products, which is dependent on the

Tab. 2: Scavenging activity (%) of EIM and TBHQ on DPPH˙ radicals

Concentration (µg/mL)	TBHQ	Control	Gamma irradiation doss (kGy)		
			4	8	12
50	$52.12^{Aa} \pm 0.02$	$41.46^{Ba} \pm 0.04$	$44.27^{Ca} \pm 0.48$	$59.75^{D}a \pm 0.19$	$44.32^{Ca} \pm 0.27$
100	$80.32^{Ab} \pm 0.15$	$56.17^{Bb} \pm 0.46$	$63.41^{Cb} \pm 0.28$	$75.67^{Db} \pm 0.61$	$59.55^{Eb} \pm 0.45$
200	$91.62^{A}c \pm 0.23$	$63.69^{Bc} \pm 0.85$	$76.56^{C}c \pm 0.39$	$91.82^{A}c \pm 0.15$	$68.29^{Dc} \pm 0.32$
400	$96.57^{A}d \pm 0.12$	$76.29^{Bd} \pm 0.25$	$85.35^{C}d \pm 0.44$	$95.34^{Ad} \pm 0.36$	$77.56^{B}d \pm 0.41$

Capital and small letters were used for comparing between means in the rows and columns, respectively.
Means with the same letters are not significantly different (p>0.05).

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Tab. 3: Antioxidant activity of EIM and TBHQ as measured by β-carotene bleaching assay

Concentration (µg/mL)	TBHQ	Control	Gamma irradiation dose (kGy)		
			4	8	12
50	$86.27^{Aa \pm 0.23}$	$46.34^{Ba \pm 0.51}$	$40.39^{Ca \pm 0.32}$	$65.58^{Da \pm 0.28}$	$44.95^{Ea \pm 0.55}$
100	$97.38^{Ab \pm 0.18}$	$59.56^{Bb \pm 0.64}$	$59.72^{Bb \pm 0.65}$	$79.15^{Cb \pm 0.31}$	$58.33^{Db \pm 0.36}$
200	$96.44^{Ab \pm 0.41}$	$72.39^{Bc \pm 0.24}$	$65.13^{Cc \pm 0.19}$	$87.57^{Dc \pm 0.54}$	$70.81^{Ec \pm 0.61}$
400	$97.62^{Ab \pm 0.27}$	$81.71^{Bd \pm 0.65}$	$85.63^{Cd \pm 0.43}$	$96.87^{Ad \pm 0.19}$	$82.56^{Bd \pm 0.32}$

Capital and small letters were used for comparing between means in the rows and columns, respectively.
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dose delivered, exposure time and the raw material used. The enhanced antioxidant capacity of a plant after irradiation is mainly attributed either to the increased enzyme activity (e.g. phenylalanine ammonialyase and peroxidase activity) or to the increased extractability from the tissues (Bhatt et al., 2007). Khattak (2012) reported that γ-irradiation of *Emblica officinalis* increased the levels of certain phytochemicals. In addition, the free radical scavenging activity was enhanced in all the radiation-treated samples up to the dose levels of 12 kGy. In brief, the study suggests that γ-irradiation is effective for the quality improvement of plant materials.

Tab. 4: Antibacterial activity of EIM against food pathogenic bacteria (zone of inhibition is expressed in mm)

γ-irradiation dose (kGy)	Bacterial strains				
	S. Typhimurium	*S. aureus*	*P. aeruginosa*	*B. cereus*	*E. coli*
0	$16.08^{Aa \pm 0.11}$	$17.95^{Ab \pm 0.12}$	$13.85^{Ac \pm 0.21}$	$15.32^{Ad \pm 0.13}$	$12.68^{Ae \pm 0.08}$
4	$16.78^{Aa \pm 0.07}$	$18.42^{Ab \pm 0.08}$	$13.77^{Ac \pm 0.16}$	$16.27^{Ba \pm 0.09}$	$13.73^{Bc \pm 0.05}$
8	$20.42^{Ba \pm 0.18}$	$22.56^{Bb \pm 0.06}$	$16.59^{Bc \pm 0.09}$	$20.13^{Ca \pm 0.17}$	$17.46^{Cd \pm 0.19}$
12	$15.98^{Aa \pm 0.13}$	$18.05^{Ab \pm 0.19}$	$14.02^{Ac \pm 0.15}$	$15.79^{Ac \pm 0.23}$	$12.92^{Ad \pm 0.17}$

Capital and small letters were used for comparing between means in the columns and rows, respectively.
Means with the same letters are not significantly different (p>0.05).

Source: Abdeldaiem, Ali and Hassanien

β-Carotene/linoleic acid (βCB)

The data in Table 3 exhibit the antioxidant activity of different EIM using the β-carotene bleaching assay. It is clear from the results that EIM from samples irradiated at 4, 8 and 12 kGy had a marked antioxidant efficacy against the oxidation of β-carotene. Otherwis, samples of EIM from sample irradiated at 8 kGy had a higher antioxidant efficacy against the oxidation of β-carotene compared with non-irradiated EIM and EIM from samples irradiated at 4 and 12 kGy. The decrease in antioxidants caused in ethanol extracts of irradiated peel of cashew nuts at 3 and 9 kGy compared to 6 kGy were attributed to the formation of radiation-induced degradation products or the formation of free radicals (Sajilata and Singhal, 2006). Quantitative differences in the constituents of nut meg oil as well as an increased amount of phenolic acids were detected after γ-irradiation, which was attributed to the degradation of tannins and consequently higher extractability of phenolic acids (Variyar et al., 1998).

Antibacterial activity of EIM against some food pathogenic bacteria

The activity of different EIM against some food pathogenic bacteria measured as zones of inhibition in millimeter is shown in Table 4. From the results it can be noticed that EIM from samples irradiated at 4, 8 and 12 kGy possess marked inhibitory activities against the studied bacterial strains. Vamanu et al. (2011) illustrated that the antimicrobial effect of ethanol and methanol extracts of two *Pleurotus ostreatus* strains had remarkable impact against the Gram-positive (*Escherichia coli* and *Listeria innocua*) and Gram-negative (*Bacillus cereus*) bacterial strains. In addition, Balakumar et al. (2011) reported that methanol extracts of bodies of *Phellinus* mushrooms had a strong antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* , *Salmonella* Typhi, *Staphylococcus aureus* and *Streptococcus mutans* . Otherwise, the ethanol and methanol extracts of *Agaricus bisporus* (button mushroom) had antibacterial activities against 10 bacterial strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella* Typhi, *Enterobacter aerogenes, Enterococcus faecium*, *Vibrio cholera*, *Bacillus subtilis* and *Bacillus cereus*) *in vitro* (Pandey et al., 2013). The data show that EIM from sample irradiated at 8 kGy had higher inhibitory activities against bacterial strains compared with other treatments. There is in a good correlation between TPC of EIM and its antibacterial activity. There was no enough research in this field to explain the effect of γ-irradiation on the antibacterial activity on mushrooms (*Agaricus ostreatus*).

Antifungal activity of EIM

The antifungal activity of EIM against some fungal strains (zone of inhibition is expressed in mm) is recorded in Table 5. The data in the present study exhibit that the EIM from samples irradiated at 4, 8 and 12 kGy had antifungal efficacies against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporium*. Balakumar et al. (2011) found that methanol extracts of *Phellinus* mushrooms had antifungal activity against different fungal species (*Penicillium* sp., *Aspergillus fumigatous*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus*). On the other hand, the results illustrate that EIM from samples irradiated at 8 kGy possess a remarkable activity against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporium* in comparison with other treatments in the present study. Again, there is in a good correlation between TPC of EIM and its antifungal potential. Furthermore, there was not enough research in this field to explain the effect of γ-irradiation on antifungal efficiencies of mushrooms.

Chemical quality index

Figure 1 shows the chemical quality index of minced BB as affected by EIM from samples irradiated at 8 kGy (3, 6 and 9%) during cold storage (4 °C). The TVBN content is an important indicator for evaluating the meat and meat products freshness. Mexis et al. (2012) mentioned that as autolysis of muscle proteins proceeds, compounds of alkaline reactions such as amines and ammonia build up producing objectionable odors. Thus, the TVBN has been used as a spoilage indicator in several animal food substrates. These data show that samples of minced BB of control and BB samples enriched with EIM (3, 6 and 9%) induced no changes in the TVBN levels. Otherwise, these results are in agree with Gomes (2002) who mentioned that irradiation produced characteristic volatile compounds apparent as irradiation odor. The major volatile compounds

Tab. 5: Antifungal activity of EIM against fungal strains (zone of inhibition is expressed in mm)

γ-irradiation dose (kGy)	Fungal strains		
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporium*
0	16.14^{Aa}±0.29	17.23^{Ab}±0.07	18.62^{Ac}±0.23
4	18.13^{Ba}±0.07	19.63^{Bb}±0.21	18.84^{Ac}±0.13
8	20.78^{Cc}±0.18	23.15^{Cb}±0.12	22.71^{Bc}±0.07
12	15.89^{Aa}±0.13	16.87^{Ab}±0.38	18.43^{Bc}±0.16

Capital and small letters were used for comparing between means in the columns and rows, respectively.
Means with the same letters are not significantly different (p>0.05).

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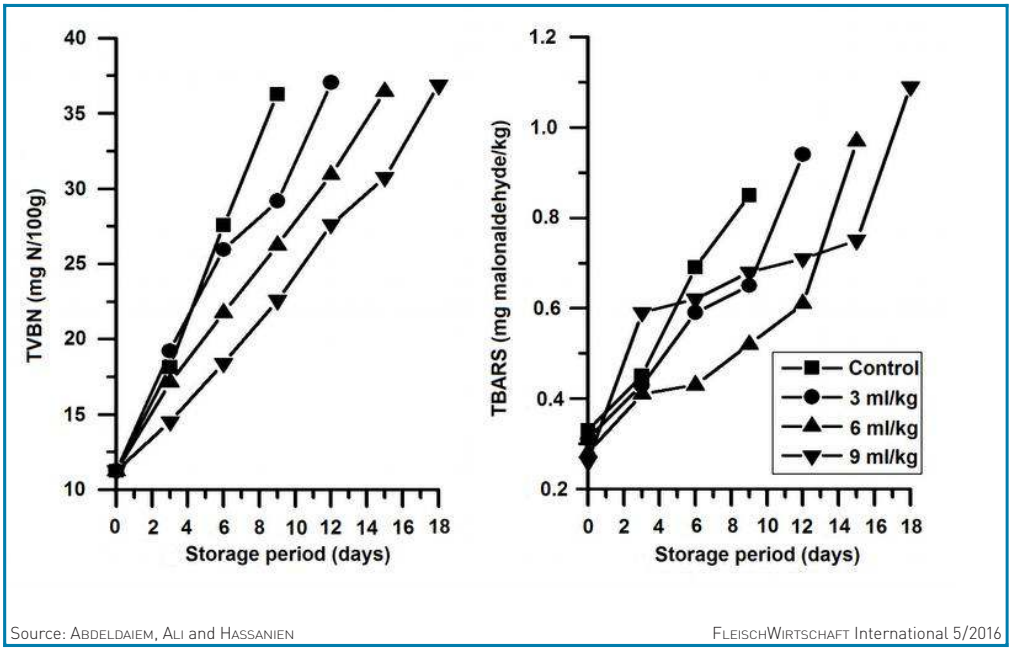

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Fig. 1: Chemical quality index (TVBN and TBARs) of BB as affected by EIM at different concentrations during storage.

responsible for off-odor in irradiated meats were mainly sulphur compounds (Ahn and Nam, 2004).

On the other hand, the results record that the TVBN values in the BB control samples and the EIM-enriched BB samples were increased significantly during the increasing storage period. Thus, the values of the TVBN of the BB reached to 36.27, 37.03, 36.45 and 36.88 (mg N/100g) for the control BB and the EIM-enriched BB by different concentrations (3, 6 and 9%), respectively. Tawfik et al. (2007) reported that the TVBN values of beef burger steaks irradiated at dose levels 3 and 4 kGy were less than the accepted limits.

The TBARS value is an index of lipid oxidation measuring the malondialdehyde (MDA) content. MDA forms through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez et al. 1997). The results show that EIM enrichment at different concentrations (3, 6 and 9%) followed in slightly decreased TBARS values compared with control during cold storage. The TBARS values of control and EIM-enriched BB increased with the increasing storage period, but the rate of increasing was slight in the EIM-enriched samples proportionally with the increasing concentration of EIM. Gan et al. (2013) mentioned that the high antioxidant activity in ethanol extracts of mushrooms can potentially be used as a source of natural antioxidants due to the presence of phenolic compounds. The antioxidant activity of phenolic compounds is associated with the hydroxyl group linked to the aromatic ring, which is capable of donating hydrogen atoms with electrons and neutralizing free radicals, so this mechanism blocks further degradation to more active oxidizing forms, such as MDA (Oliveira et al., 2011). With the storage time increasing the overall lipid oxidation increased too and the rate of lipid oxidation was faster in the EIM-enriched BB samples than in the control BB which may be mainly attributed to the strong antioxidant effect of methanol extracts of mushrooms (Yang et al. 2002; Vamanu, 2011; Pandey, 2013).

Microbiological load as affected by different concentration of EIM

In bio-preservation, the storage life is extended, and/or the safety of food products is enhanced by using natural compounds. Antimicrobial compounds present in foods can extend the shelf life of unprocessed or processed foods by reducing the microbial growth rate or viability. The shelf lifes of meat and meat products are strongly influenced by the initial microbial quality. The aerobic mesophilic bacterial counts in the control BB and the EIM-enriched BB samples at different concentrations (3, 6 and 9%) were recorded during the cold storage. Following the results in Figure 2, the initial total bacterial count of the control sample was 4.89 $\log_{10}$ cfu/g. The storage considerably increased the total microbial counts in the control sample which reached 7.63 $\log_{10}$ cfu/g after 6 days of cold storage. The maximum acceptable count for meat and meat products is 10^7 cfu/g as recommended by the International Commission on Microbiological Specification for Foods. The enrichment of minced BB with EIM had no significant effect on the initial total bacterial counts, wherein the results were 4.87, 4.84 and 4.82 $\log_{10}$ cfu/g for the BB samples treated with levels of 3, 6 and 9% of EIM at the start of the storage period, respectively. During the storage period of the control and the EIM-enriched (3, 6 and 9%) BB samples, the total bacterial counts were significantly increased reaching 7.63, 7.49, 7.26 and 7.51 $\log_{10}$ cfu/g after 6, 9, 12 and 15 days of storage, respectively. The initial total psychrophilic count was around 3.68, 3.66, 3.63 and 3.61 $\log_{10}$ cfu/g for the control BB samples and the EIM-enriched (3, 6 and 9%) BB, respectively. During the cold storage the aerobic psychrophilic count significantly increased for the control and the EIM-

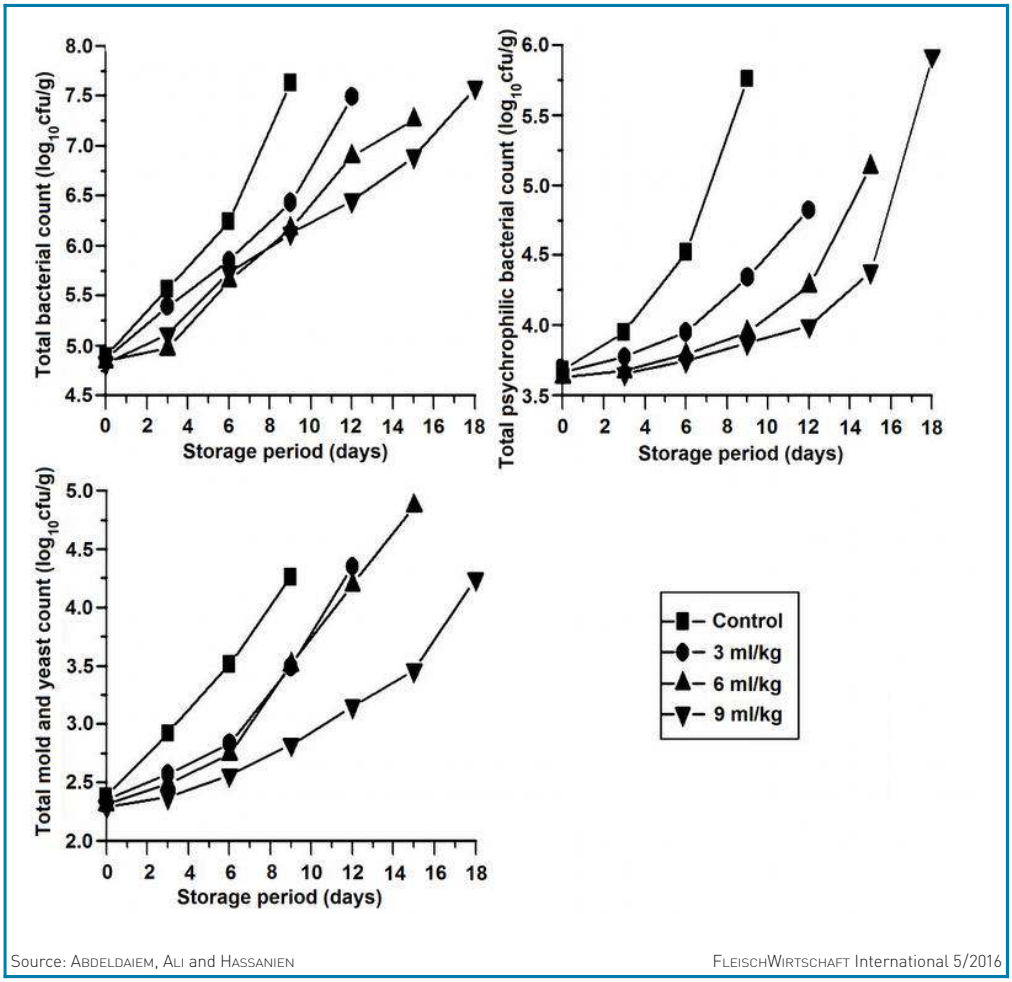
Tab. 6: Sensory attributes for appearance, flavor and texture of BB as affected by EIM during cold storage

Storage period (days)	Appearance				Flavor				Texture			
	Control	EIM (%)			Control	EIM (%)			Control	EIM (%)		
		3	6	9		3	6	9		3	6	9
0	9.2 ± 0.03^{Aa}	9.2 ± 0.04^{Aa}	9.1 ± 0.02^{Aa}	9.1 ± 0.04^{Aa}	9.5±0.02Aa	9.5 ± 0.03^{Aa}	9.4 ± 0.04^{Aa}	9.2 ± 0.03^{Aa}	9.3 ± 0.05^{Aa}	9.2 ± 0.03^{Aa}	9.1 ± 0.02^{Aa}	9.1 ± 0.03^{Aa}
3	8.7 ± 0.05^{Bb}	9.2 ± 0.05^{Aa}	9.2 ± 0.06^{Aa}	9.2 ± 0.03^{Aa}	9.1 ± 0.02^{Ba}	9.4 ± 0.03^{Aa}	9.5 ± 0.04^{Aa}	9.5 ± 0.04^{Aa}	8.9 ± 0.05^{Bb}	9.2 ± 0.03^{Aa}	9.1 ± 0.02^{Aa}	9.1 ± 0.02^{Aa}
6	7.6 ± 0.02^{Cc}	8.9 ± 0.02^{Bb}	9.2 ± 0.05^{Aa}	9.2 ± 0.04^{Aa}	9.1 ± 0.03^{Ba}	9.4 ± 0.03^{Aa}	9.3 ± 0.06^{Aa}	9.3 ± 0.03^{Aa}	8.6 ± 0.02^{Bb}	8.9 ± 0.02^{Bb}	9.1 ± 0.03^{Aa}	9.1 ± 0.02^{Aa}
9	3.2 ± 0.02^{De}R	8.7 ± 0.02^{Bb}	9.1 ± 0.06^{Aa}	9.1 ± 0.03^{Ba}	3.1 ± 0.03^{Ce}R	9.3 ± 0.02^{Aa}	9.3 ± 0.05^{Aa}	9.3 ± 0.05^{Aa}	3.1 ± 0.03^{Ce}R	8.5 ± 0.02^{Bb}	8.9 ± 0.04^{Bb}	9.1 ± 0.02^{Aa}
12		4.3 ± 0.04^{Cd}R	8.9 ± 0.02^{Bb}	9.1 ± 0.02^{Aa}		3.4 ± 0.05^{Be}R	9.1 ± 0.05^{Aa}	9.1 ± 0.03^{Aa}		4.3 ± 0.05^{Dd}R	8.8 ± 0.03^{Bb}	8.9 ± 0.04^{Bb}
15			4.5 ± 0.04^{Cd}R	8.9 ± 0.02^{Ab}			3.5 ± 0.05^{Ce}R	8.9 ± 0.02^{Bb}			3.5 ± 0.02^{Ce}R	8.9 ± 0.05^{Bb}
18				4.2 ± 0.02^{Bd}R				4.5 ± 0.02^{Bd}R				4.6 ± 0.02^{Cd}R

Capital and small letters were used for comparing between means in the columns and rows, respectively.
Means with the same letters are not significantly different ($p<0.05$); R= Rejected

Source: Abdeldaiem, Ali and Hassanien FleischWirtschaft International 5/2016

enriched samples which reached to 5.76, 4.82, 5.13 and 5.92 log_{10} cfu/g after 9, 12, 15 and 18 days of storage, respectively. Meanwhile, the total mold and yeast counts at the start were 2.38, 2.35, 2.31, and 2.29 log_{10} cfu/g for the control and the EIM-enriched (3, 6 and 9%) BB, respectively. During the cold storage period, there was a gradual increase in the total mold and yeast counts reaching 4.26 log_{10} cfu/g after 6 days of storage for the control sample. The total mold and yeast counts slightly increased during the cold storage period for EIM-enriched (3, 6 and 9%) BB reaching 4.95, 4.87 and 5.16 log_{10} cfu/g after 12, 15 and 18 days of storage, respectively. These results could be attributed to the antibacterial and antifungal impacts of EIM as reported by Balakumar et al., (2011). Otherwise, the result indicates that the enrichment with 9% of EIM was effective in inhibiting the spoilage bacteria growth and extending the cold storage life of minced BB to 18 days compared to 6 days for the control.



Source: Abdeldaiem, Ali and Hassanien — FleischWirtschaft International 5/2016

Fig. 2: The microbiological load of BB-enriched with EIM during cold storage

Sensory evaluation

Te sensory attributes for appearance, flavor and texture of the BB as affected by enrichment with EIM at different concentrations during cold storage are tabulated in Table 6. All samples at the start had high scores ranged from 9.1 to 9.5, which means that all samples at the start were of excellent quality. During the storage period, there was a significant loss in the BB quality for all samples, which were rejected by the panelists after 6, 9, 12 and 18 days for the control and the EIM-enriched (3, 6 and 9% of EIM) BB, respectively. The scores indicated that the BB samples were rejected when their total bacterial count exceeded $1x10^7$ cfu/g. Following the results, the EIM-enriched BB samples were able to keep their good quality characteristics in terms of sensory assessment.

Conclusion

Mushrooms have been a part of the human diet for thousands of years. Mushrooms could be used as an easily accessible source of natural antioxidants which is essential in fighting against diseases and as a possible food supplement. The impact of γ-irradiation on mushrooms (*Agaricus ostreatus*) was hardly studied. In general γ-irradiation increased the levels of bioactive compounds in mushrooms at 4 and 8 kGy dose levels. The results show that a methanol extract of γ-irradiated mushrooms at dose level of 8 kGy extended the shelf-life of minced BB to 18 days compared to 6 days of control. The present data will certainly help to ascertain the potency of mushrooms as a potential source of natural antioxidants to be used for nutraceutical and functional food applications. However, further research is needed to identify individual bioactive components with biological activities in γ-irradiated mushrooms.

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