Shelf-life of E-beam treated hamburgers added with tomato powder as source of lycopene

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Abstract---**Ready-to-eat hamburgers enriched with 4% dried tomato powder to supply lycopene were manufactured and treated by electron-beam (E-beam) radiation. This work is the first, to our knowledge, to use dried tomato from surplus generated by agricultural tomato processes as a way to increase cost-effectiveness and reduce environmental pollution. The effect of E-beam irradiation on hamburger shelf-life was determined. Microbiological counts, lycopene concentration, texture, colour and sensory analyses were performed after different storage periods. The treatment guarantees the hygienic quality of the meat product, extends its shelf-life to 28 days (4 kGy) and provides the daily lycopene amount recommended as healthy.**

Keywords---***Lycopene, hamburger, Electron beam***

I. INTRODUCTION

Manufacture of agricultural tomato products generates large amounts of surplus material. Recycling them would make production more cost-effective and would reduce environmental pollution. Tomato contains highly biologically active compounds such as carotenoids, including lutein, β-carotene, phytoene, phytofluene and lycopene. Using this tomato surplus in meat products makes these foods an excellent source of natural compounds.

Studies of the health benefits of lycopene have focused on its antioxidant properties due to its efficiency as a quencher of singlet oxygen. These properties have been associated with a protective effect in cardiovascular disease [1], a decrease in inflammatory response [2] and decreased risk of osteoporosis in postmenopausal women [3]. Population studies have also shown that high intake of lycopene is inversely associated with the incidence of certain types of cancers [4, 5, 6].

Numerous studies have examined the addition of antioxidants to meat products to maintain their properties during storage or improve their health benefits. However, few studies have looked at the addition of tomatoes as a source of lycopene [7, 8, 9, 10, 11, 12].

In addition to nutritional value, consumers demand from meat products high quality, convenience, innovation, safety, natural flavour and taste, and extended shelf-life. In fact, demand is growing for foods that are easy to manufacture and distribute, as well as fast and easy to eat or to cook. The preparation of ready-to-eat (RTE) foods involves such operations as cutting, slicing and packaging, all of which can compromise the sanitary quality and shelf-life of the final product. These processes introduce the risk of contamination by food-borne pathogens, some of which can grow at refrigeration temperatures, such as *Listeria monocytogenes* [13].

To avoid these problems, the industry has begun to treat food products with non-thermal technologies to control microbiological growth without modifying sensorial quality [14, 15, 16, 17]. Irradiation is effective at sterilising food, but it is known to cause several changes in lipids and proteins by inducing the formation of radiolytic products. These changes alter the sensory properties of food products in ways that affect their acceptability to consumers [18].

In previous work from our laboratory, hamburgers were prepared using dry tomato peel as a source of lycopene and then treated with an electron beam E-beam [19]. The present work followed the same procedure but used surplus tomato powder as the source of lycopene. The goal was to obtain a new meat product that could be considered a functional food that was safe and economically viable.

II. MATERIAL AND METHODS

A. Preparation of Dried Tomato (DT)

Tomato was obtained from a local factory, ground up and dehydrated in a Virtis lyophiliser (model FM12XL). DT was ground up in a mill to particle sizes of 0.025–0.05 mm and stored in the dark at -30 ºC until use.

B. Hamburger manufacture

Hamburgers of 60 g were manufactured using beef meat. The meat was ground up (C10 Grinder, Falsf Co., Spain) through a plate with a diameter of 3 mm.

DT was added to ground meat (40 g kg^{-1}) and mixed (Robot-Coupe Model R6-02VB) to homogeneity. Hamburgers with a diameter of 10 cm and a height of 1 cm were moulded in a hamburger maker. All hamburgers were vacuum-packed in laminated film bags of low permeability (35 cm^3/24 h m^2 bar to oxygen, 150 cm^3/24 h m^2 bar to CO_2) and maintained at 4ºC for less than 24 h before irradiation. Batches were manufactured in triplicate.

C. Irradiation treatment

Samples were transported under refrigeration (<4 ºC) to the irradiation plant of IONISOS Ibérica S.A. (Tarancón, Cuenca, Spain), which houses a linear electron accelerator. Doses of 2 and 4 kGy were delivered using a 10-MeV electron beam. The dose absorbed by samples was checked by determining the absorbance of cellulose triacetate dosimeters simultaneously irradiated with the samples. The temperature of samples was controlled, and the increase during irradiation was always less than 2 ºC. After irradiation, hamburgers were transported under refrigeration to the laboratory. They were stored at this temperature until use.

D. Microbiological analysis

Total viable aerobic counts were determined aseptically using 10 g of hamburger. The samples were homogenised with peptone water, serially diluted and plated in duplicate on Plate Count Agar (Oxoid, Madrid, Spain). After incubation at 32 ºC for 48 h, colony-forming units (CFU) per gram were determined.

E. Lycopene quantification

Ten g of each sample was mixed with 50 ml of dichloromethane, and the mix was shaken at 37 ºC for 30 min. The dichloromethane layer, containing the lycopene and other lipid components, was removed and stored at 4 ºC in the dark. Then another 50 ml of solvent was added to the sample. The extractions were repeated until the dichloromethane had no colour. Solvent fractions containing lycopene were mixed and immediately evaporated under vacuum at room temperature. Samples were kept frozen in a nitrogen atmosphere until quantification. Quantification of lycopene was performed by HPLC as described by [20] using a Beckman System Gold binary delivery system (Beckman Instruments, Fullerton, CA, USA) equipped with a UV–Vis photodiode array. All extractions and quantifications were performed in triplicate.

F. Cooking of hamburgers

All hamburgers were cooked during 2 min on each side using a grill preheated to 150 ºC. The temperature in the meat interior was close to 60 ºC. This treatment was sufficient to obtain a good final degree of doneness. Texture, lycopene concentration and sensory analyses were performed on cooked hamburgers.

G. Texture profile analysis

The textural properties of cooked hamburgers were evaluated using the Stable Micro System Mod. TA.XT 2i/25 texturometer. Textural profile analysis (TPA) was performed using one portion of each sample measuring 1 cm high and 2.5 cm in diameter. The portion was compressed twice to 50% of its original height. Hardness (N) and chewiness (N cm) were determined. At least seven replicate measurements were performed on each batch.

H. Colour analysis

Meat colour was measured at the surface of raw hamburgers with a Chroma Meter CR-200 colourimeter (Minolta Co., Osaka, Japan) using the Space colour CIE L*a*b*. The average value for each hamburger was the mean of 25 determinations.

I. Sensory analysis

Samples were evaluated by 50 untrained assessors, who were served three freshly cooked hamburgers (one sample of each batch treated with 0, 2 or 4 kGy). A hedonic test was carried out using a non-structured, 10-point scale (0 = extremely dislike, 10 = extremely like) in which the assessors evaluated different attributes: odour, colour, texture, taste and overall acceptability.

The sensory analysis was performed under white fluorescent lights in individual booths. Unsalted crackers and room temperature water were provided to clean the palate between samples.

J. Statistical methods

Statistical analysis of results was carried out using one-way analysis of variance, followed by Duncan's multiple range test to determine significant differences (Statgraphics 5.0 Plus).

III. RESULTS AND DISCUSSION

A. Microbiological analysis

In the non-irradiated batches, initial bacterial counts were close to 10^5 CFU g^{-1}. These counts increased to more than 10^8 CFU g^{-1} after 10 days of refrigerated storage. This value has been suggested as the end of the useful shelf-life, because values above this threshold indicate spoilage [21]. In the batches irradiated with 2 kGy, inital counts of 10^3 CFU g^{-1} increased to 10^6 CFU g^{-1} after 21 days. The batch irradiated with 4 kGy maintained a microbial count below 10^2 CFU g^{-1} during the longest storage period studied (28 days). According to approved procedures for irradiating uncooked, chilled meat [22] these counts indicate that doses of 2 and 4 kGy are adequate for guaranteeing hygienic quality of the meat product.

B. Lycopene concentration

The levels of this carotenoid were determined in dry tomato and in cooked hamburgers. Cooked hamburgers were analysed in order to evaluate the actual amounts of functional ingredient that would be available to the body upon consumption. The lycopene concentration quantified in the hamburgers was as expected, given that the added DT contained 104.6 mg of lycopene/100 g. The levels detected in hamburgers ranged from 3.9 to 4.8 mg/100 g across all batches. No significant differences ($p < 0.05$) were found between samples irradiated at different doses or stored for different periods. Similar results were reported in fresh meat products when dry tomato peel was used as the source of lycopene [19]. The results in the present study suggest that consumption of one conventional hamburger of 150 g would provide 5.8-7.2 mg of lycopene. A daily intake of 5–7 mg of lycopene by healthy human beings may be sufficient to combat oxidative stress and prevent chronic disease [23]

C. Texture profile analysis

Neither of the irradiation doses caused significant differences in the textural properties of the hamburgers (Table). These results disagree with those of other studies on irradiated fresh meat products [24. 25]. Those previous studies indicated that irradiation promotes structural changes in meat proteins, increasing the hardness of the product. In our case, it is possible that the soluble fibre in the DT acts as a gelling agent that stabilises the texture and minimises radiation-induced changes. This would be similar to the effect observed by Cabeza et al. [16], who found no significant differences in texture parameters in cooked ham irradiated at 1, 2, or 5 kGy. Nevertheless, prolonged storage of the hamburgers in the present study led to an increase in hardness and, consequently, chewiness. However, these increases were not detected in the sensory analysis (see below).

Table. Influence of irradiation and storage on texture and colour of hamburgers prepared with DT.

		Texture		Colour	
Irradiation doses (kGy)	**Storage (Days)**	**Hardness (N)**	**Chewiness (N cm)**	**L***	**Hue Angle**
0	0	42.86±6.22A	17.85±1.72B	39.60±3.45A	29.65±3.11A
	15				
	28				
2	0	48.50±6.43aA	21.70±2.21aA	37.88±2.49aA	29.68±3.83aA
	15	50.28±7.72aA	27.13±6.19aA	36.70±4.27aA	31.12±2.23aA
	28				
4	0	43.43±2.38bA	18.56±1.33bB	35.94±2.04aA	29.53±2.46aA
	15	55.69±8.60aA	30.75±5.32aA	34.33±1.42aA	31.07±2.20aA
	28	45.31±4.15b	26.05±3.67a	37.74±2.51a	30.11±2.81aA

Different capital letters mean differences between the irradiated batches at the same storage time ($p < 0.05$)
Different lowercase letters mean differences in the same batch through the storage time ($p < 0.05$).

D. Colour analysis

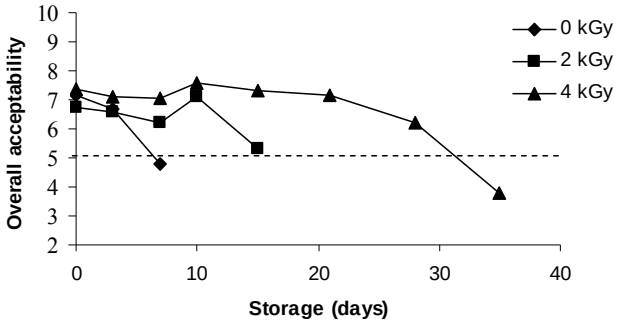
Irradiation has been reported to modify the colour of fresh meat because the input energy alters the chemical environment of the meat and destabilises myoglobin [26]. The result is a brown colour considered 'characteristic' of irradiated ground meat. Indeed, this colour change was observed by several authors [19, 25, 27] working with ground meat and hamburgers. However, in the present study, no significant differences were found between the non-irradiated hamburgers and those treated with 2 or 4 kGy (Table). This is probably because the added DT contains lycopene, the strong colour of which masks the typical appearance of irradiated meat. Colour analysis was not performed on cooked hamburgers, since the Maillard reaction and fat melting are known to mask the colorant effects of lycopene [10]

E. Sensory analysis

Sensory evaluation was performed using a non-structured hedonic scale on which the maximum score was 10 points and a score of 5 was defined as the limit of acceptability (Figure).

All the samples, both none irradiated and irradiated, showed good sensory quality from the first day of the analyses. The results indicate that the DT masked the radiation-induced changes in taste and colour. The assessors commented that the tomato taste was highly acceptable because they identified it as the usual flavour associated with hamburgers served in a restaurant. In this sense, the red colour due to the colorant powder of lycopene may have contributed to the acceptability of the hamburgers.

Figure. Overall acceptability of hamburgers supplemented with DT and subjected to E-beam irradiation. The dashed line indicates the limit of acceptability (score of 5).



The limit of acceptability was reached after different storage periods. The sensory quality lasted for 7 days at 0 kGy, 15 days at 2 kGy and 30 days at 4 kGy. These differences probably reflected microbial spoilage in the case of non-irradiated samples, and the development of off-flavours that the DT could no longer mask in the case of irradiated meat.

IV. CONCLUSIONS

Surplus tomato from agricultural manufacturing can be used as a source of lycopene in fresh meat products. The addition of 4% DT to one hamburger of 100 g gives a concentration of lycopene appropriate for a healthy diet.

E-beam treatment is effective at increasing the shelf-life of hamburgers enriched with DT as a source of lycopene. The treatment guarantees the hygienic quality of the meat product and extends its shelf-life to 28 days (4 kGy). The limiting factor is the off-flavour produced during storage as a consequence of irradiation.

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