

COMPARATIVE STUDIES ON GAMMA RADIATION AND HIGH PRESSURE INDUCED EFFECTS ON MINCED BEEF

Y. HASSAN^a, L. MÉSZÁROS,^b A. SIMON,^b E. TUBOLY,^b
CS. MOHÁCSI-FARKAS^c and J. FARKAS^{b*}

^bDepartment of Refrigeration and Livestock Products' Technology,

^cDepartment of Microbiology and Biotechnology,

Faculty of Food Science, Szent István University, H-1118 Budapest, Ménési út 45. Hungary

(Received: 18 May 2001; accepted: 1 December 2001)

The total viable cell count of bacteria in vacuum-packaged chilled minced beef has been decreased equally, by approx. two log-cycles, as an effect of 1.5–2.0 kGy gamma radiation or 200–300 MPa high hydrostatic pressure (UHP) treatment for 20 min. Coliform bacteria could be eliminated to non-detectable levels by the same treatments. The shelf-life of both untreated and non-thermally pasteurised samples were limited mainly by growth of lactic acid bacteria. At about equal bactericidal effect, more drastic changes of texture and colour occurred in UHP-pasteurized minced beef samples than in the radiation-pasteurized ones. Whereas radiation pasteurisation caused minimal changes in appearance, texture and DSC-thermograms of minced beef, UHP-pasteurisation of the raw samples proved to be strongly discolouring by denaturing the muscle pigments and causing extensive denaturation of the myofibrillar proteins. The water holding capacity of irradiated samples decreased, while that of high pressure treated ones increased as compared to the untreated control. Near infrared spectrometry and electronic nose measurements gave promising results to make distinctions non-destructively on changes of various physical-chemical changes and quality parameters as a function of pasteurising treatments and/or storage.

Keywords: gamma irradiation, high hydrostatic pressure, minced beef

Investigations in the past decades proved that ionising radiation and ultra-high hydrostatic pressure (UHP)-treatment are effective processes to inactivate non-sporeforming bacteria most important from the point of view of microbiological safety and storability of meat and meat products (CHEFTEL & CULIOLI, 1997; FARKAS, 1998). A direct comparison of their non-microbiological effects is largely lacking. The aim of our studies was to compare the effects of these treatments on some microbiological and physical-chemical properties of raw minced beef.

^a Permanent address: AECS, P.O. Box 6091, Damascus, Syria

* To whom correspondence should be addressed.

Fax: 36-1-372 6321; e-mail: huto@omega.kee.hu

1. Materials and methods

1.1. Meat and sample preparation

Boneless fresh beef was purchased from the local market in Budapest, chopped into approx. 2 cm³ cubes then minced for one minute using an electrical mincer (Robot Coupe R502). The minced beef was filled into small plastic pouches formed from 'Multibarrier 4' laminated foil, with 100 µm thickness and O₂-transmittance of 5 cm³/m²/24 h atm/, at 23 °C, 50% R.H. (produced by WIPAK Co., Finland) and sealed under vacuum (-900 mbar for 8 s) by a MULTIVAC Model A300 machine (Haggenmüller, Walfertschwenden, Germany) in 40 g units for irradiation and 20 g units for UHP treatment. The packages were refrigerated (+5 °C) until they were treated on the same day, and were stored after treatment at the same temperature.

1.2. Radiation treatment

The samples were transported in a thermally isolated ice-box and irradiated with 1.5 kGy and 2.0 kGy gamma radiation doses, respectively, in a self-shielded ⁶⁰Co radiation source (Type RH-gamma-30; IZOTOP, Moscow, Russia) at the Central Food Research Institute, Budapest, under chilled condition. The dose rate was 3.2 kGy h⁻¹.

1.3. Ultra-high hydrostatic pressure treatment

Isostatic pressurization was performed in a hydrostatic pressure vessel type "FoodLab 900" (Stansted Fluid Power Ltd., Stansted, U.K.) at 200 and 300 MPa for 20 min, while preventing the raise of the temperature over 20 °C using a cooling system (Haake C40-F6). Ethanol/castor oil (15%) was applied as pressurising medium. Pressure build-up lasted 15–30 s, pressure release took 40–55 s.

1.4. Bacteriological examination

Samples were investigated bacteriologically on the first, the second and the 15th day of refrigerated storage. After 1 min blending using a Stomacher (Interscience, France) in sterile 0.1% peptone-water, aliquots of decimal dilution series of this stock suspension were inoculated onto PCA Agar (Merck 1.05463) to determine the total viable cell (TVC) counts and into portions of a liquid medium (Fluorocult Laurylsulphate Broth with Durham tube, Merck 1.12588) to determine coliform counts by the MPN method. Expecting lactic acid spoilage of the vacuum packaged samples, the counts of lactic acid bacteria (LAB) were also estimated at the 15th day of refrigerated storage using double-layer MRS medium (Merck 1.10660). Incubation temperatures were 30 °C for TVC and LAB count, and 37 °C for coliforms. Bacterial populations were calculated as log₁₀ CFU g⁻¹.

1.5. Instrumental measurement of external colour

Surface colour of the samples was measured with six repetitions by a Minolta Chromameter type CR200 directly after the non-thermal treatments and at the second and the 15th day of refrigerated storage. The measurements were conducted first through the packaging foil then the plastic pouch was removed and colour readings were taken after 30 min to see the effect of anaerobic and aerobic conditions on the tristimulus colour values L^* , a^* and b^* .

1.6. Measurement of the water-binding capacity

After 10 days of refrigerated storage, 2 g of minced beef samples were spread on a sheet of filter paper (11 cm diam. 'Rundfilter', MN 640d, Ast. 205011) then covered with a plastic foil and pressurized between two glass plates with a 500 g weight for 5 min before the moistened area of the filter paper and the meat surface area were compared by an image analyser of the Physics Department of our University using its "Turbosegment" computer programme.

1.7. Differential scanning calorimetry (DSC)

DSC analyses were performed during the second week of refrigerated storage by a "MicroDSC III" type micro-calorimeter (SETARAM, France), where approx. 750 mg minced meat was investigated scanning in the 5 to 95 °C temperature range with 1 °C min⁻¹ heating rate using distilled water as reference sample. To observe irreversibility of transitions, after the first heating and re-cooling, a second heating run was performed.

1.8. Near infrared reflectance (NIR)-spectra

NIR analyses of minced beef samples were conducted on the 14th day of refrigerated storage using a "Spectralyzer" type 10–25 (PMC, Switzerland) scanning NIR spectrometer. Log 1/R spectra were recorded in the wavelength range of 1000 to 2498 nm in 2 nm steps. Evaluations were performed according to KAFFKA and GYARMATI (1998), using PolarQualification System (PQS) Program. "Quality points" of the samples were determined in the "quality plane". The quality point is defined as the center of the spectrum (of the spectral points) represented in polar coordinate system.

1.9. "Electronic nose" investigation

At the same time as the sensory analyses and NIR-spectrometry, 2 g of each sample were tested in standard headspace vials by a chemical sensor array ("SAMSELECT" type electronic nose, Daimler-Benz Aerospace, Rostock, Germany) using headspace autosampling. After normalising the data, principal component analysis (PCA) and the above polar qualification system using "sequence optimisation" were applied to create classification models from the sensor signal responses of the sensor array as described by KISKÓ and SEREGÉLY (2000).

1.10. Sensory testing

Sensory evaluation has been performed on the raw samples by a 6-member panel on the 14th day of refrigerated storage using scores from 1 (completely unacceptable) to 5 (excellent) for texture noted by finger pressure, colour and odour. The scores were ranked and rank sums were calculated for statistical evaluation according to Kramer's method (KRAMER, 1960).

1.11. Statistical analysis

Colour and water holding capacity data were evaluated by one-way analysis of variance with a variance ratio (F) test and least squares differences to compare means and to identify significant differences ($P < 0.05$) among treatments.

2. Results and discussion

2.1. Bacteriological examination

The logarithmic total viable cell counts estimated at the beginning and after 2 and 15 days of refrigerated storage are illustrated by Fig. 1. On the basis of acidic smell noted, and the fact that vacuum packaging stimulates the growth of lactic acid bacteria instead of obligately aerobic proteolytic psychrotrophs, on day 15th also the counts of lactic acid bacteria were selectively estimated. Their counts are shown also on Fig. 1.

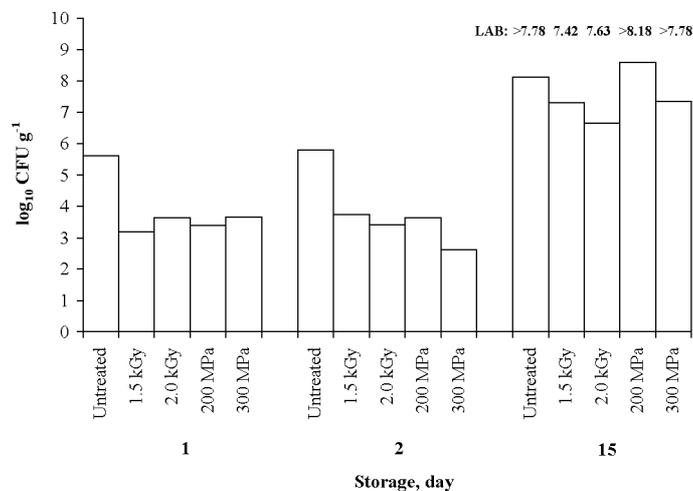


Fig. 1. Average total viable cell counts (TVC) of meat (stored at 5 °C up to 15 days). Counts of lactic acid bacteria (LAB) estimated at the 15th day only are shown by the numbers on the top of TVC-columns

Figure 1 shows that at the beginning of refrigerated storage, both irradiated and UHP-treated samples contained approx. two log cycles less total viable cell counts than the untreated controls. No significant differences were observed directly after treatments between the survivor levels of the 1.5 and 2.0 kGy or, the 200 MPa and 300 MPa samples, respectively. It is assumed that the survival levels directly after treatments were caused by bacterial spores, which are resistant to both the radiation- and UHP treatment levels applied. This reduction of the initial TVC is comparable to that observed for 3.5 kGy irradiated raw ground beef patties by LUCHSINGER and co-workers (1997). THAYER and BOYD (1993) reported 0.27 ± 0.03 kGy as D_{10} -value of *E. coli* O157:H7 in vacuum packaged chilled ground beef, thus at least 6D reduction of this pathogen could be expected by the 2 kGy irradiation dose applied by us.

On the basis of UHP-resistance data of *Listeria monocytogenes* in minced beef published previously (MÉSZÁROS et al., 1999), two-three log-cycles reduction of the viable cell counts could be expected by the 300 MPa, 20 min UHP treatment applied.

During 15 days of refrigerated storage, regrowth of survivors occurred and lactic acid bacteria became the dominating component of the spoilage association and the limiting factor of shelf life, which apparently did not exceed two weeks at 5 °C. The coliform bacteria which occurred in the untreated samples in a level of 6 thousands CFU g⁻¹ have been inactivated to a non-detectable (less than 3 CFU g⁻¹) level by both types of non-thermal pasteurization and they could not be recovered from the stored samples either during the entire storage period of 15 days. Their disappearance from the untreated sample at the end of the storage test might be due to their sensitivity to the antibacterial metabolites of the abundant growth of lactic acid bacteria.

2.2. Colour changes

Immediately after the radiation treatment, irradiated samples appeared to be slightly more reddish by visual observation than the control samples whereas UHP-treated samples were easily distinguishable from the controls by their paleness (loss of redness).

The results of instrumental colour measurements directly after irradiation and at the 2nd and 15th day of refrigerated storage are summarized in Tables 1 and 2. These data show that the more reddish hint of the freshly irradiated samples as compared to the controls was expressed basically by a decreased yellow hue (b^* value), however, this difference disappeared during the storage when this colour parameter decreased as compared to its initial values. The paleness of the UHP-treated samples was expressed by a significant increase of their lightness value (L^*), and a drastic increase of their yellow hue (b^*). When the samples were measured after unpacking, a significant increase of the red hue (a^* value) of all samples occurred, apparently due to the oxygenation of the muscle pigment, and a clear increase of the yellow hue (b^*) of the UHP-treated samples was observed. In general, irradiated samples showed much less colour difference from that of the controls.

Table 1. Changes of tristimulus colour values of vacuum packaged minced beef as an effect of irradiation or UHP treatment and refrigerated storage. Measurement through the packaging foil

Treatment	Tristimulus colour values during storage at 5 °C								
	for 0 day			for 2 days			for 15 days		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Untreated	46.16 (1.28)	15.51 (0.70)	4.38 (1.41)	45.39 (1.55)	16.01 (1.08)	1.89 (0.37)	48.16 (2.66)	16.83 (0.79)	2.54 (0.61)
1.5 kGy	44.44 (0.89)	16.20 (0.42)	2.32 (0.33)	45.29 (1.02)	16.16 (0.89)	2.34 (0.31)	46.85 (0.43)	17.11 (0.54)	2.24 (0.16)
2.0 kGy	43.73 (0.57)	15.05 (0.76)	2.48 (0.52)	44.79 (1.04)	16.00 (0.35)	1.85 (0.19)	46.02 (0.77)	17.53 (0.25)	2.27 (0.12)
200 MPa, 20 min	52.27 (0.78)	17.16 (0.90)	8.73 (0.44)	49.74 (0.86)	14.45 (2.81)	5.53 (2.11)	50.43 (0.99)	17.05 (0.70)	3.03 (0.42)
300 MPa, 20 min	57.47 (1.24)	16.06 (1.10)	8.03 (0.77)	55.45 (0.74)	11.04 (1.58)	9.13 (1.76)	56.07 (1.60)	16.30 (1.26)	4.78 (0.51)

Standard deviation values in brackets

Number of replicates : 6 (for untreated samples 12)

Table 2. Changes of tristimulus colour values of vacuum packaged minced beef as an effect of irradiation or UHP treatment and refrigerated storage. Measurement, 30 min. after unpacking

Treatment	Tristimulus colour values during storage at 5 °C					
	for 0 day		for 2 days			
	L*	a*	b*	L*	a*	b*
Untreated	47.70 (1.33)	19.80 (1.45)	8.70 (0.44)	46.53 (1.35)	20.24 (1.24)	8.10 (0.77)
1.5 kGy	46.19 (1.37)	20.18 (0.77)	8.06 (0.44)	46.01 (1.02)	20.84 (1.07)	8.56 (0.58)
2.0 kGy	45.67 (0.63)	18.24 (0.54)	7.19 (0.51)	45.92 (0.88)	19.59 (1.11)	7.96 (0.52)
200 MPa, 20 min	52.64 (0.54)	18.81 (1.06)	9.67 (0.37)	51.72 (0.52)	17.48 (3.05)	9.46 (0.55)
300 MPa, 20 min	56.95 (1.03)	18.47 (0.89)	9.98 (0.41)	56.89 (0.52)	13.30 (2.18)	9.81 (0.46)

Standard deviation values in brackets

Number of replicates: 6 (for untreated samples 12)

Regarding both a^* and b^* values, the colour of UHP-treated samples was less stable than that of the untreated or irradiated samples. It was suspected that the drastic colour changes of the UHP-treated samples, producing a colour similar to the cooked meat, may be related to an UHP-induced denaturation of the protein component of the muscle pigments, and even promoting oxidation of ferrous myoglobin into ferric myoglobin (CHEAH & LEDWARD, 1996).

2.3. Water-binding

The results of the measurement of the exudated moisture of various samples at the 10th day of refrigerated storage are shown in Table 3. Using the relative size of the exudation spot on the filter paper as compared to the surface of the meat sample, this measure showed that water binding of minced beef decreased as an effect of irradiation, whereas it increased in the UHP-treated samples.

2.4. Differential scanning calorimetry

DSC-thermograms of both untreated and non-thermally pasteurized minced beef samples showed endothermic transitions, partly due to lipid meltings (within the 10 to 40 °C range) and endotherms which are assumed to be related mainly to heat denaturations of its major proteins (in the temperature range of 40 to 85 °C) (Fig. 2).

Table 3. Effects of irradiation and UHP-treatment on exudativeness of the minced beef samples measured at the 10th day of refrigerated storage

Treatment	Relative percentage ^a of exudation surface (%)	As compared to the untreated (%)
2.0 kGy	209 a (24.2)	131 a (15.2)
1.5 kGy	175 b (11.2)	110 b (7.0)
Untreated	159 b c (12.5)	100 b c (7.9)
200 MPa, 20 min	139 c d (6.3)	88 c d (4.0)
300 MPa, 20 min	114 d (12.3)	72 d (7.7)

Number of replicates = 3

Standard deviation values are in brackets

^a Relative percentage of exudation surface = moist surface/meat surface·100

Averages followed by a same letter in the coloumn are not significantly different at $P \leq 0.05$ level.

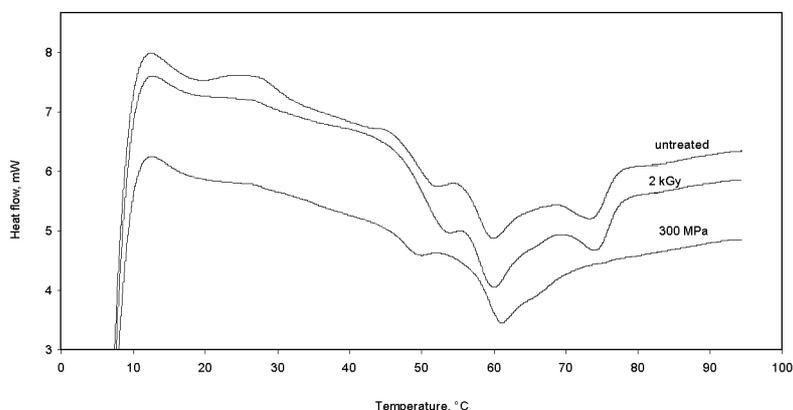


Fig. 2. DSC thermograms of the untreated (top), the 2 kGy (middle), and the 300 MPa, 20 min. (bottom) samples taken on the second week of refrigerated storage

The descending character of thermograms reflect changes in specific heat as a function of increasing temperature. In relation to the changes described in the previous sub-chapters, these protein denaturation patterns are worthwhile to be evaluated. As can be seen from Fig. 2, the DSC scans of untreated and irradiated samples were practically undistinguishable, where the first peak between 45 and 55 °C can be attributed mainly to the heat-denaturation of myosin, and myosin subunits, the composite transition showing a distinct large peak and a shoulder between 55 and 68 °C can be considered as the heat-denaturation of connective tissue (collagen and other stromal proteins) and sarcoplasmic proteins including various enzymes and myoglobin, whereas the distinct peak between 68 and 78 °C can be attributed to denaturation of actin, actinins and troponins (FINDLAY & BARBUT, 1990). A shoulder in the range of 78 to 85 °C shows that a transition process of even more heat resistant entities (F-actin, actomyosin?) occurred in this temperature range.

UHP treatments caused drastic changes of the DSC thermograms (Fig. 2, bottom graph). A significantly decreased “myosin peak” was shifted to several degrees lower temperature than in the control, and a diminished “actin peak” was shifted (200 MPa, 20 min) to lower temperatures or disappeared almost completely (300 MPa, 20 min). The low heating rate in our micro-DSC instrument tends to reduce somewhat the temperature of the endotherms as compared to the high heating rates of regular DSC equipments (ARNTFIELD et al., 1990). The beef component producing the transition represented by the main peak around 60–62 °C seems to be the most pressure-tolerant, while that part of the thermograms which originally showed conformation changes induced between 65 and 70 °C were also less-and-less distinct by the severity of the high pressure treatments. Since DSC only measures net changes, eventual concomitant coagulation (aggregation) processes, which should produce exotherms, could be apparently masked by the denaturation endotherms due to greater enthalpy changes involved in the

denaturation processes (FINDLAY & BARBUT, 1990). Thermograms obtained during a second heating run of the re-cooled samples did not show any peaks in the protein denaturation range, illustrating that the endothermic transitions observed between 40 and 95 °C during the first heating were all irreversible processes (WRIGHT et al., 1977). These observations are also in agreement with those of our previous studies on UHP effect on three different types of meat (beef, pork and turkey) (MÉSZÁROS & FARKAS, 2000).

Subtracting the second scans from the first scans resulted difference-thermograms. The surfaces between the thermograms and those lines which can be drawn between the 30 and 80 °C points of the difference thermograms, normalized to the unit mass of the thermo-analysed samples may represent “total enthalpies” of transitions and might be used as relative measures of the non-denatured/non-coagulated parts of total proteinaceous components of the minced beef samples left in them after the non-thermal pasteurizing treatments. These values are given in Table 4, which shows clearly that the UHP-treatment decreased progressively the proportion of the non-denatured part of certain protein components.

Table 4. Estimated “total enthalpies” of transitions between 30 °C and 80 °C points of the difference thermograms

Treatment	Sample mass (mg)	J	J g ⁻¹	Enthalpies	
				cal	cal g ⁻¹
Untreated	750	2.14	2.85	0.511	0.681
1.5 kGy	750	2.02	2.70	0.483	0.644
2 kGy	753	2.13	2.83	0.509	0.676
200 MPa	750	1.63	2.17	0.389	0.587
300 MPa	750	1.30	1.74	0.311	0.415

2.5. Near infrared reflectance measurements

Quality point analyses of the NIR reflectance spectra conducted on the 14th day of the refrigerated storage in a selected wavelength range of 1250–1300 nm from the whole spectra resulted in “quality points” of both UHP-treated and irradiated samples which had distinguishably different locations from those of the control samples, however, the two types of non-thermal treatments caused shifts of quality points into opposite directions (Fig. 3), providing an additional clue that UHP treatment and irradiation produced physical-chemical changes differing according to the type of non-thermal pasteurization treatments, as observed also in the dissimilar types of changes observed in both the colour- and water binding measurements. The mechanisms involved in these NIR spectrometric changes are not at all clear yet. They may be related also to the denaturation processes reflected also in the change of the DSC-thermograms of the UHP treated samples. They might be, however, influenced by the divergent bacteriological status of various samples at the time of the NIR measurement.

2.6. "Electronic nose" measurements

Electronic nose measurements and their chemometric analysis (PCA) conducted the same time as the NIR spectrometry, resulted in also distinct clustering of chemosensor responses between the control, the irradiated and the UHP-treated samples, reflecting probably different volatile compositions and concentrations of samples, due to their different bacteriological status (Fig. 4).

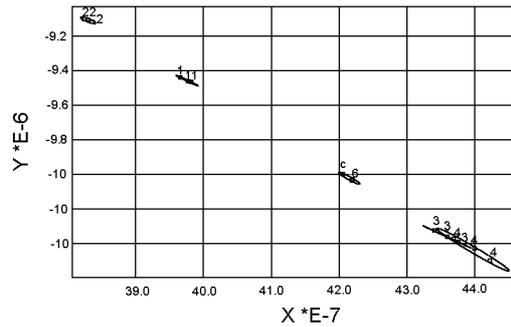


Fig. 3. Positions of quality points of three replicate measurements of untreated control (C), UHP-treated (1 = 200 MPa, 20 min), UHP-treated (2 = 300 MPa, 20 min), and irradiated (3 = 1.5 kGy; 4 = 2.0 kGy) minced beef samples, calculated on the basis of their NIR reflectance spectra, measured at the 14th day of refrigerated storage

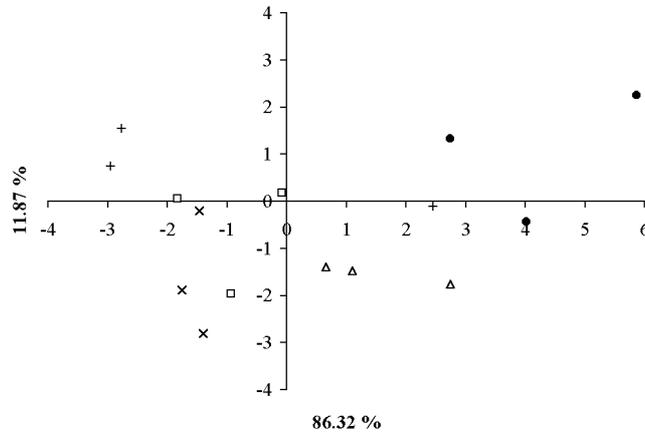


Fig. 4. Positions of quality point-clusters of untreated, UHP-treated and irradiated samples calculated from sensor-array responses of a "SAMSELECT" electronic nose, measured at the 14th day of refrigerated storage (●: control; Δ 1.5 : kGy; □ : 2 kGy; X : 200 MPa; + : 300 MPa)

Table 5. Average sensory scores and rank sums of samples tested at the 14th day of refrigerated storage

Treatment	Colour		Smell		Texture ^a	
	Score	Rank sums	Score	Rank sums	Score	Rank sums
Untreated	4.2	11.5	2.3	15.5	3.5	13.0
1.5 kGy	4.0	13.0	2.5	14.0	3.7	11.5
2 kGy	4.2	11.5	2.2	18.0	3.5	13.0
200 MPa	1.7	24.0	1.3	22.0	2.7	22.0
300 MPa	0.3	30.0	1.8	20.5	1.0	30.0

^a Texture tested by finger pressure

Number of panelists: 6

Rank sums between 10 and 26 do not differ significantly from each other at $P \leq 0.05$ level.

2.7. Sensory evaluation

Table 5 shows the average sensory scores and their rank sums, respectively, from sensory analyses taken at the 14th day of refrigerated storage.

It can be seen that the sensorial colour scores of both the untreated and the irradiated samples were still acceptable, whereas the intense loss of original colour due to the UHP-treatment was highly unacceptable to the panelists.

The odour of all samples was less than acceptable due to the extensive growth of the lactic acid spoilage flora. UHP treatments were again the worst ones, probably due to a less effective diminution of lactic acid bacteria from the original flora than that might occur in case of radiation treatments.

Regarding texture scores, the panelists distinguished clearly the 300 MPa, 20 min UHP-treated samples from the control and the irradiated ones due to the hard, rubbery texture of the UHP-treated samples in relation to their pressure induced coagulation.

3. Conclusions

Our results show that at about the same bactericidal effect, more drastic changes of colour and texture occurs in UHP-pasteurized minced beef samples than in radiation-pasteurized ones, and strengthen the statement of MACFARLANE (1985) that "of all foods and food constituents, muscle and muscle proteins are probably the most responsive to pressure". Our results are in agreement with the experiences of CHEAH and LEDWARD (1996) that in pressurized minced pork above 300–400 MPa loss of protein structure occurred, myosin and actin both denatured as well as many of the sarcoplasmic proteins. Myoglobin was irreversibly denatured at pressures ≥ 400 MPa.

Due to its high protein content, complex structure and sensitive redox system, raw meat seems to be easily affected by UHP. DSC proved to be an effective method to monitor structural changes induced by UHP and to assess protein denaturation induced by this non-thermal treatment. The observed non-thermal denaturation/coagulation might result, however, welcome changes of functional properties for some technologies where increased water-binding and better sliceability is required.

Further studies are started to compare the effects of these non-thermal physical pasteurization techniques on lipid oxidation and on the SDS-PAGE patterns of salt-soluble proteins and on the allergenicity of raw minced beef and mechanically deboned turkey which will be published elsewhere. It is planned to study whether UHP-induced discolouration of muscle pigments could be prevented by curing the meat with nitrite addition, considering the more heat-stable colour of nitroso-myoglobin than that of the original, non-nitrosated muscle pigment.

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This study was supported in part of the National Science Funds (OTKA), Budapest (T 032715). and the International Atomic Energy Agency, Vienna, by granting a fellowship for the first author. The authors thank Prof. K. KAFFKA, Dr. Zs. SEREGÉLY and Ms. K. ASCHENBRENNER for the NIR- and electronic nose-measurements.

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