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Influences of gamma irradiation and storage on the capsaicinoids of sun-dried and dehydrated paprika

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Abstract

A study was conducted to investigate the changes in the pungent components (capsaicin, dihydrocapsaicin, homodihydrocapsaicin, isodihydrocapsaicin and nordihydrocapsaicin) of paprika as a function of drying method, gamma irradiation and storage period. Sun-dried and dehydrated paprika samples were irradiated by using a ^{60}Co gamma irradiator at five doses (0, 2.5, 5.0, 7.5 and 10 kGy) in polyethylene bags and stored at ambient temperature for 10 months. The capsaicinoid contents of the samples were analyzed by HPLC every 2 months within the 10 months. The major pungent components, capsaicin and dihydrocapsaicin, significantly ($P < 0.01$) increased with increasing irradiation doses. The increases of capsaicin, dihydrocapsaicin and homodihydrocapsaicin contents were about 10% with the dose of 10 kGy. In contrast, a significant ($P < 0.01$) decrease was observed in these components with storage. The levels of all capsaicinoids were significantly ($P < 0.01$) higher in dehydrated paprika than in sun-dried paprika. Nordihydrocapsaicin was found only in fresh red pepper. Although isodihydrocapsaicin was not detected in paprika during the first five months of the storage period, it was detected from the 6th month of the storage period. Hence, isodihydrocapsaicin might be used to identify paprika which has been stored for longer than six months.

Keywords: *Capsicum annuum*; Capsaicinoids; Drying method; Gamma irradiation; Storage

1. Introduction

Pungent spice paprika (*Capsicum annuum* L.) is one of the oldest, most important and widely used food flavourings and colorants. It is also used as raw material for the pharmaceutical industries (Daood et al., 2002; Deli, Molnár, Matus, & Toth, 2001; Ladron-Guevara et al., 2002; Reeves, 1987). The amounts and characteristics of flavouring, colouring and essentially pungent compounds in paprika spices are important with regard to quality (Peusch, Muller-Seitz, Petz, Muller, & Anklam, 1997). The pungency of paprika is directly related to the pungency of *Capsicum* fruits, which are processed into the paprika.

Pungent components, peculiar to the fruits of *Capsicum* plants, are the capsaicinoids. All of the identified capsaicinoids in the *Capsicum* fruits are vanillylamides of branched fatty acids, with 9–11 carbons, (Fig. 1) of which capsaicin (vanillylamide of 8-methylnontrans-6-enoic acid) and dihydrocapsaicin (vanillylamide of 8 methylnonanoic acid) occur in quantities greater than 80%. The remaining derivatives are found in very small amounts (Krajewska & Powers, 1988; Perucka & Materska, 2001; Thomas, Schreiber, & Weisskopf, 1998).

Capsaicinoids have strong physiological and pharmacological properties. In addition to its widespread use as a neuropharmacological tool, the medicinal value of capsaicin has been evaluated in the treatment of painful conditions, such as rheumatic diseases, cluster headaches, painful diabetic neuropathy and post-herpetic neuralgia. Even used at low levels in the diet, capsaicinoids decrease the myocardial and aortic cholesterol levels. Today, capsaicinoids are being studied as an effective treatment for a number of sensory nerve fibre disorders, including arthritis, cystitis, and human immunodeficiency virus. (Perucka & Materska, 2001;

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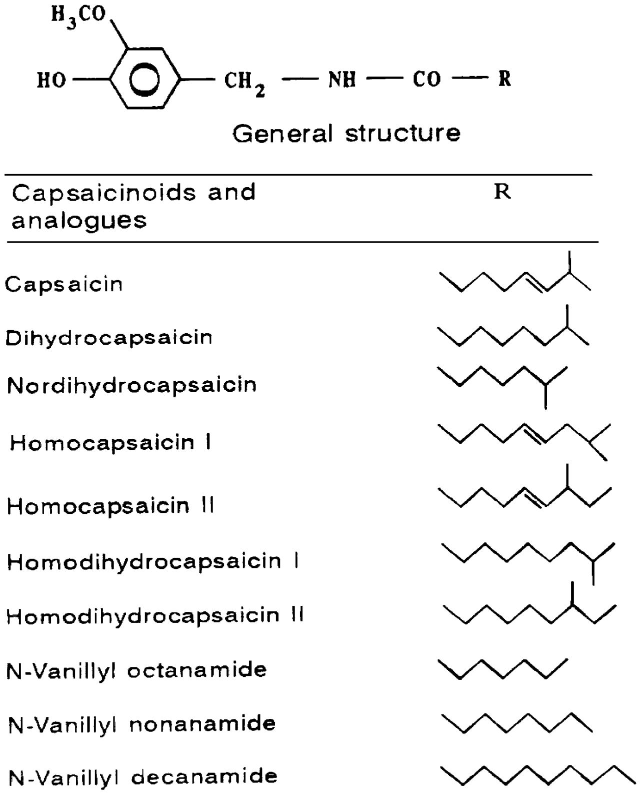

Fig. 1. Structural formulae of capsaicinoids (Contreras-Padilla & Yahia, 1998).

Robbins, 2000; Tsuchiya, 2001). They are also reported as having antioxidant and antibacterial effects on certain groups of bacteria (Dorantes et al., 2000; Erdemoglu, 2001; Henderson & Slickman, 1999).

Capsaicinoids are produced in glands on the pepper placenta and on the white ribs that run down the middle, and along the sides, of a pepper. As a result, the glands and white ribs are the hottest parts of a red pepper. Since the seeds are in close contact with the ribs, they are also often hot (Bosland, 1992; Dong, 2000). The capsaicinoids are synthesized through the cinnamic acid pathway, and it is thought that their degradation is aided by the action of peroxidase (Contreras-Padilla & Yahia, 1998).

Govindarajan (1985) and Bosland (1994) determined that there are great diversities in the contents and composition of capsaicinoids among fruits of *Capsicum* species, and even among cultivars. The environment, especially the climate, light, soil, moisture, fertilization and temperature during plant growth, is considered to have an impact on capsaicinoid levels, as does the age of the fruit (Estrada, Pomar, Diaz, Merino, & Bernal, 1999; Estrada, Bernal, Diaz, Pomar, & Merino, 2002; Titze, Hiepler, Seitz, & Petz, 2002). In general, the fruits of *C. annuum* contain 0.0003–0.01% and the fruits of *Capsicum frutescens* contain 0.3–1% capsaicinoids (Perucka & Oleszek, 2000). Processing the harvested *Capsicum* fruits also plays an important role on the level of capsaicinoids, in that both the drying conditions and the number of seeds included have an influence on pungency (Titze et al., 2002).

In order to measure the pungency of the hot pepper and paprika, The Scoville Organoleptic Test, the first reliable measurement of pungency, was developed by W.L. Scoville in 1912. This test involves a taste panel of five individuals, who differentiate among diluted samples that give the burning sensation. Since then, several methods have become available for the identification and quantification of capsaicinoids; however, HPLC is considered the most reliable and rapid method (Collins, Wasmund, & Bosland, 1995; Peusch et al., 1997; Wall & Bosland, 1998; Yao, Nair, & Chandra, 1994)

Because of contamination throughout the processing and storage in improper conditions, paprika has a great number of molds, yeasts, bacteria and insects. This restricts or decreases its commercial value because of the strict hygienic demand in spices. Therefore, they are obligatorily sterilized or fumigated in some way. (Buckenhüskes, 2001; Narvaiz, Lescano, Kairiyama, & Kaupert, 1989; Subbulakshmi et al., 1991; Sharma, Padwal-Desai, & Nair, 1989). Because of their low moisture content, spices are microbiologically stable products; however, once in contact with water-rich foods, microorganisms can proliferate quickly. For this reason, the irradiation of spices is one of the safest, most efficient, and widely used methods for obtaining optimum sanitation. Additionally, paprika is not usually used immediately after processing; rather, it is stored for various periods of time before use.

Many studies have been carried out on the capsaicinoids of *Capsicum* fruits, investigating growing conditions, physiological aspects and derived products such as paprika and oleoresins (Artik, Kadakal, & Yemis, 2001; Contreras-Padilla & Yahia, 1998; Peusch et al., 1997; Titze et al., 2002; Zewdie & Bosland, 2000; Zewdie & Bosland, 2001). However, effects on the capsaicinoids of post-harvest applications, such as drying, milling, heating, irradiation and storage, have rarely been considered. Post-harvest studies of capsaicinoids have mostly concentrated on the extraction methods and their efficiency.

The objective of the present study was to determine the comparative effects of gamma irradiation, and the period of storage, on the capsaicinoid stability of sun-dried and dehydrated paprika.

2. Materials and methods

2.1. Materials

Full ripe *C. annuum* fruit, an unnamed Turkish paprika cultivar, was harvested by farmers from their own fields during the harvesting season in the province of Kahramanmaras, Turkey. The harvesting season of pepper begins in September and continues until the end of October. Kahramanmaras is one of the most intensive pepper production areas. It is located in Southern Turkey, is situated at around 650 m above sea level, and receives about 700 mm annual rainfall. For each experimental trial, 100 ± 10 kg of ripe pepper pods were sampled after harvest.

2.2. Processing of paprika

Pepper pod samples were processed into paprika by using two different paprika processing systems used commercially in Turkey. Paprika processing was carried out at two local plants, named Musan and Godeler Co. (Kahramanmaras, Turkey).

2.3. Sun drying

In Turkey, farmers traditionally use the sun-drying method, due to its low cost. Pepper samples were spread out on fields and left under sunlight exposure for 5–7 days. After drying, the moisture content of pods had dropped to between 12% and 14%. Impurities of dried pods were cleaned and seeds were removed. The seeds of 30% of the sample were re-added to the deseeded dried pepper before milling. The milling process consisted of coarse grinding by two hammer mills. Flakes were separated into two classes (either the size of <1 mm or 1–3 mm) by mechanically shaken sieves, while the coarse particles were sent to another hammer mill. Paprika with the dimensions of 1–3 mm was used for this study.

2.4. Oven drying

Washing equipment, a chopper, a holed washing cylinder, and drying equipment were used in the drying process. Stalks and seeds were removed before the pepper was chopped into a 9 mm square shape. Then, the chopped pepper was dehydrated for 90 min by a hot air stream in a tunnel drier. The temperature of the air at the entrance of the tunnel was about 70 °C. After drying, the moisture content of the chopped pepper dropped to between 11% and 12%. After the addition of 30% of the seeds, they were crushed by using a hammer mill, and subsequently classified into the flakes and powder, as explained previously.

2.5. Salting and polishing

Ten per cent of salt and 8% of oil (olive oil and cotton seed oil (1:1)) were added to the sun-dried and dehydrated paprika flakes. This process is a common traditional application in Turkey.

The final product was packaged in polyethylene bags before undergoing gamma irradiation and subsequent storage.

2.6. Irradiation of paprika

Paprika samples were irradiated at the Nuclear Agriculture and Animal Science Research Center, Ankara, Turkey. The irradiation process of samples was carried out in a ^{60}Co gamma irradiator (Gamma Cell 220), with the average absorbed dose being 2.5, 5.0, 7.5 and 10.0 kGy, and the maximum dose rate being 0.042 kGy/min. Treatments were performed on the eight samples at each step, due to the restricted volume of the irradiator cell at the ambient temperature. The absorbed doses of samples were confirmed at each step by putting a Harwell Amber Perspex dosimeter in the centre, and the exterior of the eight bags was wrapped into a cylindrical shape, using sticky ribbon.

The irradiation treatment was conducted in duplicate for every dose. All of the irradiation treatments were completed in 3 days.

Samples were stored at room temperature under dark conditions. Capsaicinoids of the samples were extracted within 10 months, specifically at the end of each 2-month storage period.

2.7. Extraction of capsaicinoids

Capsaicinoids were extracted from the samples of paprika and red pepper by applying the technique described by Collins et al. (1995).

The capsaicinoids were extracted from 1.2 g of samples in 12 ml acetonitrile by being heated at 80 °C for 4 h. Suspensions were periodically shaken every 30 min throughout the extraction process. The suspended material was allow to cool and settle. The supernatant was filtered into a 2 ml glass vial by using a 0.45 μm membrane filter (Millipore), and then used for HPLC injection.

2.8. Chromatography

Liquid chromatography was performed, using a Varian HPLC solvent delivery system (9010) equipped with auto sampler (Marathon), fluorescence detector (Varian 9070) and Star software for data processor. The separation was performed on a Nucleosil 5 C_{18} column (250 × 4.6 mm ID) coupled with a Nucleosil 5 C_{18} guard column (4 × 4.6 mm ID).

The following HPLC operating condition, used by Peusch et al. (1997), was employed. The eluent was a mixture of acetonitrile/water/acetic acid (100:100:1 v/v/v), and the flow rate was 1.2 ml/min. The fluorescence detector was set at 280 nm excitation and 320 nm emissions. Injection volumes were 20 μl, while run time and temperature were 23 min and 20–22 °C, respectively.

2.9. Identification and quantification of capsaicinoids

Standards of capsaicin (98%), dihydrocapsaicin (90%) and a mixture of capsaicinoids (60% capsaicin) were purchased from Sigma Chemical Co. (St. Louis, MO) and were used for retention time verification and instrument calibration. Quantification was achieved by the external standard method.

2.10. Calculation of scoville heat unit (SHU)

Scoville heat unit of samples was calculated by the method of Todd, Bensinger, and Biftu (1977). The method involved multiplying the concentration of each capsaicinoid by the individual dilution factors which cause burning sensation.

2.11. Statistical analysis

The experiment was conducted as a randomized plot with a factorial design in the drying methods, irradiation doses, and six periods of storage ($2 \times 5 \times 6$), using duplicate samples. Data were subjected to analysis of variance, and appropriate means separation was conducted using Duncan's multiple range test analysis by SAS software.

3. Results and discussion

3.1. Capsaicinoids of red pepper

In the present study, the capsaicinoids and SHU of red pepper were analyzed to determine the pungency of raw material and an assessment of the capsaicinoid stabilities during the paprika processing. The capsaicinoid composition and SHU values of red pepper are given in Table 1. Five principle capsaicinoid components were determined in the sample, with the total concentration of 529 mg/kg, all of which provided 8366 SHU. A number of previous authors found that the capsaicinoid levels and pungency values of *C. annuum* fruits have a broad range of variance (Dong, 2000; Perucka & Oleszek, 2000; Peusch et al., 1997). According to the Bosland (1994) classification, the present value is included in the class of medium pungency. The capsaicinoid concentration, and indirectly its pungency, is inevitably dependent upon species and varieties. However, it should be considered that the attributes are also highly influenced by a number of extrinsic factors, such as fruit age, water stress, temperature, soil and fertilizer (Estrada et al., 2002; Titze et al., 2002).

Due to different burning sensations of individual capsaicinoids (Krajewska & Powers, 1988), the composition of capsaicinoids is just as important as the overall capsaicinoid level. In the present study, capsaicin and dihydrocapsaicin were the major components of the sample, with both of them representing 84.9% of the capsaicinoids. The capsaicin to dihydrocapsaicin ratio was 1.55. Identical findings on *C. annuum* fruit, with an explanation of the characteristics peculiar to species, have been found in previous articles (Collins et al., 1995; Estrada et al., 2002). The other minor components were nordihydrocapsaicin, homodihydrocapsaicin, as well as an unknown component. It should be pointed out that there was no major change in the proportion of capsaicin to dihydrocapsaicin, although the individual level of capsaicinoids was highly changeable.

3.2. Effect of drying method on the capsaicinoids of paprika

Both paprika processing methods caused a significant ($P < 0.05$) decrease in the total capsaicinoid level (Table 1). However, the loss of capsaicinoids in the dehydration method was less than that of the sun drying method. The levels of all capsaicinoids in sun-dried and dehydrated paprika, except for isodihydrocapsaicin, were significantly ($P < 0.05$) different. Individual capsaicinoids had different stabilities during processing. Even though the most stable component was capsaicin, it decreased 21.5% in dehydrated paprika and 24.6% in sun-dried paprika. Dihydrocapsaicin was the second most stable component, after capsaicin. The other capsaicinoids were more or less sensitive to the processing in both methods. Of particular interest, nordihydrocapsaicin and the unknown component had the highest sensitivity to the manufacturing of paprika, because they could not be detected in either the dehydrated or sun-dried paprika. The other substantial decrease was recorded in homodihydrocapsaicin content. On the other hand, while isodihydrocapsaicin could not be detected in fresh red pepper, it was detected in paprika processed by both drying methods. In a few other studies, however, isodihydrocapsaicin was indeed found to be present in the fruit of several *C. annuum* varieties (Collins et al., 1995; Zewdie & Bosland, 2000).

In the present study, the decrease in the capsaicinoid levels during processing may not occur only as a result of the sun drying or dehydration processes. In paprika production, the milling process and the dilution of paprika with seed, salt and oil may have little effect on the capsaicinoids decreases. However, the oxidation reac-

Table 1
Capsaicinoids composition of fresh red pepper and changes during whole process in sun-dried and dehydrated paprika (mg/kg dry matter)

Capsaicinoids	Fresh fruit	Sun-dried paprika	Loses (%)	Dehydrated paprika	Loses(%)
Nordihydrocapsaicin	7.73 ± 0.350^{a}	nd	100	nd	100
Capsaicin	273 ± 5.98	$206^{b} \pm 4.54$	24.6	$214^{a} \pm 3.14$	21.5
Dihydrocapsaicin	176 ± 4.87	$123^{b} \pm 2.80$	30.5	$135^{a} \pm 2.10$	23.7
Homodihydrocapsaicin	11.9 ± 0.462	$4.03^{b} \pm 0.115$	66.0	$4.78^{a} \pm 0.116$	59.7
Iso-dihydrocapsaicin	nd	$3.94^{a} \pm 1.01$	–	$4.39^{a} \pm 1.05$	–
Unknown	60.3 ± 2.74	nd	100	nd	100
Total	529 ± 14.3	$336^{b} \pm 6.92$	36.5	$358^{a} \pm 4.71$	32.3
SHU[b]	8366 ± 223	$5377^{b} \pm 111$	35.7	$5722^{a} \pm 75.1$	31.6

Values in a line followed by different superscript letters are significantly ($P < 0.05$) different (Duncan's multiple range test). nd: not detected.

[a] Mean value ± standard error ($n = 3$ for fresh fruit, $n = 60$ for sun-dried and dehydrated paprika).

[b] SHU = scoville heat unit.

tion of capsaicinoids is speeded up by the catalytic effect of peroxidase in the longer drying period (Contreras-Padilla & Yahia, 1998). Hence, the sun-drying process results in higher capsaicinoid decreases.

This study indicated that; in terms of capsaicinoids stability, the dehydration process may be more preferable than the sun drying process. This may be due to the faster drying rate, which restricts peroxidase activity.

3.3. The effects of gamma irradiation on the capsaicinoids of paprika

In recent years, gamma irradiation of paprika has been necessary process because of demands for safe and high quality products. In the present study, the effect of gamma irradiation on the pungency of paprika and related compounds was investigated in detail. It can be clearly seen from Table 2 that all components of capsaicinoids, with the exception of isodihydrocapsaicin, were significantly ($P < 0.05$) increased with the maximum legal dose of 10 kGy. However, isodihydrocapsaicin was not affected by irradiation doses. The increases of capsaicinoid levels were estimated to be about 10%. Likewise, Subbulakshmi et al. (1991) reported that the pungency of irradiated paprika was greater than in that of the control sample. The capsaicinoids increase with the effect of irradiation treatments can be explained by changing the conformation of the molecules and/or accompanying compounds which affects the extraction yield. However, the level of isodihydrocapsaicin did not change with gamma irradiation. Doses up to 5 kGy of gamma irradiation led to greater increases of capsaicin and dihydrocapsaicin levels. With doses of more than 5 kGy, the levels of the same components did not show any statistical differences. In other words, both 7.5 and 10 kGy doses had similar effects on capsaicin and dihydrocapsaicin.

3.4. The effects of storage on the capsaicinoids of paprika

Table 3 summarizes the changes in the level of individual capsaicinoids of paprika due to storage period. Under ambient storage, the level of each capsaicinoid in paprika was significantly ($P < 0.01$) decreased with storage. Generally, all capsaicinoid components decreased almost 30% within ten months of storage. The maximum decrease was recorded in dihydrocapsaicin. On the other hand, isodihydrocapsaicin, which could not be detected in the early stages of storage, was only detected at 6 months of storage. Furthermore, its concentration rose sharply after 6 months of storage, and this phenomenon may be the result of the high storage temperature. The last two stages of the 10-month storage period coincided with the months of June and August, when the average

Table 2
Quantitative changes in the capsaicinoid composition of irradiated paprika (mg/kg dry matter)

Irradiation doses (kGy)	Capsaicin	Dihydrocapsaicin	Homodihydro-capsaicin	Iso-dihydrocap-saicin	Total Capsaicinoids	SHU[b]
Control	$197^{c} \pm 6.95$	$121^{d} \pm 4.53$	$4.15^{c} \pm 0.217$	$4.26^{a} \pm 1.86$	$327^{d} \pm 10.9$	$5225^{d} \pm 173$
2.5	$205^{b} \pm 5.67$	$126^{c} \pm 3.75$	$4.25^{bc} \pm 0.199$	$3.54^{a} \pm 1.35$	$338^{c} \pm 8.77$	$5413^{c} \pm 140$
5.0	$211^{ab} \pm 5.62$	$130^{bc} \pm 3.87$	$4.36^{abc} \pm 0.176$	$3.92^{a} \pm 1.41$	$349^{bc} \pm 8.61$	$5584^{bc} \pm 138$
7.5	$219^{a} \pm 6.65$	$134^{ab} \pm 4.26$	$4.58^{ab} \pm 0.209$	$4.37^{a} \pm 1.79$	$362^{a} \pm 10.1$	$5785^{a} \pm 161$
10	$217^{a} \pm 5.44$	$133^{a} \pm 3.71$	$4.68^{a} \pm 0.177$	$4.73^{a} \pm 1.80$	$359^{ab} \pm 8.23$	$5740^{ab} \pm 131$

Values in a column followed by the different superscript letters are significantly ($P < 0.05$) different (Duncan's multiple range test).

[a] Mean value ± standard error ($n = 24$).

[b] SHU = scoville heat unit.

Table 3
Quantitative changes in the capsaicinoid composition of paprika during storage (mg/kg dry matter)

Duration of storage (months)	Capsaicin	Dihydrocapsaicin	Homodihydrocapsaicin	Iso-dihydrocapsaicin	Total capsaicinoids	SHU[b]
Control	$244^{a} \pm 4.46$	$150^{a} \pm 2.66$	$5.38^{a} \pm 0.142$	nd[c]	$400^{a} \pm 7.01$	$6390^{a} \pm 112$
2	$237^{a} \pm 5.18$	$146^{b} \pm 2.99$	$4.92^{b} \pm 0.197$	nd[c]	$388^{a} \pm 8.16$	$6207^{a} \pm 130$
4	$210^{b} \pm 3.08$	$130^{c} \pm 2.37$	$4.38^{c} \pm 0.155$	nd[c]	$344^{b} \pm 5.50$	$5497^{b} \pm 87.7$
6	$207^{b} \pm 4.86$	$128^{c} \pm 3.88$	$4.36^{c} \pm 0.239$	nd[c]	$339^{b} \pm 8.85$	$5423^{b} \pm 141$
8	$184^{c} \pm 2.76$	$113^{d} \pm 1.97$	$3.56^{d} \pm 0.126$	$4.535^{b} \pm 0.310$	$305^{c} \pm 4.94$	$4885^{c} \pm 78.9$
10	$176^{d} \pm 2.32$	$106^{e} \pm 1.90$	$3.83^{d} \pm 0.134$	$20.438^{a} \pm 1.493$	$306^{c} \pm 7.78$	$4893^{c} \pm 76.4$

Values in a column followed by the same superscript letters are not significantly ($P < 0.05$) different (Duncan's multiple range test).

[a] Mean value ± standard error ($n = 20$), nd: not detected.

[b] SHU = scoville heat unit.

temperature is much higher than in other months. However, this hypothesis must be researched in detail to determine whether there is a relationship between storage time and conditions with this compound. With respect to the ratio of capsaicin to dihydrocapsaicin, there was an increase, especially during the last two stages of storage. Therefore, it may be possible to express an isomeric transformation from dihydrocapsaicin to isodihydrocapsaicin.

In terms of both capsaicin and dihydrocapsaicin, there were relationships between the drying method and storage stability. Changes in the amounts of these principal pungent components were less in the dehydrated paprika during storage, whereas they changed notably in sun-dried paprika after 2 months of storage.

4. Conclusion

1. Fresh red pepper, the raw material of paprika, has moderate levels of the following capsaicinoids: capsaicin, dihydrocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin and an unknown component. The principal components are capsaicin and dihydrocapsaicin, with the proportions being 51.6% and 33.4%, respectively.
2. All capsaicinoid contents were decreased in paprika manufacturing. Nordihydrocapsaicin and the unknown compound of red pepper were completely destroyed during paprika processing. In terms of capsaicinoids, the dehydration method was less destructive than, and therefore superior to, the sun drying method.
3. Gamma irradiation resulted in an increase of about 10% in the capsaicinoid content of paprika.
4. Nevertheless, the paprika capsaicinoids were gradually decreased to almost 70% of their initial concentration throughout the ten months of storage. On the other hand, isodihydrocapsaicin, which could not be detected at the early stages of storage, was determined only after 6 months of storage. Therefore, this compound may be a useful marker for the indication of storage time, and/or the conditions of paprika during longer storage.

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