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DNA COMET ASSAY TO IDENTIFY DIFFERENT FREEZING TEMPERATURES OF IRRADIATED LIVER CHICKEN

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ABSTRACT

The cold chain is a succession of steps which maintain the food at low temperature. The thawed food never be frozen again and the best solution being to consume it quickly to avoid the microorganism growth which causes decay and nutrients damage. One of most important point is that freezing process, unlike irradiation, do not destroy microorganisms, only inactive them as long as they remain in a frozen state. The Comet Assay is an original test used to detect irradiated foods that's recognize the DNA damage and can then be used to control the overall degradation of the food and in a certain extend to evaluate the damage caused by irradiation, different forms of freeze and storage time on liver chicken cells. Different freezing temperatures were used, deep freeze -196°C and slow freeze -10°C. Samples were irradiated in a ^{60}Co irradiator with 1.5, 3.0 and 4.5 kGy radiation doses. Fast freezing technique induces a low percent of DNA degradation comparing to slow freezing technique. This procedure could be a good choose to chicken freezing processing.

1. INTRODUCTION

The industrially freezing food process used, mainly for meat, are: air-freezing property, freezing plating, forced air circulation, dip freezing, liquid spray and liquid nitrogen freeze [1]. The freezing involves a temperature decrease up to -18°C or below the crystallization of water and solutes [2]. In the freezing process, the free water crystallizes and the size of ice crystals are dependent on the velocity and temperature of freezing [3]. Small size solutes molecules are able to migrate and concentrate among the ice crystals or between the segments of a same single crystal. The crystals ice performing mechanical action that could cause the membranes rupture and DNA degradation [4].

As the rate of freezing, is accepted that the fast freezing get final products with better frozen quality due formation of small ice crystals between the structures of cells, in the intercellular and intracellular spaces, the crystals size are so small that not occur cells damage [5]. In slow freezing larger crystals are formed, causing the breakdown of cell membranes due the crystals size formed in the intercellular space. Other causes of membranes rupture are the cellular injury caused by the increase of osmotic pressure and irreversible precipitation or denaturing of cell colloidal constituents. The ice crystals location from the tissue and cell suspension is in function of freezing rate, temperature and nature of the cells. The slow freezing rate from plant tissues, animals or cellular suspensions (microorganisms, red blood cells) often cause

crystals formation, mainly in the extracellular location [6]. Conditions that lead preferential crystals formation in the extracellular result in larger ice crystals associate with maximum water deslocation and cells shrinking in a frozen state. All tissues types, animals, plants or microorganisms cells, display a uniform crystals ice distribution when frozen quickly under very low temperatures [7].

The thawed food should not be frozen again, must be consumed quickly to prevent the proliferation and acceleration of microorganisms growth that provide deterioration and consequently, nutrients loss [8]. The "Comet Assay" test is originally used to identify irradiated foods, identifies cell damage, perhaps is possible to verify an abuse of temperature and identify in a cellular level which freezing speed is better for food preservation [9, 10].

The ionizing radiation is a process of food conservation through the microorganisms elimination which does not occur when freeze the food. Radiation avoids or delay process of infestation, depreciation or food contamination. Food irradiation can contribute to a safer and more plentiful food supply by inactivating pathogens, eradicating pests and by extending shelf-life, but causes damage in the DNA of the cells [11]. To identify irradiated food many methods has been studied and discussed in the literature [12, 13, 14, 15, 16, 17].

2. Experimental

2.1. Samples

Liver chicken: **1**°- *in natura*; **2**°- frozen at -10°C for 3 hours; **3**°- frozen at -196°C for 5 seconds and maintained at -10°C for 3 hours.
The samples were obtained in local market in São Paulo, Brazil. The samples were stored in a insulated jars (+/- 4ºC) during transportation.

2.2. Irradiation

The samples of each dose were irradiated in IPEN-CNEN/SP, at dose levels of 1.5; 3.0 and 4.5 kGy using a ^{60}Co (Gammacell 220, A.E.C.L., dose rate: 2.64 kGy/h) Harwell Amber 3042 Dosimeters were used for the measurement of radiation dose.

2.3. Methodology

All the samples were analyzed using the Comet Assay described by Cerda *et al.* 1997[18] and modifications as proposed by Duarte *et al.* 2009 [19], 5 g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. Cell suspension (100 µl) was mixed with 600 µl of low-melting agarose (0.8% in PBS). 100 µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lyses buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 min. The slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. SyBr Gold staining (15µL SG + 150mL PBS) at 4°C. The observation was with the samples still humid, the DNA fragment migration patterns of 100 cells for each dose level were evaluated with a fluorescence microscope (With excitation filter 420-490nm [issue 510nm]). The comets tails lengths were measured from the middle of

the nucleus to the end of the tail with 40x increase for the count and measure the size of the comet. The samples with 5.0 g were packed in plastic bags and labeled.

3. Results and Discussion

The "Comet Assay" Test is sensitive cell's DNA disruption, originally used to identify foods submitted to irradiation process. The "comet" size was related to the food degradation, so is possible and commonly used a scale of degradation depending on the size of the comet, varying the type 10 (minor or no degradation) to type 50 (greater degradation) (Fig. 1). Similar results were obtained by other authors [11, 9, 19].

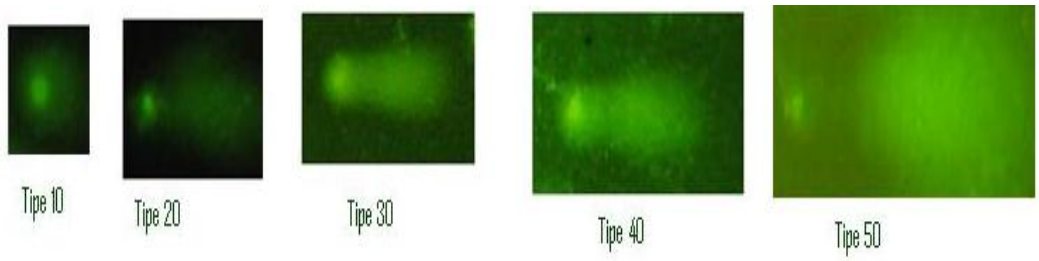

Figure 1. Typical scale of comet size and type

Using the scale of Figure 1 as reference, an estimate of cell degradation can be made according used freezing method. Differences could be checked in slow freezing and fast freezing with or without the irradiation interference in freezing process. In non-irradiated samples can be compared the effect of freezing at -10ºC (slow freezing) and liquid nitrogen -196ºC (fast freezing) (Fig. 2).

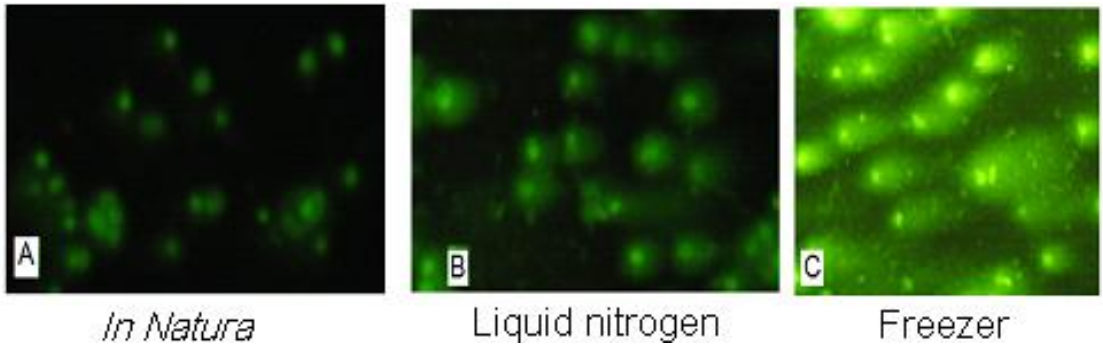

Figure 2. Liver chicken cells *in natura* not irradiated, freeze with liquid nitrogen and freeze in a usual freezer.

In unirradiated samples submitted to liquid nitrogen method, preserve 90% of intact cells very closer like *in natura* samples. However the freezer let only 10% of intact cells (Fig. 3). The viability of liquid nitrogen process justify for maintain 80% more comets type1 than the freezer process. Similar results were obtained by other authors [20, 21].

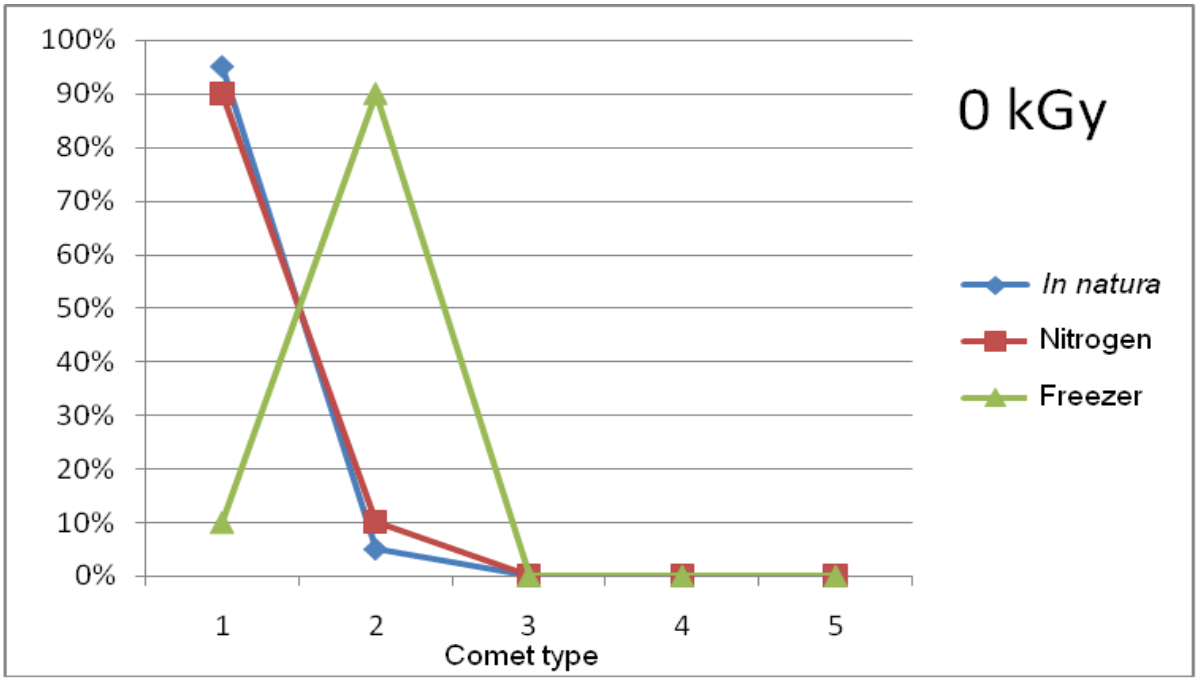

Figure 3. Percent and types of comets in not irradiated samples

With the increase of radiation dose the percent of comets type approach each other. But in all cases the nitrogen was the better method choise figure 4 and figure 5. Has be clear that both methods are very good to maintain the original characteristics, the irradiation treatment does not cause more severous damage that cold process cause to the liver chicken samples (Fig. 6 and Fig. 7).

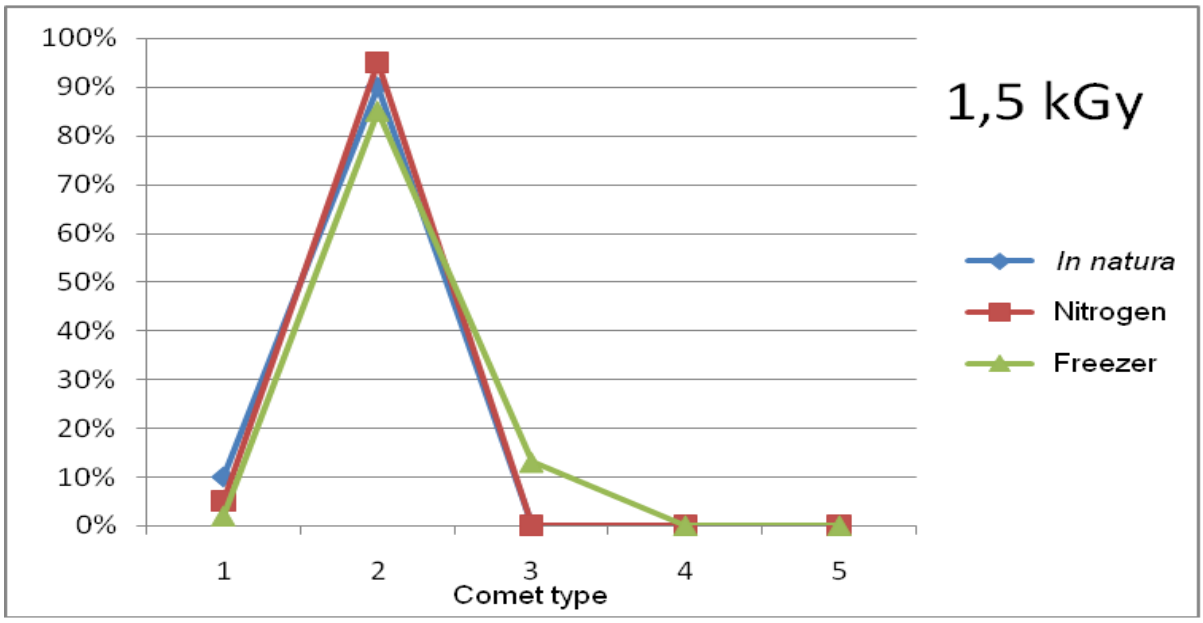

Figure 4. Percent and types of comets irradiated with 1.5kGy.

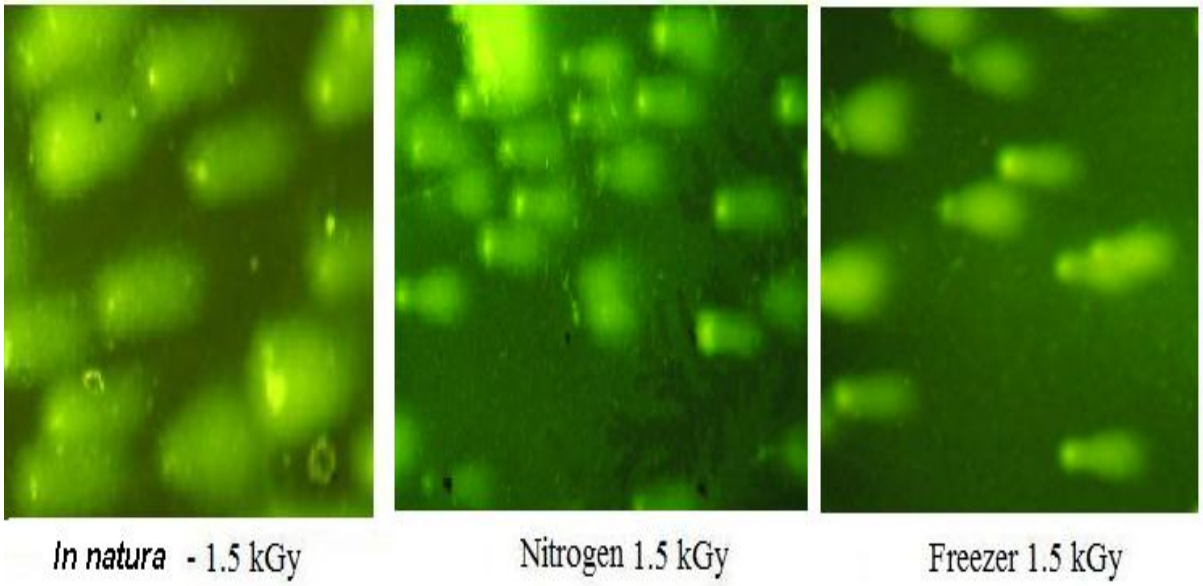

Figure 5. Chicken cells *in natura*, liquid nitrogen and usual freezer. All the sample irradiated with the dose 1.5 kGy.

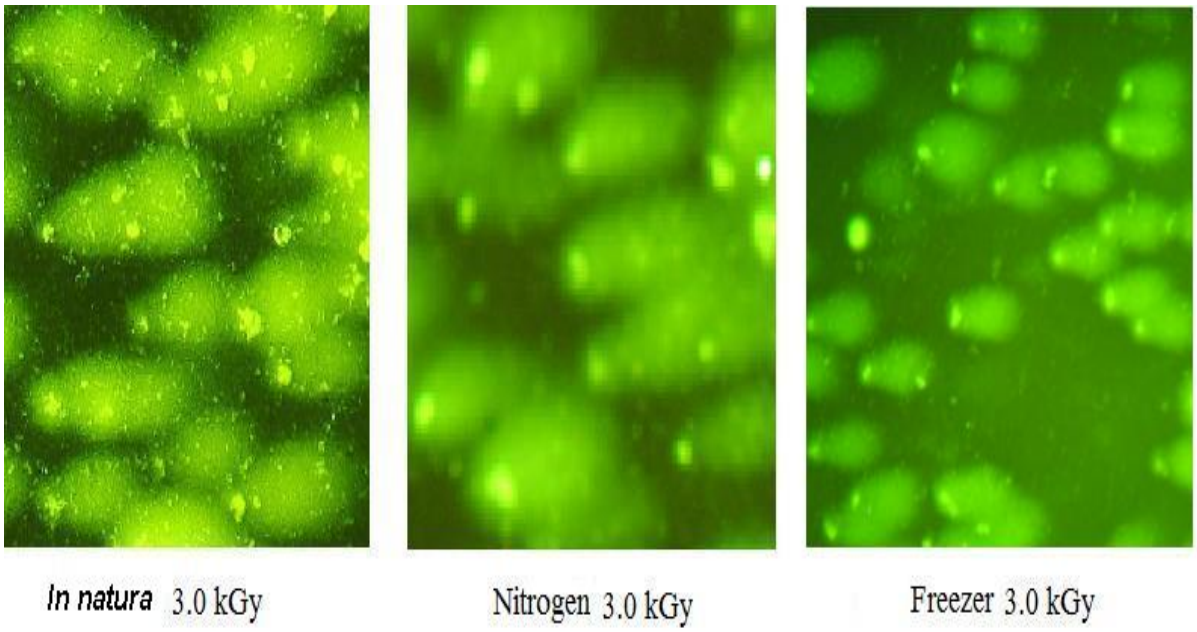

Figure 6. Chicken cells *in natura*, freeze with liquid nitrogen and freeze in a usual freezer. The entire sample irradiated with the dose 3.0 kGy.

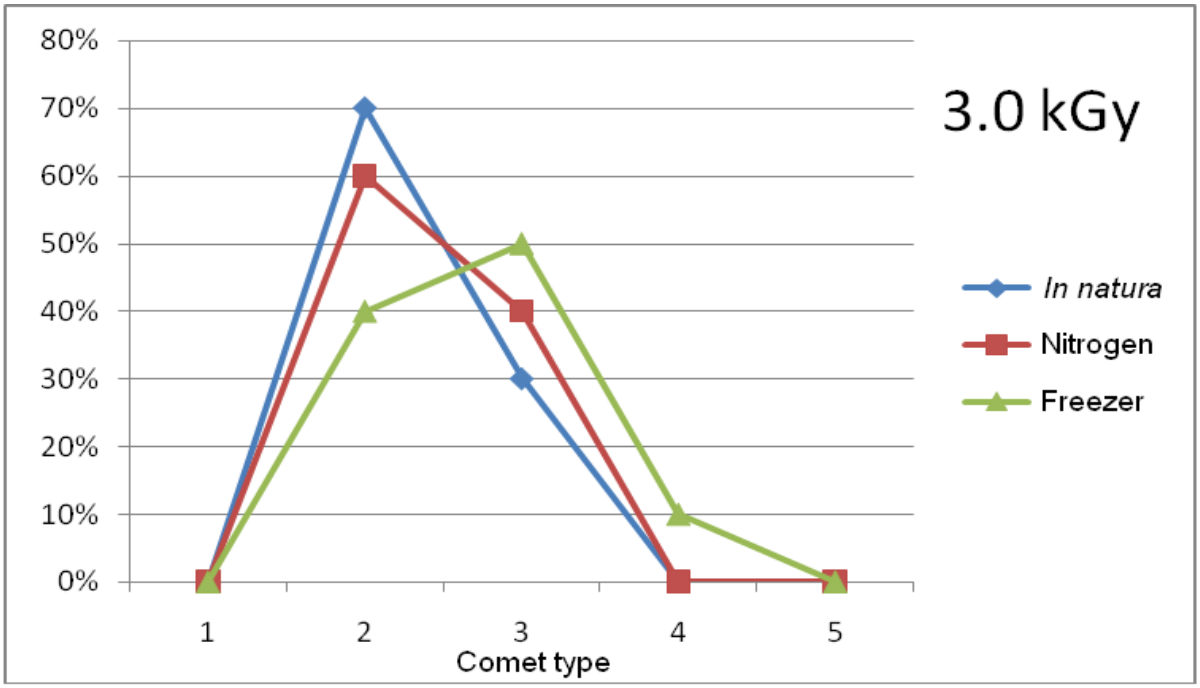

Figure 7. Percent and types of comets irradiated with 3.0kGy.

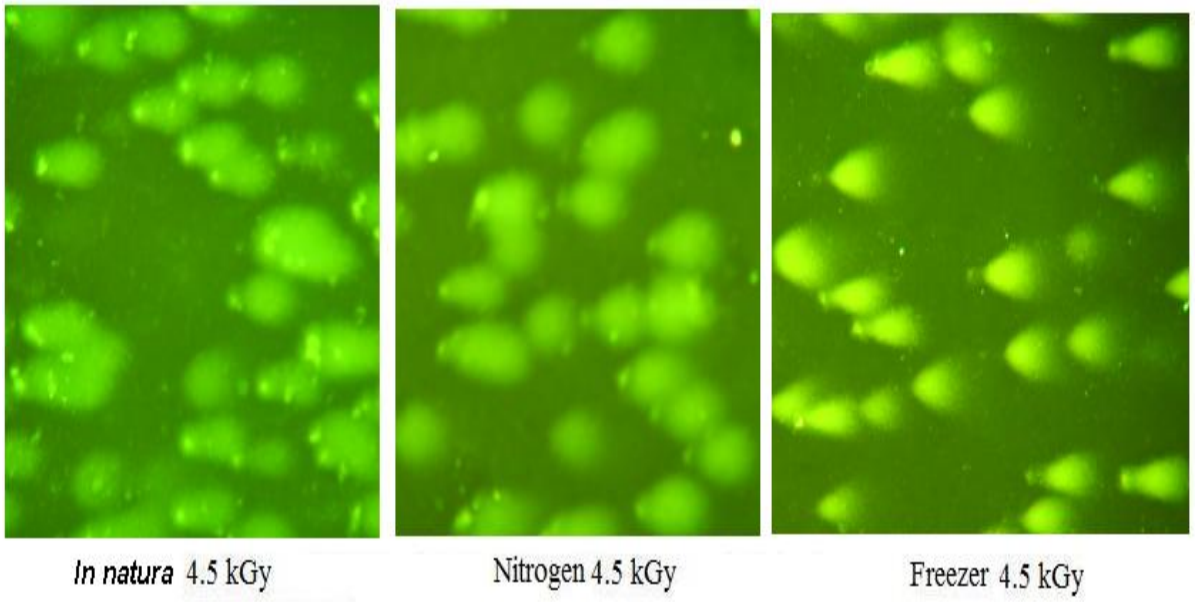

Figure 8. Chicken cells *in natura*, freeze with liquid nitrogen and freeze in a usual freezer. All the sample irradiated with the dose 4.5 kGy.

The higher degradation was recorded in the irradiated sample with 4.5 kGy and frozen in freezer (Fig. 8 - Fig. 9). This work enforces the theory that irradiation, freezing, thawing and refreezing were responsible factors for cellular degradation.

Our results are in accordance with similar studies [14, 15], who got similar results with irradiated frozen hamburger. Several authors [14, 15, 17, 18] using DNA Comet Assay to detect irradiated foods showed that DNA damage was a function of irradiation treatment and time-temperature abuse [10]. Using this technique, an effective screening of DNA fragmentation induced by radiation is obtained.

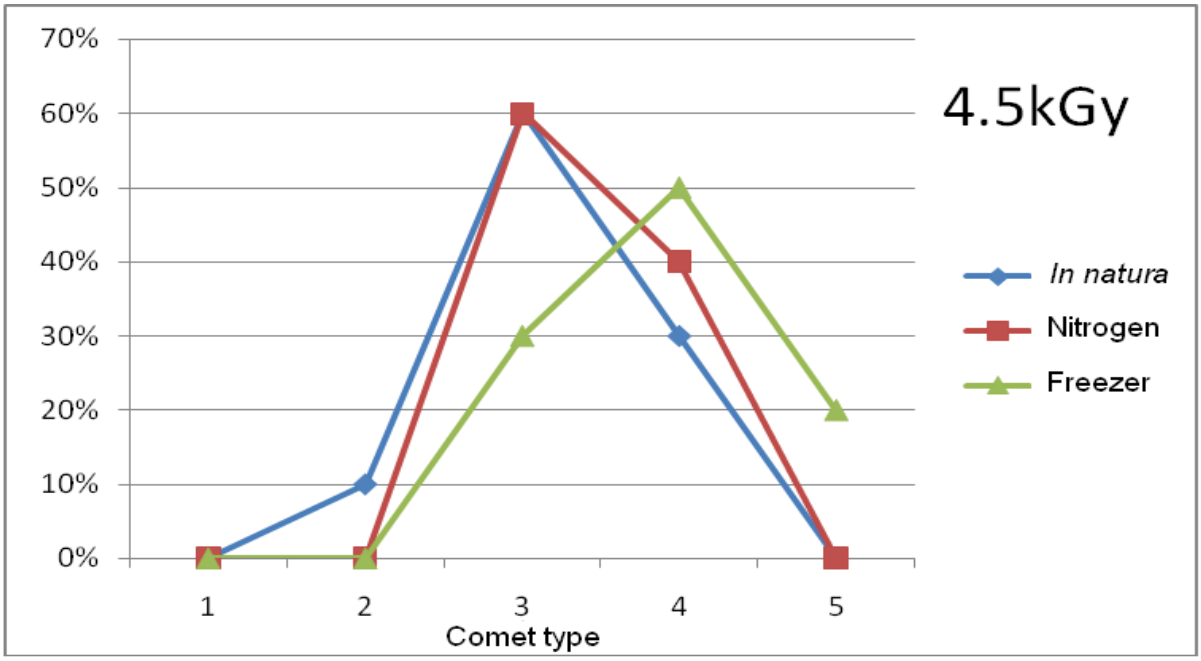

Figure 9. Percent and types of comets irradiated with 4.5kGy.

4. Conclusion

Freezing process is actually a widely practice used for food storage in various segments of industries and consumers. Irradiation process has an important role in increasing food shelf life. One of the most important points is that freezing process, unlike irradiation, does not destroy microorganisms, but only inactivated them as long as they remain in a frozen state. It was possible to verify that the best method of freezing is the use of liquid nitrogen at -196°C to replace the normal method of freezing done in freezer at -10°C. The storage time was 3 hours in freezer, possibility to viewing the beginning of degradation process, without physical-chemical changing linked to irradiation. All samples where the food was frozen in normal freezer (slow freezing), is the sample what had the higher degradation in any irradiation dose.

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