THE IMPACT OF PHYSICAL-CHEMICAL PARAMETERS OVER THE FORMATION OF PAPAIN NANOPARTICLES CROSSLINKED BY RADIATION

Gabriela N. Fazolin[1*], Gustavo H. C. Varca[1], Ademar B. Lugão[1]

1- The Institute of Energy and Nuclear Research (IPEN-CNEN/SP)
*gabrielafazolin@usp.br

Key Words: *papain nanoparticle, gamma radiation, crosslinked protein, physical-chemical parameters.*

With the advancement of research and use of nanotechnology in biomedical areas and application in different treatments the need for stable systems with low toxicity and null adverse effects becomes important. Within this context papain appears as an alternative biomolecule which is a proteolytic enzyme extract by the fruit of *Carica papaya* Linnaeus. Its already successfully applied in the pharmaceutical and biomedical area as a drug carrier and debriding agent for wounds. Recently, papain was developed in nanoscale using 50 Mm phosphate buffer (pH 7,2 ± 0,2), ethanol (20%, v/v) as cosolvent and ^{60}Co as radiation source for gamma radiation (10 kGy) to promote protein crosslinking. This technique promotes papain nanoparticle sterilization and crosslinking in a single step showing that radiation is a promising technique for the development of nanoparticles for biomedical use. However, important parameters such as the influence of physical-chemical parameters over nanoparticle formation remains unexplored and unclear. Therefore, this work aimed to evaluate the influence of pH and temperature over papain nanoparticle formation including buffer molarity and different temperatures throughout the synthesis and irradiation process. Nanopapain was synthetized as above-mentioned however phosphate buffer was used in a range of 1 to 50 mM and ultrapure water in presence of ethanol and irradiated at 10 kGy. The influence of temperature was evaluated by performing the synthesis at -20, 0 and 20 °C. Additionally, it was verified the sterility of the material and its stability over time. The hydrodynamic radius and crosslinking were evaluated by dynamic light scattering and fluorescence measurements respectively. The pH range was influenced by buffer molarity, thus, lower molarities led to a final pH of 5 and the higher molarities around 7. Bityrosine formation was influenced by the pH range and increased when synthesis was performed near optimal pH (7 for papain), although particle size didn't undergo any changes as a result of the pH changes. On the other hand, enzymatic activity was less affected by the process maintain about 80% of the native enzymatic activity only when synthesis was carried out in 50 mM phosphate buffer. With regard to temperature as the temperature increased the levels of crosslinking also increased in the order of 20>0ºC>-20ºC. In conclusion nanoparticle size was not influenced by the temperature shift. Concerning enzymatic activity samples irradiated under ice-cold conditions presented decreased bioactivity loss if compared to the samples irradiated at room temperature. After an evaluation of the results, the study revealed that samples synthetized with buffer molarity of 50 Mm (pH 7,2 ± 0,2) and 0ºC would promote higher crosslinking levels and less enzymatic activity decrease.

Processo FAPESP nº 2017/20177-3