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Extractable proteins from irradiated field natural rubber latex

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Abstract

In this study field natural rubber latex was irradiated with different doses near a ^{60}Co gamma source to reduce the water-soluble protein content in the final product. The protein content of the films obtained by casting method was extracted with phosphate buffer solution, pH 7 and was measured using Micro BCA Protein Assay kit. Also was measured protein in the serum samples of field NRL. The concentration of extractable proteins increased with increasing radiation dose.

Keywords: Extractable NRL proteins; Natural rubber latex proteins; Irradiated field NRL

1. Introduction

Natural rubber latex (NRL) is extracted from *Hevea brasiliensis* tree and is used to produce different kinds of rubber goods like gloves, condoms, balloons and some part of medical and dental equipments, but it is predominantly used in the production of surgical and examination gloves.

The allergy caused by latex products has become a serious problem, the sweat can remove proteins and allow contact with skin causing sensitization or allergic reactions.

The mechanisms of sensitization are further complicated by the fact that many common fruits, especially banana, avocado, contain structures which cross-react with NRL proteins. Therefore, the sequence of events in the sensitization processes cannot be established with certainty (Sunderasan et al., 1994; Yagami et al., 1995; Haque, 2001).

The American Society of Testing and Materials (ASTM) have formed a task group to study test method for measuring extractable protein (EP) in latex medical devices. There is an urgent need to manufacture gloves of low EP content to meet the requirements of Food and Drug Administration (FDA) of United States of America introduced in 1995. Some methods have been suggested to reduce the EP content in latex gloves, as using low protein latex; proper leaching during the process of production; chlorination of latex products (Lai Pin Fah, 2002).

Some groups have observed denaturation and immunochemical modifications in irradiated proteins with immunoreactivity changes by destruction of protein domains responsible for the antibody recognition as observed by Kume and Matsuda (1995) and Nascimento (1998).

The objective of this study was to extract and determine the water-soluble proteins from radiation vulcanized field NRL.

2. Experimental

It was utilized field latex (FL) emulsion conserved in ammonia with 27.9% of total solid contents, from Vietnam.

Samples of FL were irradiated in a ^{60}Co source at Takasaki Radiation Chemistry Research Establishment (TRCRE), Japan Atomic Energy Research Institute (JAERI), in different doses: 10, 20, 30, 40 and 50 kGy. After that each sample was separated into two fractions.

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The first one was centrifuged at 15 000 rpm/2 h; the separated serum was centrifuged again at 15 000 rpm/1 h to clarify the FL serum. With the second fraction of irradiated emulsion was prepared film by casting method.

The extraction of proteins in those films was made before and after leaching them with 1% NH_4OH solution for 20 min. Leaching is the removal process of hydrophilic materials from latex products by washing in water. The removal of excess of chemical compounds and water-soluble of non-rubber such as protein and added compounding ingredients results in improvement of physical properties such as tensile strength and film clarity. The effectiveness of the leaching process is critical in the determination of the overall quality of gloves produced.

Also the leaching process was carried out with 1% NH_4OH and PBS during 24 h at room temperature to investigate if could leach as much as possible amount of EP. After this process the films were submitted to extraction in PBS and EP was measured.

The extraction of EPs of each film was made using 1 g of small pieces (0.5×0.5 cm) immersed in 10 ml phosphate buffer solution (PBS) pH 7 in a shaking water bath at 37°C for 2 h.

Protein determination was carried out in serum and in the extracts of films before and after leaching process using Micro BCA (bicinchoninic acid) Protein Assay Reagent Kit from Pearce Co.

The protein standard calibration curve was obtained by using bovine serum albumin (BSA) in a concentration of 2 mg/ml. The reaction is linear from 0.5 to 20 μg/ml.

The absorbance were read at 562 nm using a UV–visible spectrophotometer Shimadzu-800, Japan.

3. Results and discussion

The concentration of extractable water-soluble proteins in serum of irradiated field NRL increased with increasing irradiation doses, as shown in Fig. 1.

Fig. 2 shows the concentration of extractable water-soluble proteins from irradiated FL films before and after leaching process. The behavior is the same as of serum, increases with increasing irradiation dose. In this figure it is possible to see that the level of EP became lower after leaching process. These results agree with those presented by Makuuchi et al. (2000).

Some proteins still remain in the latex final products and are extractable in water solution and can provoke sensitization and allergic problem.

Fig. 3 presents the percentage of protein loss during the latex film leaching process. Loss of protein decreased with increasing radiation dose.

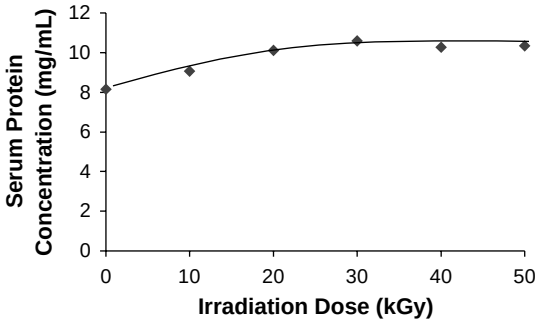

Fig. 1. Irradiated field NRL serum proteins. Dose: 0, 10, 20, 30, 40 and 50 kGy.

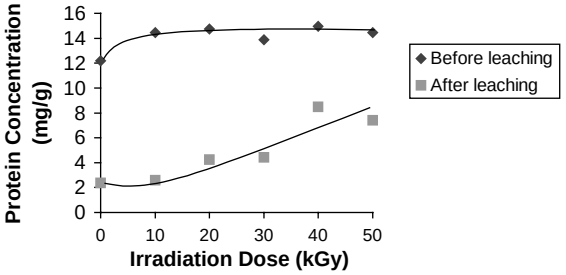

Fig. 2. Extractable proteins from irradiated field NRL films before and after leaching. Dose: 0, 10, 20, 30, 40 and 50 kGy.

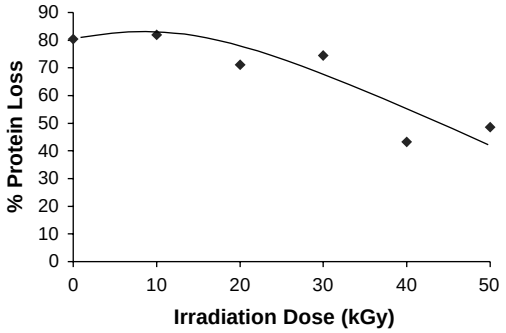

Fig. 3. Percentage loss of EP in the leaching process.

The leaching process seemed to be not good enough but could extract about 80% of proteins of no irradiated FL film and irradiated with 10 kGy. In the film of sample irradiated with 50 kGy the loss of EP was about 50%.

The amount of EP in the films submitted to 24 h leaching presented the same magnitude, 0.16 and 0.11 mg/g when leached with ammonia and PBS, respectively. So, after 24 h of leaching process the amount of EP decreased from 15 to 0.1 mg/g, proving that the leaching time has to be 24 h as already showed by Haque (2001).

4. Conclusion

- Irradiation of FL increases EPs in the serum and in the films. The concentration of EP increases with increasing radiation dose.
- After leaching process still have proteins, which are extractable with phosphate buffer solution.
- Leaching of 24 h reduced the EP from film to about 100 times.
- In the leaching process the % loss of EPs of the FL films decrease with increasing dose of irradiation.

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