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Country Report: Thailand

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1. Current status on "Radiation Treatment of Liquid Samples" in Thailand

1.1 Laboratory scale

1.1.1 Radiation processing of silk protein
1.1.2 Radiation degradation of chitin/chitosan
1.1.3 CMCS/KC hydrogel laminated PVP/KC hydrogel
1.1.4 Gamma irradiation of anionic natural polymer solution for use as latex protein scavenger
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1.1.9 Study on latex allergenic protein removal by radiation treatment of fresh field latex

1.2 Market trial scale

1.2.1 Production of "CU-LPS" for low protein HA latex industry (Far Sight Group of Company Ltd.)
1.2.2 Production of "Osan" for plant growth promoter (CU Natural Products Co.Ltd.)
1.2.3 Production of "Oligocide" for fungicide (CU Natural Products Co.Ltd.)

1.3 Commercial scale

1.3.1 Production of "Thiox" protective gloves from RVNRL (Siam Okamoto Co.Ltd.)

2. Current status on application of electron accelerators for liquid

2.1 Laboratory scale (conducted at Takasaki JAERI)
 2.1.1 Radiation processing of silk protein
 2.1.2 Depolymerization of CMC, HEC, HPC

2.2 Market trial scale: none

2.3 Commercial scale: none

3. Potential application of electron accelerators for liquid

3.1 Degradation of chitosan for use as plant growth promoter and fungicide
3.2 Depolymerization of LM pectin, CMC, HEC, HPC for HA-Latex Industry

Radiation Processing of Silk Protein
Bilateral Research Cooperation
OAEP and JAERI
December 1998 – December 2002

Thailand and Gunma Prefecture, Japan are famous for the sericultural areas. Thailand's production of silk is about 1,200 ton per year, Gunma Prefecture is also a prominent area for production of silk in Japan. Machine and hand reeling process of silk give about 10% of silk waste. The cooperated research program "Radiation Processing of Silk Proteins" was conducted to recycle the resources into new materials (non-textile application) and to avoid environmental pollution.

Silk fiber from silk worm (Bombyx mori) consists of 2 bundles of fibril, this part is 70 – 80 % fibrous protein or fibroin and the rest 20 – 30 % is amorphous matrix called sericin which function as a gum to bond the 2 fibroin filaments together.

Topics of interest:

- Radiation degradation of silk protein
- Production of fine silk powder by radiation (dry method)
- Antioxidant effect of silk protein on lipid peroxidation
- Antibacterial activity of irradiated silk protein powder
- Wound dressing hydrogel with silk protein
- Antibacterial activity of cross-linked silk protein/PVA hydrogel

1. Radiation degradation of silk protein

Silk fibroin in solid form and solution form (1% in $CaCl_2$ / EtOH / H_2O after dialysis) were gamma irradiated from 0-160 kGy. Solubility in the mentioned solvent was observed for irradiated solid fibroin. The irradiated samples at 160 kGy in oxygen and air could completely dissolve in the solvent in 5.3 and 6.5 hours respectively, whereas the unirradiated sample did not dissolve in > 100 hours at 40° C Specific viscosity of fibroin solution was observed to decrease rapidly with the dose. It was also cofirmed that silk fibroin undergo radiation degradation by SDS PAGE and size exclusion chromatography.

2. Production of fine particle silk powder by radiation (dry method)

Conventional method for production of fine particle silk powder for cosmetic materials is by dissolving silk fibroin in $CaCl_2$ or LiBr solution, then dialysis and dry.

Production of fine silk powder could be done by radiation. Irradiation of silk fibroin by electron accelerator (1MeV, 1 mA) from 250 –1000 kGy revealed that the irradiated silk fibroin could be milled to fine powder (<90 microns) whereas unirradiated sample could not yield such fine powder. The dose required to obtain about 90% yield was 750 kGy in oxygen. Irradiation in nitrogen gave somewhat lower yield at the same dose. This process need no solvent and it is called "dry process". Noted that at the dose of 750 kGy, the dissolution of irradiated fibroin in water at room temperature, 1 hour was about 60%.

3. Antioxidant effect of silk protein on lipid peroxidation

Since silk protein is biocompatability, in viro study on inhibitory effect of silk protein on lipid peroxidation was studied. Lipid peroxides are the products of chemical damage occurred by oxygen free radicals to the lipid component of cells. This oxidative damage is believed to cause cancer, aging, coronary artery disease, toxicity induced by thermal, chemical and radiation. Malondialdehyde (MDA) is one of the compounds formed from lipid oxidation. Analysis of MDA can be done with high sensitivity and simple by thiobarbituric acid (TBA) test. In this experiment bovine adult serum was used as a lipid source.

Freeze dried silk silicin was Irradiated by 2 MeV, 1 mA EB of for 1000 – 1500 kGy and solid silk fibroin for 1000 – 2000 kGy at room temperature. After dissolving in water, soluble parts were separated.

Lipid peroxidation in bovine adult serum was induced by gamma irradiation at 10 kGy in the presence or absence of silk protein or bovine serum albumin (BSA) at various concentrations. MDA was determined immediately after induced by irradiation by TBA test. It was found that irradiated silk siricin could depress the concentration of MDA formation greater than that of BSA but in less extend than unirradiated silicin while irradiated fibroin had no effect on the suppression.

4. Antibacterial activity of irradiated silk protein powder

Irradiated silk fibroin (500 kGy) was converted to silk fibroin .HCl by soaking in 1N HCl for 72 hour, after washing and drying it was pulverized and brought into solution of 5 – 15 %. Aseptic filtration was done for the solution and 400 μl was mixed with 100 μl containing E.coli, B.subtilis and S. aureus K. and nutrient broth. After incubation at 37° C for 78 hour, O.D. at 650 nm was measured by a spectrophotometer. The amount of silk fibroin powder needed to inhibit growth of bacteria of interest was calculated from the solubility of silk fibroin. The result was expressed in minimum inhibitory concentration (MIC).It was found that 5% of silk fibroin powder was enough to inhibit growth of E.coli and S. aureus but no effect on B.subtilis. The MIC was calculated from the solubility of 500 kGy irradiated silk fibroin powder to be 2.4 mg/litre.

5. Wound dressing hydrogel with silk protein

Silk fibroin from $CaCl_2$ / EtOH / H_2O dissolution after dialysis with concentration of 3 % was mixed with various concentrations of PVA solution. Dissolved air in the solutions was expelled by N_2 stream and casted films were gamma irradiated to 10 – 120 kGy. Gel fraction, swelling, gel strength of film samples were evaluated in comparison with PVA and silk fibroin hydrogel alone. The appropriate formula of hydrogel which showed high water absorption and gel srength was 1% silk / 3% PVA at ratio of 1:3 and irradiation at dose of 30 kGy.

6. **Antibacterial activity of cross-linked silk protein/PVA hydrogel**

Agar disk diffusion method was conducted for 3 % fibroin solution and 3 % PVA solution (1:1) hydrogel film at doses of 20 - 50 kGy on E.coli, Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis. Width of clear zone of growth inhibition for these bacteria was found to increase with the irradiation doses. The hydrogel prepared from solution blend of 3% silk protein, 3% PVA and irradiated at 50 kGy showed the highest antibacterial activity on E.coli, Bacillus subtilis and S.aureus.

Conclusion

The results obtained from the activities conducted provide a basic knowledge not only to develop the technique of radiation prcessing for utilization of silk protein as biopolymer and biomaterial but also to recycle of natural resource. JAERI and OAEP will cooperate with private companies for goal of commercialization.

Gamma Irradiation of Anionic Natural Polymer Solution for Use as Latex Protein Scavenger

The need for low water extractable protein (EP) medical rubber gloves in the US and EU market has a strong impact to glove manufacturing industry since last 5 years. While US. FDA mandate the amount of EP in medical glove to be less than 50 ppm, it is expected that this value may be lower when synthetic polymer gloves are more available in the world market. This may be considered as a non–tariff barrier and not actual Allergy Type I (Immediate Type Allergy) which is life threatening and cause health care workers to have a risk from anaphylactic shock because the number of people died from the incident was only 15 so far. It is, however very important to tackle this problem seriously especially for a rubber gloves export country like Thailand.

Objective

To develop a simple yet efficient and low cost process to produce low allergenic protein HA Latex from fresh field latex (FFL).

Process

Natural radiation degradable polymers which carry negative charge above pH 7 are put to the test for latex protein removal after radiation degradation. The aim of degradation is to reduce their molecular weight so that they become more soluble in water while they form complex with positively charge latex protein. As all of the water soluble protein in latex serum and at rubber particle surface are well associated so in the centrifugation process of FFL to HA latex almost all of the EP will be removed to skim latex.

Negatively charged natural radiation degradable polymers

There are a number of negatively charged natural radiation degradable polymers but only few of them meet the requirement of resisting relatively high temperature in the centrifugation machine and most importantly is economical available. Examples are low methylated (LM) pectin, hydroxy ethyl cellulose (HEC), hydroxy propyl cellulose (HPC) etc.

Irradiation of the polymer solution

Irradiation of the polymer solution (1.5-2.5 %) was done batchwise using 20 L stainless steel drum on turn table . Required dose was 3-5 kGy depend on the initial M.W. of the polymer used. The final M_v of approximately 15-20 kDa was suitable to use as latex protein scavenger.

Results

Addition of 0.25 – 1.2 part per hundred rubber (phr) of the irradiated polymer into FFL prior to centrifugation could produce HA latex which comply with ISO 2004 standard. Mechanical stability time (MST) of the concentrated latex increased gradually not different from that of the control latex and reached 650 second in approximately 3 weeks. The EP content in the obtained HA latex was 20-50 ppm.

Gamma Radiation Degradation of Chitosan For Use as Plant Growth Promoter and Fungicide

Radiation degradation of chitosan is well known to be effective and easy to control process. Two steps process has been adopted for chitosan depolymerization for market trial production scale inThailand. The first step is solid powder irradiation at dose of 100 kGy to reduce M.W. to about 2-2.5x10^5, the second step is 10% solution in 2.5% HOAc irradiation to further reduce M.W. down to oligochitosan (repeating unit, n is 7-14). Collection of water soluble chitosan (monomer to hexamer) can also be done by dissolving the irradiated solid powder in water and succesively precipitate the chitosan by MeOH. The yield is rather small about 0.65% but it is a kind of by product from the process.

Low M.W. chitosan has a wide application as fungicide. It has been put to good use for salak (Salacca wallichiana) fruit coating to eliminate some fungi on the skin as well as to preserve its texture by reduce respiration and reduce water evaporation.

Oligochitosan solution has been widely used as plant growth promoter in variety of decorative plants, flowering plants, vegetable, tissue culture protocorm like body, hydroponics, grape and others. Oligochitosan has been mixed with shrimp feed to bond the granule, to reduce bacteria attack and to enhance shrimp growth.

Water soluble chitosan may find applications via its anti-microbial activity such as addition into hydrogel wound dresser etc. Its activity of anti-tumor may be a great potential to develop the production process of this functional material to a larger scale.