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Contrastive Study on Properties of Acid and Microorganisms Coagulated Natural Rubber during Accelerated Storage

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The properties of natural rubber latex coagulated by acid and microorganisms during accelerated storage were contrasted, respectively. The molecular weight, gel content, Mooney viscosity, Wallace initial plasticity and the plasticity retention index of natural rubber latex during the accelerated storage period were analysed. The results indicate that prolonged storage time leads to a gradual increase in the molecular weight, gel content, crosslink density and Wallace initial plasticity, whereas plasticity retention index reduces. Wallace initial plasticity exhibits significant correlation, while plasticity retention index indicates a negative correlation with both molecular weight and gel content, respectively. The molecular structure and properties of natural rubber latex coagulated by microorganisms are significantly different from those of natural rubber coagulated by acid. The molecular weight, gel content and Wallace initial plasticity of natural rubber latex coagulated by microorganisms are higher than those of natural rubber latex coagulated by acid, while plasticity retention index and protein content of natural rubber latex coagulated by microorganisms is lower than that of natural rubber latex coagulated by acid.

Keywords: Natural rubber; microorganisms coagulated; acid coagulated; properties

Natural rubber (NR) is a renewable resource obtained from latex of the *Hevea brasiliensis* tree. It is a natural material of primary importance in the tyre industry and many other applications including general rubber goods owing to its characteristics of flexibility and fatigue resistance[1]. In 2015, 12 million tonnes of NR is reported to have been consumed[25]. As an important biosynthesised polymer, NR exhibits excellent chemical and physical properties with a wide range of applications[2-4]. To date, no substitutive material matches its excellent combination of properties that compliment production of automobile and aircraft tyres[5-7].

Natural rubber latex (NRL) is a complex colloidal suspension containing 30 – 45% vol of rubber particles and 10 – 20% vol of lutoids[8]. NR comprises long-chain branched cis-1,4-polyisoprene as well as biomolecules such as proteins, lipids and carbohydrates[9]. Besides polyisoprene, NR is rich in various nonisoprene components such as carbohydrates, proteins and lipids, constituting a favorable medium for the development of microorganisms[10]. It is well known that coagulation of NR latex suspensions into an elastic gel marks the first step towards producing dried NR products, while acid coagulation is among the most

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commonly used methods. However, large amounts of effluents with high chemical oxygen demand and ammonia will be produced by coagulating NR latex with formic and acetic acids, which poses a severe threat to the environment. Based on natural coagulation of fresh latex in plantations, a biologically assisted coagulation method was developed by adding sugar or even microorganisms into fresh latex to reduce environmental pollution. It was also found that the quality of rubber obtained from biologically assisted coagulation is significantly improved and suitable for the manufacture of high-performance tyres.

NR is a linear polymer with excellent resilience. However, the Mooney viscosity and Wallace initial plasticity (P_0) of NR increases gradually during storage due to intermolecular crosslinks. Moreover, there has been much attention on this issue over past decades within the rubber industry. Intermolecular crosslinks are believed to develop from the carbonyl or epoxy groups in NR molecules[11-14]. The variations of molecules and mechanical properties of acid-coagulated NR（NR-a） during accelerated storage are reported to result in gradual increase of the molecular weight, gel content and size of NR-a, subsequently affecting its mechanical properties[15-16]. It is well known that there are more than 5% of non-rubber substances such as proteins, lipids, organic acids and carbohydrates associated with NR particles and it has been proven that these non-rubber substances have significant effects on the molecular structure, physical properties and vulcanisation characteristics of NR. Therefore, it is logical to perceive that the structural variations of NR during storage are related to the existence of these non-rubber substances. There are few reports about the variation of structure and properties of NR coagulated with microorganisms (NR-m) during storage in literature.

Most studies have been focused on the structure, chemical composition and properties of raw NR, with little information on initiating product manufacture. A better understanding of this stage would help control the structure of the gel and improve the quality of NR products. In this work, the variation of molecular weight, gel content, P_0, Wallace plasticity retention index (PRI) and crosslink density of NR-m and NR-a were contrasted during the accelerated storage phase. Further, the relationship between molecular structure and physical properties were also discussed.

EXPERIMENTAL

Preparation of NR Samples

Acid coagulated NR (NR-a). About 300 mL of fresh NR latex with a dry rubber content of 0.33 g/mL from a local NR plantation field was coagulated with 20 mL 5% acetic acid. The coagulum was pressed to about 2 mm thick with a self-made simple device and dried in an oven at 70°C to obtain a sample.

Microorganisms coagulated NR (NR-m). About 300 mL of fresh NR latex with a dry rubber content of 0.33 g/mL from a local NR plantation field was kept at room temperature until it coagulated, then the coagulum was pressed to about 2 mm thick and dried in an oven at 70°C to obtain the sample.

Storage Experiment

NR samples were pressed to about 3.2 ~ 3.6 mm thick, put into sealed containers containing the desiccant, phosphorus pentoxide (P_2O_5) and heated in an oven at 70°C. The heating times were zero, six, 12, 24 and 36 h, respectively.

Characterisations

Plasticity and Plasticity Retention Index. The P_0 and PRI were determined according to *ISO 2007:1991* and *ISO 2930:1995*, respectively.

Molecular Weight and Gel Content. About 0.1g dried NR-m or NR-a samples were dissolved in 100 mL toluene for three days at room temperature in the dark without stirring. The soluble and insoluble fractions in toluene were separated with a sieve of aperture size of 45 µm and collected as sol and gel fractions. The insoluble gel phase was dried at 70°C. The weight of the dried gel was used to evaluate the gel content. The gel content was determined based on the percentage ratio between the gel and original rubber fractions. The sol phase was used to determine the molecular weight.

Intrinsic Viscosity and Molecular Weight. The intrinsic viscosity of the sol phase was determined by an Ubbelohde viscometer at 25°C. The value of intrinsic viscosity was adopted to calculate the molecular weight.

Crosslink Density. Crosslink density of dried gel from NR-m and NR-a samples were assessed using the NMI20-Analyst (Niumag Corporation, Shanghai, China). Magnetic field strength was set at 0.5 Tesla and determining temperature was 95°C, while the corresponding resonant frequency was 21.9 MHz.

FTIR Analysis. Fourier transform infrared spectroscopy (FTIR) was performed using a Perkin-Elmer spectrum (Nicolet 6700, Amercia), one FTIR spectroscopy in attenuated total refraction (ATR) mode.

RESULTS AND DISCUSSION

Effect of Storage on the Molecular Weight and Gel Content of NR

NR can be characterised by the molar mass distribution, the molecular weight of its polyisoprene chains and its gel content. Molecular weight is a parameter used to evaluate the average length of polyisoprene chains[8]. Gel content corresponds to the part of NR which is not soluble in an organic solvent. It can be seen that the molecular weight and gel content increase with storage time (*Figures 2* and *3*).

Upon tapping, the polyisoprene molecular chain in NR latex either breaks away or is crosslinked according to different conditions. A number of researchers have postulated that crosslinking reactions are caused by the abnormal groups and non-rubbers assumed to exist in NR such as epoxide[12-14], aldehyde[17-19], lactone[20], and proteins or amino acids[21]. Among these findings, it is widely accepted that aldehyde groups on the main chain of the NR molecule are responsible for crosslinking *via* the aldol condensation reaction. A study on fractionated NR revealed that aldehyde content increased in line with molecular weight of the rubber[9]. When two separating molecules react *via* aldol condensation, the condensation is termed intermolecular. If the union is between atoms or groups of the same molecule, the reaction is termed intramolecular condensation. Intermolecular condensation leads to gel formation, while intramolecular condensation leads to the increase of molecular weight. A bimolecular aldol condensation between rubber aldehyde groups occurring in the dry rubber phase can be expressed as follows (*Figure 1*)[22]. Alkaline conditions are favored for aldol condensation.

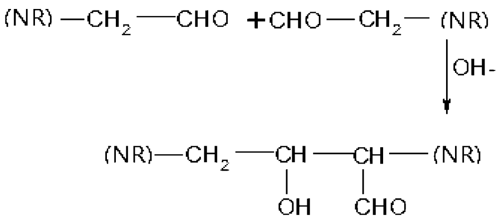

Figure 1. Bimolecular aldol condensation.

The pH varies when the latex is coagulated by different methods. Microbial coagulation results in a pH higher than seven, so the molecular weight and gel content are relatively high. However, the pH of acid coagulation lies generally in the range of 4.4 – 5.0, which is unconducive to rubber molecular crosslinking by aldol condensation. With increase in storage time, the degree of condensation reaction increases, resulting in an increment of molecular weight and gel content. From *Figures 2* and *3*, the difference in molecular weight of NR-a and NR-m is significantly more than that of gel content, which indicates that intramolecular condensation takes place easily during storage.

Effect of Storage Time on the Crosslink Density

Marinho and Tanaka[23] point out that the NR molecule crosslinks during storage was formed by chemical or physical crosslinking. As shown above, the chemical crosslinking reaction is probably the aldol condensation between aldehyde groups on the long-chain fatty acid of phospholipid molecules at the terminal group of rubber molecules. A three-dimensional (3D) network that forms during storage has been widely recognised to have a significant effect on mechanical properties of NR, *i.e.* high tensile and tear strength, good elasticity, and so forth. The network

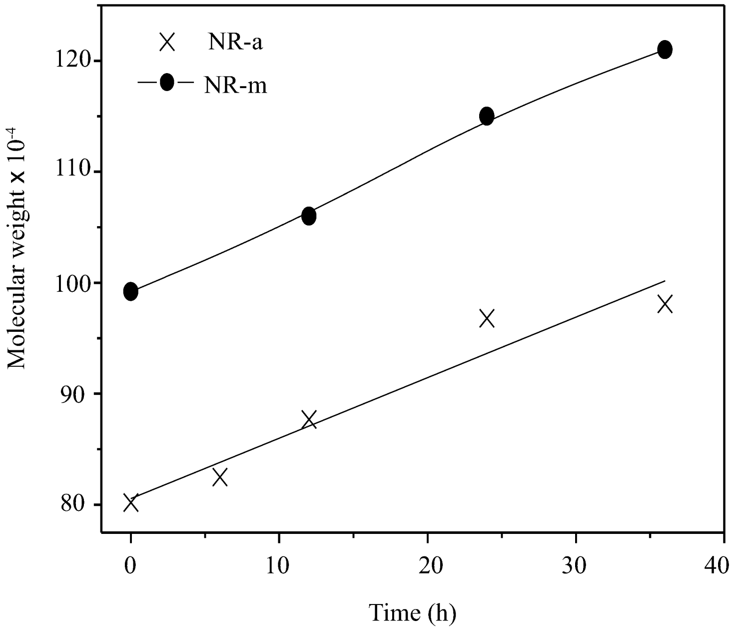

Figure 2. Effect of storage time on the molecular weight of NR-a and NR-m.

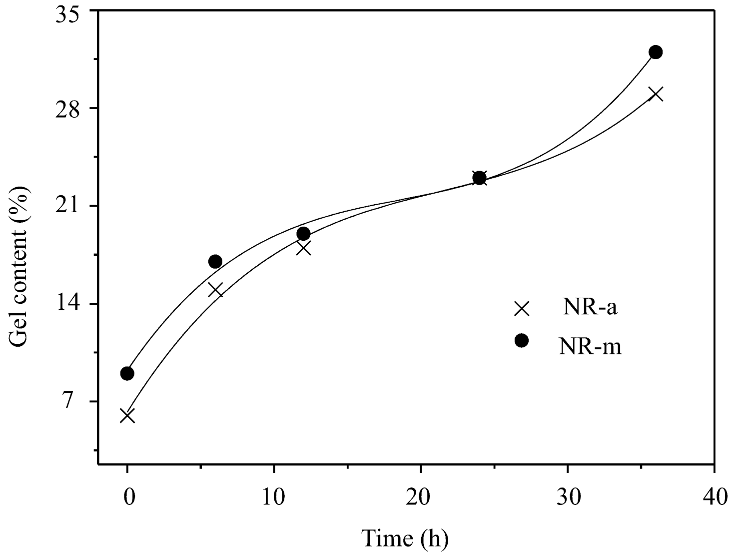

Figure 3. Effect of storage time on the gel content of NR-a and NR-m.

may be formed as the rubber molecules react with each other to yield chemical linkages between the molecules[24]. A fundamental structure of NR was proposed to have two terminal groups, *i.e.* the initiating-end linking to proteins and the terminating-end linking to phospholipids, consisting of phosphoric ester and two molecules of long-chain fatty acid esters. As both functional groups of NR may be active for a reaction, the 3D networks may form during storage as a result of the reactions between the functional groups of NR chains, as shown in *Figure 4*.

Table 1 shows the crosslink density (XLD) of NR-m and NR-a stored for zero to 36 h determined by a magnetic resonance crosslink density spectrometer. After storage for 36 h, the XLD of NR-m and NR-a increased. More intermolecular crosslinks occurred during the storage period, leading to an increment of crosslink density in the gel phase.

Effect of Storage Time on the Properties of NR

The change in the six properties (dirt content, ash content, nitrogen content, volatile matter content, P_0 and PRI) of the standard NR with storage time was monitored. The first four properties reflect the purity of NR, P_0 indicates the plasticity and reflects the mechanical property, and PRI shows the aging property of NR[15]. It can be seen from *Figure 5* that P_0 of NR increases with the storage time. *Figure 5*, illustrates a significant variation in P_0 over the first 12 h of accelerated storage. The increment of P_0 decreases after 12 h and levels off after 25 h of storage. In contrary to P_0, the PRI decreases with increasing storage time (*Figure 6*).

NR latex coagulated microbially, uses minerals and nutrition present in the latex or added accordingly, to promote reproduction

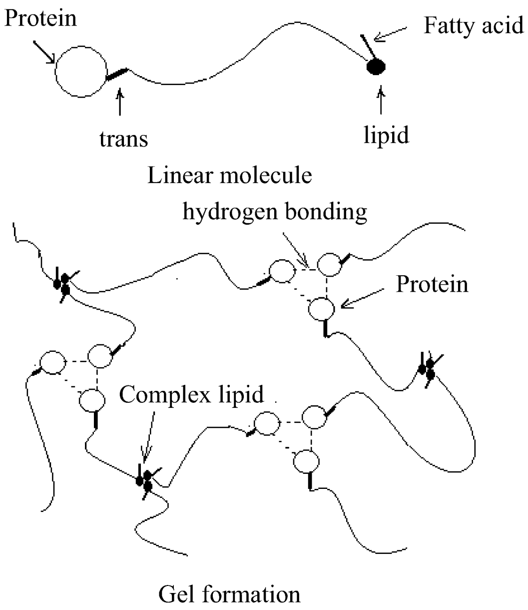

Figure 4. Possible gel structure of NR.

TABLE 1. CROSSLINK DENSITY OF NR-m AND NR-a

Storage time (h)	XLD (10^{-5}mol/cm^3) (NR-m)	XLD (10^{-5}mol/cm^3) (NR-a)
0	4.70	4.23
6	4.85	4.39
12	5.02	4.52
24	5.15	4.61
36	5.32	4.80

of microorganisms. Non-rubber component latex groups are then produced to a greater degree of conversion as non-rubber component metabolites may promote crosslinking of rubber molecules. Resultantly, there is an increase in molecular weight, gel content, and the P_0 value.

NR latex contains 1 – 3% protein, and some of these proteins act as antioxidants. Most proteins are retained during the acid coagulation process, hence the products exhibit good antioxidant performance with a higher PRI value. In the microbial coagulation process, microbial metabolism consumes a part of the protein and some non-rubber components, reducing the content of natural anti-aging ingredients while leading to decreased antioxidant performance. Therefore, NR-m exhibits increased sensitivity to heat and oxygen than NR-a, which enables easy degradation and a lower PRI value. This value reduces further with prolonged storage time.

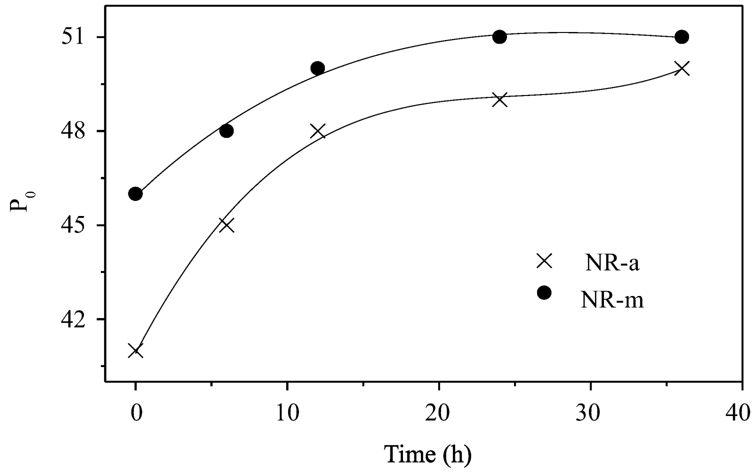

Figure 5. Relationship between P_0 and storage time.

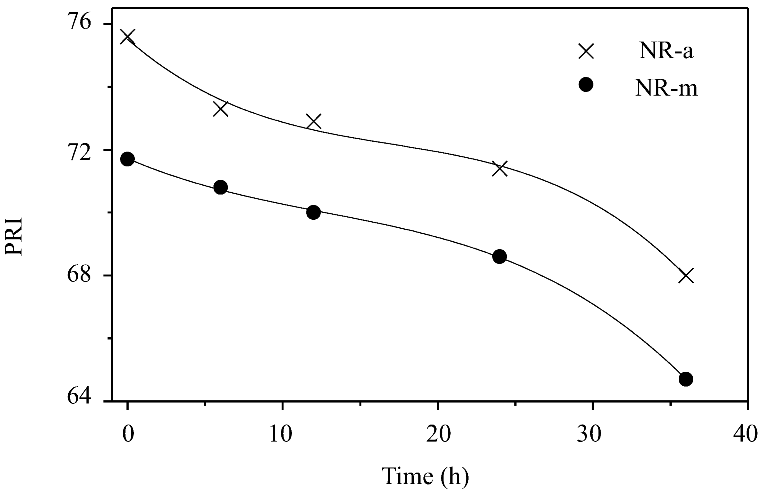

Figure 6. Relationship between PRI and storage time.

Relationship between Structure and Properties of NR

From the results above, values of crosslink density, molecular weight and gel content for all samples increase as the storage time increases. In order to reflect the relationship between structure and property of NR, *Figures 7* and *8* reflect the relationship between P_0 and molecular weight or gel content. *Figures 9* and *10* illustrate the relationship between PRI and molecular weight or gel content.

P_0 value indicates the plasticity of rubber. As the P_0 value increases, NR deformation reduces. This is a significant consideration for raw NR production, subsequently used in the manufacture of rubber products. It can be seen from *Figures 7* and *8* that P_0 values are exponentially increasing with an increment in

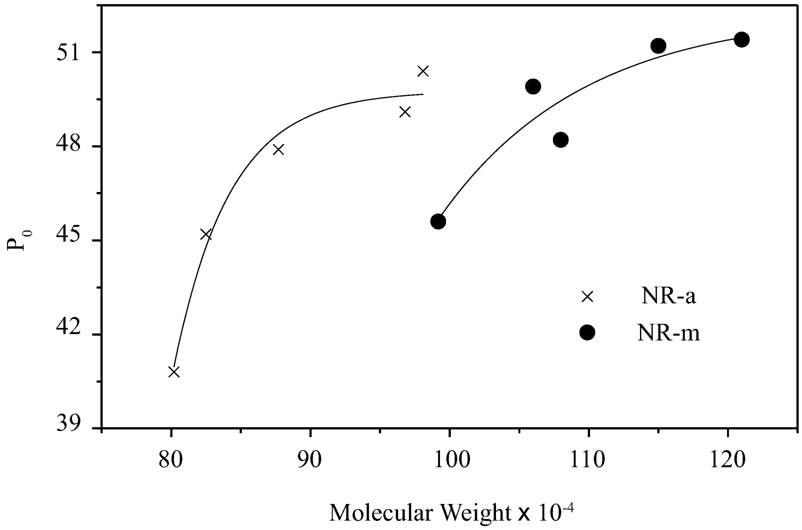

Figure 7. Relationship between P_0 and molecular weight.

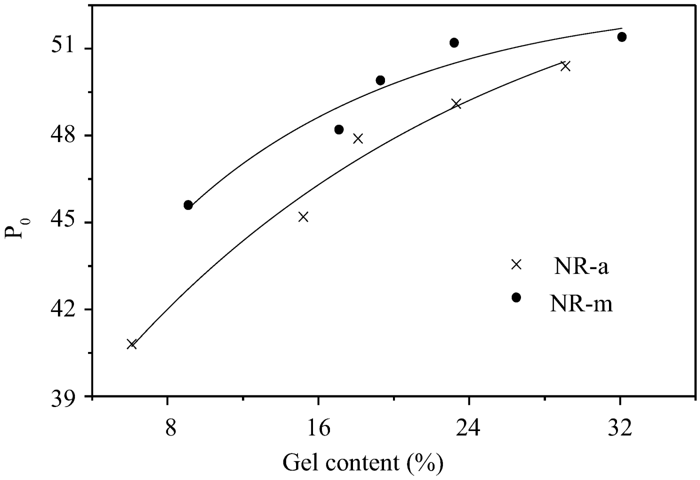

Figure 8. Relationship between P_0 and gel content.

molecular weight and gel content in both NR-a and NR-m. The P_0 of NR is determined by the bulk viscosity of the material. The larger the molecular weight of the material, the greater its viscosity. A higher crosslink density of NR, makes it tougher to deform owing to a higher value of P_0. Comparing equal values of molecular weight and P_0, NR-m shows higher molecular weight and lower gel content. This indicates that there exists a difference in the crosslink networks of NR coagulated by acid and microorganisms. NR-a demonstrates a higher content of protein, and perhaps more physical crosslink bonds by means of hydrogen bonding between protein, while in NR-m there exists more chemical crosslink bonds by aldol condensation.

Figures 9 and *10* are relationships between PRI contrasted against both molecular weight and gel content. It can be seen from *Figure 9* that PRI values have negative relationships with increasing molecular weight and gel content in both NR-a and NR-m. PRI is a measure of the aging properties of materials. During the aging process, the physical crosslinking bonds are easier to fracture due to its lower bond energy. Therefore during storage, greater molecular weight coupled with increased physical crosslinking bonds worsens aging performance.

The Differences of Molecular Structure between NR-a and NR-m

The FTIR spectra of NR-a and NR-m are shown in *Figure 11*. The absorptions at 2960 cm^{-1}, 2917 cm^{-1}, 2852 cm^{-1}, 1448 cm^{-1} and 1376 cm^{-1} are attributed to methyl and methane. The absorption at 1663 cm^{-1}, 1083 cm^{-1} and 835 cm^{-1} are vibrations of carbon-carbon double bonds. The absorption at 1575 cm^{-1} is the vibration of acid amide II of a few proteins in NR. The FTIR spectra of NR-m and NR-a are nearly the same. The only difference between them is that the absorption bands of the proteins in NR-m at about 1575 cm^{-1} is weaker and nearly disappeared, which indicates that the protein content of NR-m is lower than that of NR-a. There are about 1 – 3% natural proteins existing in natural latex. During the acid-coagulating process, most of the proteins remain in the NR-a. But during the microbial-coagulating process, microorganisms assimilate most of the proteins by metabolism.

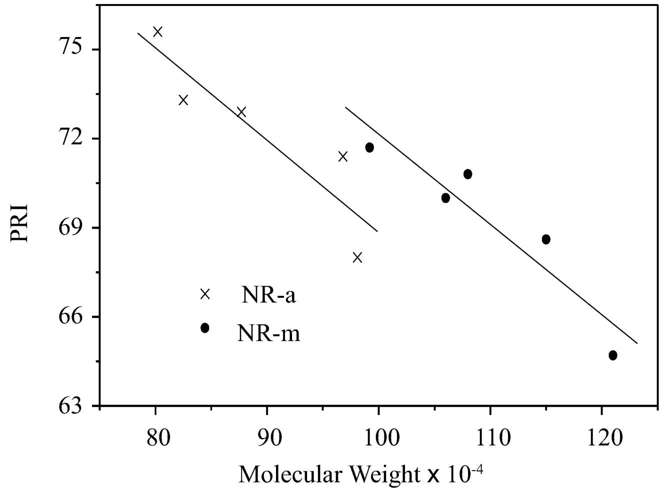

Figure 9. Relationship between PRI and molecular weight.

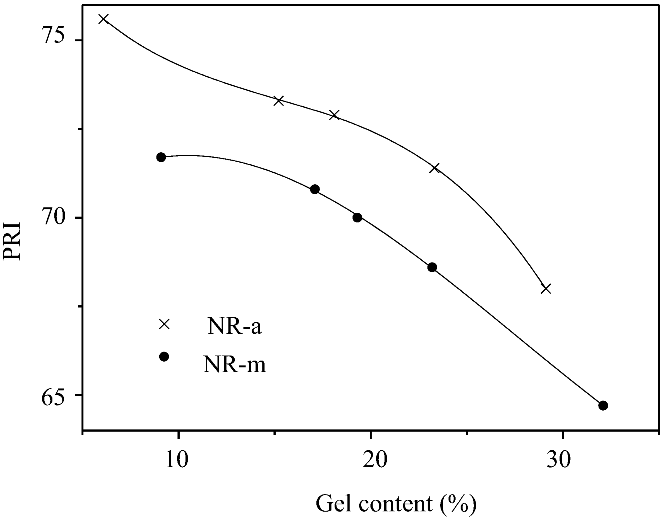

Figure 10. Relationship between PRI and gel content.

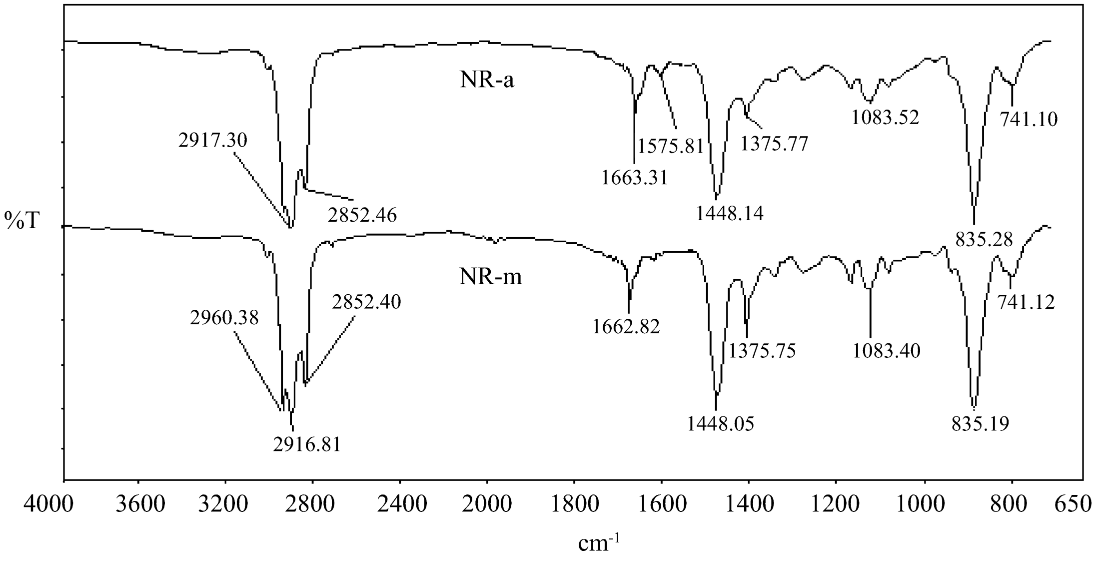

Figure 11. *FTIR spectra of NR-a and NR-m.*

The differences between NR-m and NR-a are embodied by molecular structures and their properties. Compared to NR-a, the molecular weight, gel content and P_0 of NR-m are higher; whereas the PRI is lower. During the microbial-coagulating process, NR latex is coagulated under a higher pH value without acid, which accelerates crosslinking reactions *via* aldol condensation. In this case, the molecular weight and gel content of NR-m increased, leading to an increment of P_0. The proteins in NR are natural antioxidants. On the contrary, due to the lower protein content of NR-m, the antioxidation and PRI of NR-m are lower.

CONCLUSIONS

With prolonged storage time, the molecular weight, gel content and P_0 of NR increases continuously, whereas the PRI reduces. There is a close relationship between molecular weight and properties of NR. In both NR-a and NR-m, P_0 increases with the increment of molecular weight and gel content, while PRI shows a negative correlation with both molecular weight and gel content. There exists a difference in the rate of change in these parameters between NR-a and NR-m. There are significant differences in structure and properties between NR-a and NR-m. The molecular weight, gel content and P_0 of NR-m are higher than those of NR-a. Compared to NR-a, the PRI of NR-m is lower, reflecting poor antioxidation and lower protein content.

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