Effect of Preparation Conditions on Bonding Strength of Soy-based Adhesives *via* Viscozyme L Action on Soy Flour Slurry

Nairong Chen, Qinzhi Zeng, Jiuping Rao, and Qiaojia Lin*

To evaluate the effects of preparation conditions of a 'green' soy-based adhesive(SBA), Viscozyme L was employed to hydrolyze the polysaccharides in defatted soy flour (DSF) for preparing SBAs, and plywood bonded by SBAs with *Pinus massoniana* veneer was then produced. Effects of enzymolysis pH, temperature, time, and additive amount of the Viscozyme L on water-insoluble substances content (WISC) and bonding strength (boiling-water test) of SBAs were investigated. Results showed that bonding strength increased first then decreased as enzymolysis pH and temperature were increased. WISC decreased with increasing pH and decreased first then increased as temperature increased. WISC decreased and bonding strength improved slowly with the increasing time. Bonding strength improved slowly as additive amount of Viscozyme L increased. WISC decreased as the added amount of Viscozyme L increased and then decreased slowly at the added amount of Viscozyme L of about 50 FBG and beyond. SBAs prepared by Viscozyme L action on soy flour slurry decreased WISC and improved bonding strength. The suitable preparation conditions of SBA for plywood are as follows: enzymolysis pH 5.2, temperature 50 °C, and time 20 min, and the additive amount of Viscozyme L depended on the application condition.

Keywords: Soy-based adhesive; Defatted soy flour; Viscozyme L; Preparation conditions; Bonding strength; Water-insoluble substances

*Contact information: College of Materials Engineering, Fujian Agriculture and Forestry University, Fuzhou, Fujian province 350002, P. R. China; *Corresponding author: Linqj208@163.com*

INTRODUCTION

Adhesives are used in diverse applications ranging from furniture to housing and from aerospace to packaging. Most of these adhesives are based on petroleum as the raw material. However, petroleum, a fossil resource, is finite and non-renewable. In addition, toxic components such as phenol and formaldehyde, which are often released from petro-based adhesives, are probably carcinogenic to humans (Lin *et al.* 2012). As a result, increased attention has been paid in the past decade to development of adhesives using renewable resources (*e.g.* biomass). Other factors contributing to this tendency are the biomass with the features of abundant availability, relatively low cost, and significant environmental benefits, *etc*.

Defatted soy flour (DSF), which is a biomass derived from soybean, contains approximately 50% protein, 40% carbohydrates, and other minor components (Karr-Lilienthal *et al.* 2005; Bainy *et al.* 2008). The soy-based adhesives (SBA) prepared from DSF are widely being studied nowadays for application in wood based panels. Many attempts, using chemicals or resins as modifiers, have been carried out to modify soy

protein in DSF for preparation SBAs. Polyethylenimine epichlorohydrin resin (Li *et al.* 2004; Gu and Li 2011); poly (acrylic acid) solution and sodium dodecyl sulfate (Gao *et al.* 2012); combinations of an acid, a salt, and alkali (Chen *et al.* 2012; Lin *et al.* 2012); 2-octen-1-ylsuccinic anhydride (Qi *et al.* 2013); and epoxy resin (Chen *et al.* 2013b), melamine-formaldehyde resin and their mixture (Lei *et al.* 2014) have been used to modify the soy protein in DSF, and positive results were obtained. Previous studies by the authors have shown that the properties (*e.g.*, bonding strength, water resistance) of SBAs are also influenced by its carbohydrates, because the carbohydrates in unmodified SBAs are mainly polysaccharides with lower chemical activities in the curing process (Chen *et al.* 2013a). However, substitution of polysaccharides for monosaccharides (*e.g.*, glucose) improves the properties. It was concluded this was a result of the Maillard reaction occurred between proteins and monosaccharides. Therefore, hydrolysis the polysaccharides in DSF can be expected to apply for preparation of 'green ' SBAs.

Viscozyme L is a commercially available enzyme mixture, containing a wide range of carbohydrase activities, including cellulase, hemicellulase, xylanase, arabanase, and β-glucanase activities; it can effectively hydrolyze plant cell wall polysaccharides (Yu *et al.* 2003; Guan and Yao 2008). Viscozyme L has been used widely in scientific research, such as on nano-fiber (Campos *et al.* 2013), fermentation (Hwang *et al.* 2013), and assisted extraction of proteins (Latif and Anwar 2011), sugars (Kim *et al.* 2014), and oils (Rovaris *et al.* 2013). Rosset *et al.* (2012) reported that protein extraction, after pretreatment with Viscozyme L of DSF, was mainly affected by the temperature of extraction, whereas the content of reducing sugars was affected by the enzyme concentration. Then they used the DSF pretreated by Viscozyme L to prepare silken tofu, and observed that the treated tofu had more glucose and galactose than the control. The total reducing sugar (glucose equivalents) content in treated tofu was approximately four times higher than that in the control, but the soy odor and surface uniformity of tofu showed no preference for one over the other (Rosset *et al.* 2012). However, all these studies are involved in the food engineering, and little information is available about the preparation conditions of SBAs by hydrolyzing the polysaccharides in DSF using Viscozyme L.

In this study, Viscozyme L was applied in DSF slurry to prepare SBAs with the objective of determination its most suitable preparation conditions. The effects of Viscozyme L action with pH, temperature, time, and its additive amount on water-insoluble substances content (WISC) and bonding strength (after a boiling-water test) of SBAs were evaluated. The present results will guide research into the further development of green SBAs.

EXPERIMENTAL

Materials

Hydrochloric acid solution (30%) was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (China), and used as received. DSF was obtained from Shandong Wonderful Industrial Group Co., Ltd. According to the supplier's instructions, 98% of the DSF was passed through a 200 mesh screen. Viscozyme L (from *Aspergillus aculeatus*) was a gift from Novozymes (Denmark). The activity of Viscozyme L was 100 fungal beta-glucanase units (FBG)/g. *Pinus massoniana* veneer 300 mm × 300 mm in size, 1.2 to 1.3 mm thickness and with a moisture content of 10 to 12 (wt.%) was supplied by Jianyang Luban Wood Industry Co. Ltd (China).

Methods

SBAs preparation

DSF (20 g) and distilled water (80 g) were added to a three-necked flask and stirred for 40 min in a 35 ºC water bath. The different pH values of the DSF slurry were then adjusted with hydrochloric acid solution. After the different FBG units of Viscozyme L had been added into the DSF slurry, it was enzymolized for varying times at different temperatures, to obtain a SBA. The experimental design is shown in Table 1. Half of the SBA used to evaluate WISC was stirred for 10 min at a 85 ºC water bath to inactivate the Viscozyme L, and then the SBA was cooled to room temperature; and the other half of the SBA used to evaluate bonding strength was immediately cooled in a 5 ºC water bath for 10 min, and then was stored in a refrigerator. The viscosity and solid content of the final SBA were tested by using a Brookfield Rotational viscometer at room temperature and an oven at 103 ºC, respectively. In the control experiment, only Viscozyme L was substituted by inactivated Viscozyme L to prepare control samples, and other treatments were same to SBA samples.

Table 1. Preparation Parameters of SBAs

pH	Temperature (ºC)	Time (min)	Additive amount of Viscozyme L (FBG)
4.0-6.2	45	30	100
5.2	40-75	30	100
5.2	50	5-70	100
5.2	50	20	0-200

Preparation of plywood

The SBA stored in a refrigerator was used to prepare three-ply wood (thickness of 3.0 mm) by coating 140 g/m^2 of the adhesive on each veneer layer. The assembly time, pressing temperature, pressure, and time were set as 10 min, 160 °C, 1.0 MPa, and 3.6 min, respectively. Average results are presented from the triplicate tests.

Characterization

The adhesive treated at 85 ºC for 10 min was used to determine the WISC. A 4.0 gram (m_1) portion of this adhesive was weighed into a 50 mL centrifuge tube with the addition of 40.0 grams of distilled water. The sample was stirred and centrifuged at 9000 rpm for 10 min (ANKE, radius = 15 cm, China), then the precipitate was dispersed with another 40.0 grams of distilled water and re-centrifuged at 9000 rpm for 10 min. After centrifugation, the precipitate was transferred to a weighing bottle. The bottle was then opened and air-dried in an oven at 103 ºC until a constant weight was achieved; the weight was denoted by m_2. The WISC of the SBA was calculated using the following equation:

$$\text{WISC} = m_2/m_1 \times 100\% \quad (1)$$

The results are presented as the means of three replicates.

The plywood wet strength was determined using the conditions and methods described by the Chinese National Standards GB/T 9846-2004. A piece of plywood was cut into ten 100 mm × 25 mm specimens. The bonding strength of the developed SBA was evaluated through the glue line wet strength of the plywood samples subjected to boiling-water soaking pretreatment. Under these conditions, the plywood specimens were soaked

in boiling water for 3 h and then cooled to room temperature for 10 min. A tensile testing machine (MTS, USA) with a cross-head speed of 10 mm/min was used to test the wet strength. The number of test specimens for each combination is 30 (10 × 3), the average wet strength is calculated.

RESULTS AND DISCUSSION

The carbohydrate in DSF is mainly crude fiber from cell wall polysaccharides, which are comprised of mannose, glucose, arabinose, xylose, galactose, rhamnose, and fucose, *etc*. (Slominski and Campbell 1990; Huisman *et al.* 1998; Stombaugh *et al.* 2000). Viscozyme L as an enzyme mixture can effectively hydrolyze plant cell wall polysaccharides to reducing sugars such as galactose, glucose, and arabinose (Hourigan and Chesterman 1997; Huisman *et al.* 1999; Hanmoungjai *et al.* 2002). According to the catalytic selectivity of Viscozyme L, soy protein in DSF would not be hydrolyzed during the hydrolysis process of polysaccharides; hence the WISC of SBAs would be decreased due to water-insoluble polysaccharides hydrolyzed to water-soluble monosaccharides. The variation of WISC reflects the results of Viscozyme L action on polysaccharides. The applications of an SBA is determined by its bonding strength, which can be evaluated from the wet strength of plywood produced from a veneer and SBA. Therefore, the suitable preparation conditions of SBAs would be carried out after comprehensive analysis results of WISC and bonding strength.

Effects of pH

Figure 1 shows the WISC and bonding strength of SBAs from the DSF slurry with different pH enzymolized by 100 FBG units of Viscozyme L at 45 ^{o}C for 30 min. As shown in Fig. 1(a), the WISC of all samples decreased as pH was increased and then decreased sharply at pH of about 5.8 and beyond. This could be ascribed to the higher acidic amino acid residue (glutamic acid residue, aspartic acid residue, *etc*.) content in the soy protein, because the acidic amino acid residue is readily reactive with base to give carboxylate structures, and in water media the solubility of soy protein is increased (Henn and Netto 1998). The WISCs of SBA also decreased with pH, and were significantly lower than the control samples when the pH was lower than 5.8, indicating that the water-solubility components of SBA were enhanced as a result of polysaccharides in DSF being hydrolyzed during the Viscozyme L treatment process (Qu 2011). The control samples had higher WISC at pH 4.2 and 4.6 as compared to the other control samples due to the fact that soy protein has its lowest solubility at the isoelectric point (pH=4.2 to 4.6) (Kumar *et al.* 2002); but the Viscozyme L hydrolyzed samples showed no such result. This indicates that the effects of Viscozyme L on increased solubility of polysaccharides were greater than the effects of different pH on decreased solubility of soy proteins in SBA. The maximum difference value of WISC between the SBA and control samples was 7.42% when the pH was 5.2, implying the high enzymatic activity of Viscozyme L at the pH 5.2 condition. Figure 1(b) shows that the bonding strength of all samples fluctuated slightly, at a 0.40 MPa level first, and then decreased as the pH was increased; and the bonding strength of SBA samples was significantly higher than that of the control samples. The maximum bonding strength of SBA samples were 0.42 MPa when the pH was 5.2 or 5.4, which represents an increase by 40% relative to the highest bonding strength (0.30 MPa) of the control sample at the pH 5.0 condition. These findings indicate improved water resistance

and gluability of SBA samples. This might result from its increased reducing sugar content as a result of cross-linking occurring between reducing sugar and protein during the Maillard reaction (Chen *et al.*, 2013a).

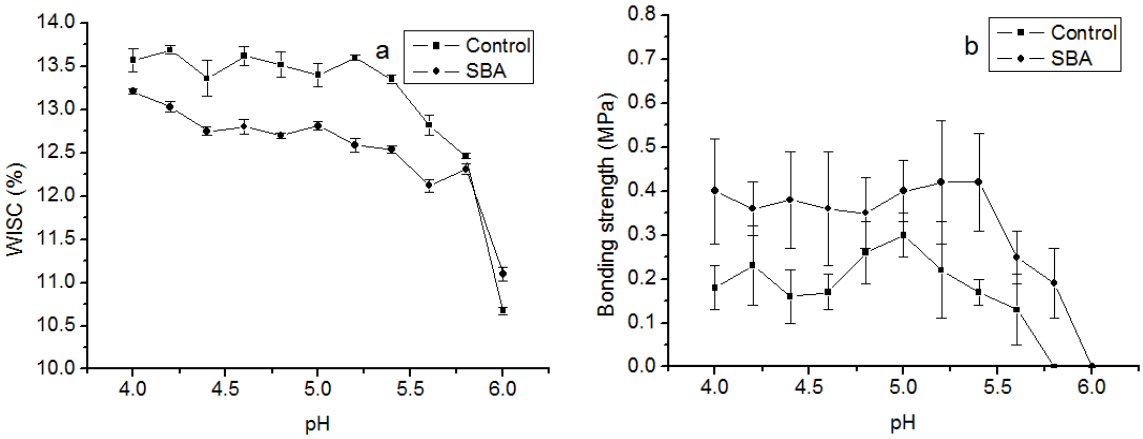

Fig. 1. Effect of enzymolysis pH on the WISC (a) and bonding strength (b) of SBAs

Effects of temperature

Figure 2 shows the WISC and bonding strength of SBAs for the DSF slurry with pH 5.2 enzymolized by 100 FBG units of Viscozyme L at different temperature for 30 min. As shown in Fig. 2(a), the WISC of all samples decreased first and then increased as temperature was increased; the lowest WISC of the SBA and control samples were 12.16% and 13.21% at the temperature of 50 ºC and 55 ºC, respectively. This is might due to the different denaturation degree of soy protein under different temperature conditions. At a low temperature (*e.g.* <50 ºC), the water-solubility of soy protein increased as temperature increased, and the WISC decreased. With further increase of the temperature (*e.g.* >55 ºC), the WISC increased. It is probable that denaturation of the soy protein occurred, such that the material aggregated together, leading to decreased water-solubility (Kim *et al.* 2001). This was also in agreement with the phenomenon found in the experiment, which showed that all the samples were easily separated into liquid and solid components when the temperature was increased beyond 55 ºC. The maximum difference of WISC between the SBA and control samples was 8.13% at 50 ºC, suggesting that the appropriate enzymolysis temperature of Viscozyme L was 50 ºC. Figure 2(b) shows that the bonding strength of the control samples slowly increased as temperature was increased, which might be attributed to the reduced Maillard reaction between polysaccharides and soy proteins, because soy proteins became denatured with increased temperature and easily aggregated together, leading to lower activities. It has been reported in previous studies that the water solubilities of the Maillard reaction products between proteins and polysaccharides are enhanced by a large number of hydroxyl radicals, resulting in the poor water resistance or gluability of cured SBAs (Chen *et al.* 2013a). The bonding strength of the SBA samples increased first and then decreased as temperature was increased; and all the bonding strength of SBA samples were higher than control samples. This suggesting that the water resistance and gluability of SBAs were different with the different Viscozyme L enzymolysis temperature. The bonding strength of the SBA samples at 50 °C and 60 °C were 0.52 MPa and 0.53 MPa, respectively, which is not significantly different. However, the bonding strength showed a significant decrease at 55 ºC and may be ascribed to the experimental error.

Hence, 50 °C, which offers a lower energy consumption, is an appropriate enzymolysis temperature for Viscozyme L.

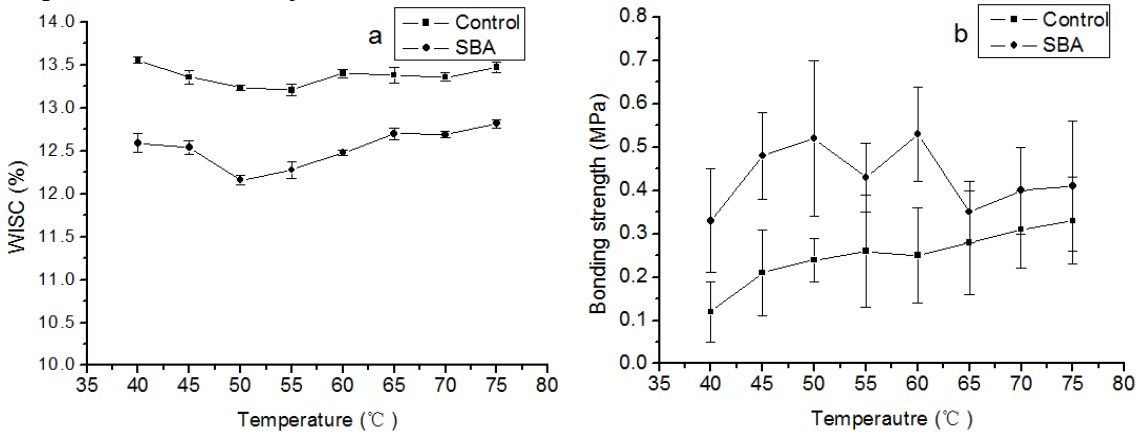

Fig. 2. Effect of enzymolysis temperature on the WISC (a) and bonding strength (b) of SBAs

Effects of time

Figure 3 shows the WISC and bonding strength of SBAs for DSF slurries with pH 5.2 enzymolized by 100 FBG units of Viscozyme L at 50 °C for different times. As shown in Fig. 3(a), the WISC values of the SBA samples were obviously lower than the control samples when time was 5.0 min, suggesting the high catalytic efficiency of Viscozyme L on polysaccharides. When increasing the pretreatment time further, the WISC values of the control samples were slowly decreased, which might due to the acid hydrolysis of polysaccharides (Lavarack *et al.* 2002). The lowest WISC of the SBA and control samples were 12.05% for 60 min and 13.14% for 70 min, respectively; and the maximum difference of WISC was 8.13% at the time of 20 min, implying that the time of 20 min is probably enough to hydrolyze polysaccharides.

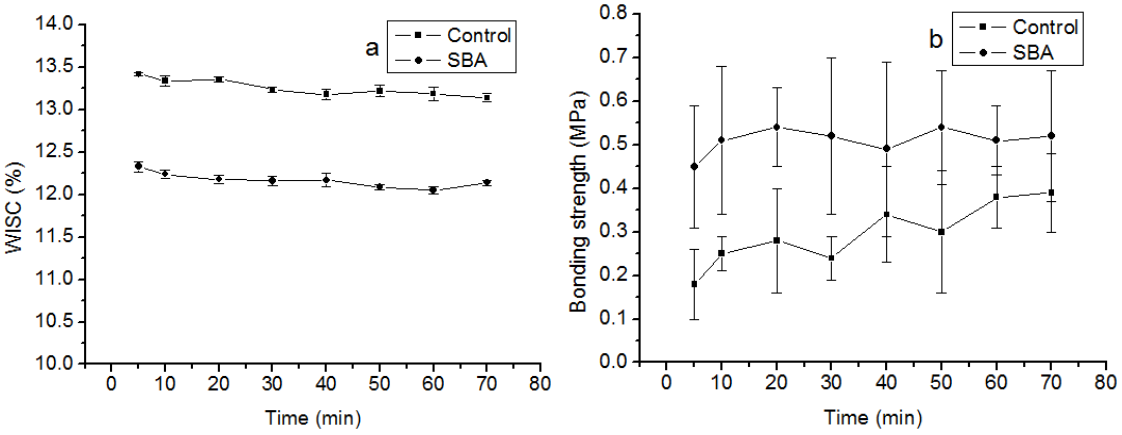

Fig. 3. Effect of enzymolysis time on the WISC (a) and bonding strength (b) of SBAs

Figure 3(b) shows the bonding strength of the control samples increased as time increased, indicating that the water resistance and gluability of control samples were improved by acid treatment, because the increased reducing sugar was produced from acid hydrolysis some of polysaccharides (Chen *et al.* 2010). All the bonding strength of SBA samples were higher than control samples; the highest bonding strength of SBA samples was 0.54 MPa at the time of 20 min; increased treatment time further, the bonding strength

fluctuated slightly at about 0.50 MPa. Based on the combination of results shown in Fig. 3(a), 20 min is judged to be the appropriate time for preparation the SBA.

Effects of Viscozyme L

Figure 4 shows the WISC and bonding strength of SBAs from DSF slurries with pH 5.2 used for enzymolysis with different units of Viscozyme L at 50 °C for 20 min. As shown in Fig. 4(a), the WISC of SBA samples could be decreased immediately when 25 FBG units of Viscozyme L was added. When the dosage of Viscozyme L was increased to 50 FBG units, the WISC was decreased to 12.16%; increasing the added amount of Viscozyme L further caused the WISC to fluctuate slightly, at a 12.10% level. This suggested that the polysaccharides in DSF could be well hydrolyzed when the additive amount of Viscozyme L was within the range from 25 to 50 FBG units.

Figure 4(b) shows that the bonding strength of all samples increased as the additive amount of Viscozyme L was increased and then increased slowly at the additive amount of Viscozyme L of about 50 FBG and beyond, which is in contrast to the variation tendency of WISC. It is possible that this is because the increasing the added amount of Viscozyme L would improve the monosaccharides content of SBAs, which may result in its enhanced water resistance and gluability during the curing process. Even so, the bonding strength of SBAs still cannot meet the requirement of the China National Standard – the plywood type I for exterior (*Pinus Massoniana* plywood ≥ 0.89 MPa). Therefore, the better properties of SBAs would be rely on the modification of monosaccharides or proteins derived from products of Viscozyme L action on DSF slurry. Nevertheless, the modification of monosaccharides or proteins was not the main aim of this research. Under the preparation conditions (pH5.2, 50 °C, 20 min, and 50 FBG units of Viscozyme L), the WISC, bonding strength, viscosity, and solid content of SBAs were 12.16%, 0.52 MPa, 795 mPa • s, and 19.3%, respectively.

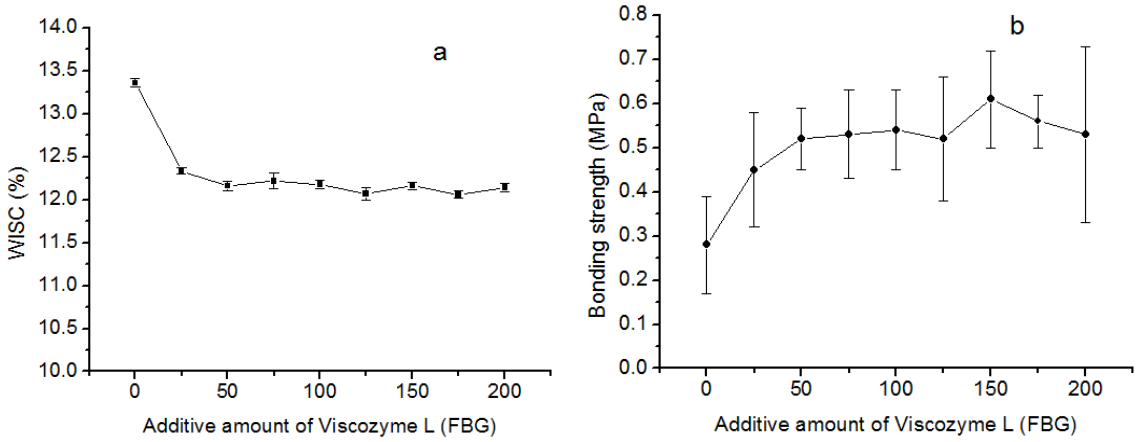

Fig. 4. Effect of the additive amount of Viscozyme L on the WISC (a) and bonding strength (b) of SBAs

CONCLUSIONS

1. The water-insoluble substances content (WISC) of soy-based adhesives (SBAs) decreased with increasing of enzymolysis pH and time of Viscozyme L. The WISC decreased first and then increased with increasing of temperature, and decreased first and then decreased slowly as the added amount of Viscozyme L was increased.

2. Bonding strength of SBAs increased first and then decreased as enzymolysis pH and temperature of Viscozyme L increased, and increased first and then increasing slowly with time, and the additive amount of Viscozyme L increased.
3. The WISC values were of SBAs lower than the control samples, but bonding strength of SBAs were higher than control samples. The suitable preparation conditions of SBAs via Viscozyme L were pH 5.2, temperature 50 ^{o}C, and time 20 min. The additive amount of Viscozyme L was dependent on the application conditions.

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