ADHESIVE PERFORMANCE OF CAMELINA PROTEIN AFFECTED BY EXTRACTION CONDITIONS

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ABSTRACT. Camelina protein (CP) adhesives were prepared from de-hulled camelina meal using alkaline solubilization (CP 8, CP 9, CP 10, CP 11, CP 12) and isoelectric precipitation. CP 12 had the highest protein yield with 46.22%, more than twice that of CP 8 (22.71%), indicating that extreme alkaline pH is necessary for high camelina protein solubility and protein yield. Extreme alkalization resulted in severe molecular dissociation of camelina protein, as indicated by the appearance of a low molecular weight band (20 kDa). Compared to CP 8, CP 9, CP 10, and CP 11, CP 12 had a completely denatured protein structure with greater amounts of exposed functional groups, which is beneficial to the adhesion strength of CP 12. CP 12 with 9% sodium chloride treatment demonstrated optimum adhesion performance with dry and wet strengths of 4.36 and 1.36 MPa, respectively, compared to 3.37 and 1.05 MPa for CP 12 without sodium chloride treatment.

Keywords. Adhesives, Biodegradable, Camelina proteins, Rheology, Thermal properties.

Camelina, which was possibly unintentionally introduced as a weed in flaxseed in North America, is an ancient oil plant that originated in Germany around 600 B.C. (Putnam et al., 1993). Worldwide, researchers in the food and bioenergy industry are interested in camelina because of its unique fatty acid profiles in oil. Camelina contains levels of polyunsaturated fatty acids as high as 90%, of which 38% of those levels are linoleic acids (18:3, omega-3) (Putnam et al., 1993), suggesting that camelina is a good candidate for the creation of high-quality and healthy edible oils. In addition, camelina oil-derived biofuel has been shown to reduce greenhouse gas emissions by up to 80% compared to aviation diesel oil refined from crude oil (Winchester et al., 2013). Camelina seed have been proven to have 29% to 49% oil content, varying by breed and growing environment (Vollmann and Eyck, 2015), and camelina meal, a by-product of camelina oil extraction, is typically comprised of 30% to 50% protein, up to 10% residue oil, and 22% fiber (Budin et al., 1995). Camelina meal is primarily fed to poultry as part of a protein feed ration; however, feeding rates are limited to no more than 10% due to antinutritive compounds, such as glucosinolates, phytic acid, sinapine, and condensed tannins (Russo and Reggiani, 2012). Nutritional drawbacks restrict the use of camelina meal as a food or feed ingredient. Therefore, new added-value co-products must be developed to make camelina more competitive with other oil crops and increase its economic value. Utilization of co-products from camelina would result in increased growing areas and production, thereby benefiting U.S. farmers and enhancing rural economic development.

Extensive study of protein-based adhesives, such as soy protein-based binders, has led to the conclusion that adhesion between proteins and wood surfaces occurs as the protein spreads, wetting and penetrating the porous wood structure to achieve mechanical interlocking. Physical attraction and chemical bonding occur between wood and protein during the setting period, followed by entanglements and cross-links caused by physical attraction and chemical bonding between protein-protein and protein-wood surfaces during thermal setting (Wool and Sun, 2005; Li et al., 2004).

Camelina proteins are extractable from camelina seed using water, alkaline, and salt as solvents (Li et al., 2014). Protein fractions of albumin, glutelins, and globulin with harvesting yields of 10%, 65%, and 12%, respectively, compared to total protein in the entire camelina seed have been extracted and showed potential for use as binders to wood substrates (Li et al., 2015). Globulin, which demonstrated higher bonding strength than glutelin, exhibited water resistance at optimum curing temperatures comparable to that of other vegetable proteins, such as sorghum or soy protein (Li et al., 2011). Camelina proteins with higher purity can be harvested with the above-described method plus a degumming step. Camelina seed usually contains 2% to 3% gum (polysaccharide) with extremely high water-holding capacity, causing the entire seed meal-water suspension to be extremely viscous. Previous research showed that a minimum 1:30 (w/w) ratio of camelina meal to water is necessary to obtain fully protein hydration due to the presence of gums in camelina.

In this study, we utilized the decortication process and pure endosperm meal for protein extraction with the purpose...
of improving camelina protein separation efficiency (solid/water ratio reduced to 1:15) and the bonding strength of camelina protein when used as a wood adhesive. A previous study used a pH of 12 for glutelin protein extraction to ensure high protein extraction yield (Li et al., 2014, 2015). However, extreme alkaline conditions hydrolyze and denature protein completely, potentially affecting the adhesion performance of camelina protein. Therefore, in this study, pH values lower than 12 were also applied to isolate camelina protein with increasingly native protein structures. Sodium chloride (NaCl) extracted globulin showed better adhesion performance than glutelin in a previous study, but the globulin accounted for only 12% of the total camelina protein (Li et al., 2014). Therefore, simultaneous extraction of globulin and glutelin protein and preservation of protein adhesion performance were also investigated to simplify the camelina protein isolation process. Specifically, the research objectives included studying the effect of pH and NaCl treatment on camelina protein extraction rate and evaluating the physiochemical properties, such as adhesion properties, protein composition, thermal properties, solubility, and morphology.

MATERIALS AND METHODS

MATERIALS

Camelina seed was provided by Montana Gluten Free Processors (Belgrade, Mont.). The seed decortication process was conducted by Sunhai Bioadhesive Technologies (SBT LLC, Manhattan, Kans.). Hexane, hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium chloride (NaCl) were purchased from Fisher Scientific (Fair Lawn, N.J.). Cherry wood veneers with dimensions of 50 × 127 × 5 mm (width × length × thickness, respectively) were provided by Veneer One (Oceanside, N.Y.).

DEFATTING

The decorticated seed was pressed with a screw press (KK8 F Universal, KernKraft, Reut, Germany) at 90°C to remove most of the oil and form pellets. The pellets had 10% residual oil and were milled with a cyclone sample mill (Udy Corp., Fort Collins, Colo.) and defatted with hexane at a solid/liquid ratio of 1:10 (w/v) for 2 h at room temperature in three cycles. The defatted camelina meal (DCM) was placed in a tray under a fume hood with a uniform thin layer (~2 mm) for 24 h to evaporate the residual hexane.

ISOLATION OF CAMELINA PROTEIN

DCM samples were mixed with distilled water at a solid/liquid ratio of 1:15 (w/v), adjusted to pH 8 to 12 at increments of pH 1 using 2 N NaOH with continuous stirring for 2 h, and centrifuged to remove insoluble materials. The supernatants were adjusted to pH 4.5, centrifuged to precipitate the camelina protein, and labeled as CP 8, CP 9, CP 10, CP 11, and CP 12. At extraction pH 12, NaCl was added to the meal-water suspension at concentrations of 0%, 3%, 6%, and 9% (w/v), stirred for 2 h, and centrifuged to remove insoluble materials. The supernatants were adjusted to pH 4.5 and centrifuged to precipitate the camelina protein. All camelina protein samples were lyophilized and ground into powder for further analysis.

CHEMICAL ANALYSIS

Nitrogen content was measured with a CHNS/O elemental analyzer (2400 Series II, PerkinElmer, Shelton, Conn.). Nitrogen was converted to protein using a factor of 6.25. All tests were performed in duplicate.

ELECTROPHORESIS (SDS-PAGE)

SDS-PAGE was performed on a 4% stacking gel and 12% separating gel with a discontinuous buffer system, as described by Laemmli (1970). A camelina protein sample was mixed with a buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue. To determine disulfide bonds in camelina protein, SDS-PAGE was carried out under reducing (2-mercaptoethanol) and non-reducing conditions. A total of 8 μg of protein was applied to the sample wells. Molecular weight standards (14.4 to 97.4 kDa) were run with the samples. Electrophoresis was performed at 40 mA and 150 V for 120 min. After running, the gel was stained in 0.25% Coomassie brilliant blue R-250 and destained in a solution containing 10% acetic acid and 40% methanol.

RHEOLOGICAL PROPERTIES

Apparent viscosities of camelina proteins were tested using a rheometer (Bohlin CVOR 150, Malvern Instruments, Southborough, Mass.) with a parallel plate (PP20, 20 mm plate diameter and 500 μm gap). Shear rate dependence of apparent viscosity measurements were tested with a shear rate of 25 s⁻¹. The testing temperature was 23°C. A thin layer of silicone oil was spread over the circumference of the sample to prevent sample dehydration during the test. All experiments were performed in duplicate, and average values were reported.

DIFFERENTIAL SCANNING CALORIMETRY

Thermal transition properties of protein samples were measured with a differential scanning calorimetry (DSC) instrument (Q200 V24.4, TA Instruments, New Castle, Del.) that was calibrated with indium and zinc before taking measurements. Samples of wet camelina proteins weighing approximately 20 mg were measured in a hermetic aluminum pan under a nitrogen atmosphere with a gas flow rate of 50 mL min⁻¹. All samples were heated from 25°C to 120°C at a heating rate of 10°C min⁻¹ in an inert environment. All experiments were performed in duplicate.

TURBIDITY

The turbidity of protein samples was determined with a UV-Vis spectrophotometer (BioMate 3, Thermo Electron Corp., Madison, Wisc.). Samples were diluted to concentration of 0.1% with deionized water and adjusted to various pH values designed. Absorbance of protein solutions was measured at 600 nm after 30 min of stirring. All measurements were done in duplicate, and average values were reported.

TRANSMISSION ELECTRON MICROSCOPE

A Philips CM 100 (FEI Co., Hillsboro, Ore.) transmission
electrophotography (TEM) was used to observe the microstructure of camelina protein. All protein samples were diluted to concentration of 0.1% in deionized water and then sonicated for 30 min in an ultrasonic stirrer (model 320, L&R Manufacturing Co., Kearny, N.J.). Diluted samples were absorbed onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort Washington, Pa.) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Burlington, Vt.) for 60 s at room temperature.

WOOD SPECIMEN PREPARATION

Cherry wood samples were preconditioned in a controlled-environment chamber (model 518, Electro-Tech Systems Inc., Glenside, Pa.) for 7 d at 25°C and 50% relative humidity (RH). Camelina proteins that were re-dispersed in distilled water at 12% (w/v) were used as wood adhesives. Camelina adhesives were brushed along the edges of two pieces of cherry wood with an application area of 127 × 20 mm until the entire application area was completely covered. The adhesive amount applied to each piece was approximately 0.06 g (dry basis). The brushing and setting procedures followed the method described by Mo et al. (2004). Brushed areas of the two pieces were affixed together at room temperature for 10 min and then pressed at a pressure of 2.0 MPa at 170°C for 10 min using a hot press (model 3890 Auto ‘M,’ Carver Inc., Wabash, Ind.).

MECHANICAL PROPERTIES

After pressing, the glued-wood assemblies were conditioned at 23°C and 50% RH for two days and then cut into five specimens, each measuring 127 mm (length) × 20 mm (width) × 5 mm (thickness). The cut specimens were conditioned for another five days at 23°C and 50% RH before the dry test. Three adhesion strengths were tested: dry strength, soak strength, and wet strength. Wood specimens for dry strength testing were prepared and tested using an Instron testing system (model 4465, Instron, Norwood, Mass.) according to ASTM Standard Method D2339-98 (ASTM, 2002c). The crosshead speed of the Instron system for adhesion strength testing was 1.6 mm min⁻¹, and adhesion strength was recorded as the tensile strength at the maximum load. Reported results were averages of five samples. Water resistance was determined by measuring wet and soak strengths according to ASTM Standard Methods D1183-96 (ASTM, 2002b) and D1151-00 (ASTM, 2002a), respectively. Preconditioned specimens were soaked in tap water at 23°C for 48 h and then tested immediately for wet strength.

**Table 1. Camelina protein adhesive yield, protein purity, and extraction rate as affected by extraction pH.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wet Adhesive Yield (%)</th>
<th>Solids Content (% d.b.)</th>
<th>Dry Adhesive Yield (%)</th>
<th>Protein Purity (% d.b.)</th>
<th>Camelina Protein Extraction Rate (% d.b.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 8</td>
<td>58.2 a</td>
<td>39.0 b</td>
<td>22.7 a</td>
<td>89.4 a</td>
<td>36.1 a</td>
</tr>
<tr>
<td>pH 9</td>
<td>63.0 a</td>
<td>40.5 bc</td>
<td>23.3 ab</td>
<td>89.3 a</td>
<td>36.9 a</td>
</tr>
<tr>
<td>pH 10</td>
<td>72.9 b</td>
<td>40.6 bc</td>
<td>29.6 ab</td>
<td>88.8 a</td>
<td>46.6 b</td>
</tr>
<tr>
<td>pH 11</td>
<td>120.0 c</td>
<td>31.9 a</td>
<td>37.1 c</td>
<td>85.0 a</td>
<td>57.8 c</td>
</tr>
<tr>
<td>pH 12</td>
<td>143.7 d</td>
<td>32.2 a</td>
<td>46.2 c</td>
<td>85.3 a</td>
<td>70.0 d</td>
</tr>
</tbody>
</table>

Means in the same column followed by different letters are significantly different at p < 0.05.

**STATISTICAL ANALYSIS**

Data from the mechanical property evaluation were taken from an average of five samples. Data from the experiments carried out in duplicate were analyzed using analysis of variance (ANOVA) at the 0.05 level according to procedures in SPSS (ver. 19.0, IBM Corp., Armonk, N.Y.).

**RESULTS AND DISCUSSION**

**Protein Extraction Yield and Purity**

As shown in table 1, high pH was required for high camelina protein extraction yield. At pH 12, the camelina protein yield (dry basis) and extraction rate were 46.22% and 70.0% (based on total protein in camelina meal), respectively, which were more than two times higher than the yield at pH 8. Camelina protein extracted at high alkaline pH typically has more negative charges due to ionization of carboxyl groups and deprotonation of amine groups. The subsequent increased electrostatic repulsion among protein promotes protein-water interaction, thereby increasing protein solubility (Valenzuela et al., 2013; Lawal, 2004). The highest solubility of camelina protein occurred at pH 12, which was consistent with previous work (Li et al., 2014). Soluble protein extracted from pumpkin, quinoa, and zein also reached the highest value when extracted at pH 12 (Popovic et al., 2011; Abugoch et al., 2008; Shukla and Cheryan, 2001). Zein protein was reported to have a high rate of nonpolar amino acid residues and a deficiency in basic and acidic amino acids, accounting for its soluble behavior (Shukla and Cheryan, 2001). However, for camelina protein, acidic (28.9%) and basic (16.6%) amino acids were shown to be similar to the soy protein profile (30.9% and 16.4%) (Li et al., 2014; Utsumi et al., 1997). Therefore, a greater number of nonpolar radical groups than polar groups could have been located on the protein surface, making camelina protein more tolerant of pH treatment as compared to soy protein (over 90% of soy protein was solubilized at pH 8) (Jiang et al., 2010). However, at extreme alkaline pH 12, non-protein matter was also extracted with the protein, resulting in a slight decrease in protein purity (85% at pH 12 compared to 89% at pH 8). In addition, the water content of camelina protein increased remarkably at pH 11 and pH 12, as proven by the decreased solids content, suggesting high water-hydration capacity due to the increased interaction between highly denatured/unfolded protein and water.

**NaCl Treatment**

NaCl treatment improved the camelina protein extraction rate (table 2). The dry protein extraction rate was increased by 10% with the addition of 9% NaCl. This increment in the...
extraction rate was attributed to two NaCl effects. First, the NaCl solution extracted globulin protein together with gluten protein. Secondly, the increased solubility of protein attributed to salt is due to the improved activity and binding capacity of chloride ions to the positively charged protein surface, thereby increasing its solubility (Inyang and Iduh, 1996). As shown in figure 1, the overall turbidity of NaCl-treated CP 12 was lower than that of control CP 12, indicating that NaCl decreased protein aggregation in the pH range of 2 to 12, resulting in increased camelina protein solubility. At low pH and mild alkaline conditions (pH < 10) with low ionic strength of the salt, the presence of ions interacted with charged groups on camelina proteins, forming an electric double layer at the crystal-solution interface and significantly increasing the apparent solubility of proteins (Lee et al., 2003; Zhang, 2012). We assume that under extreme pH treatment, excessive negative charges on the protein surface diminished the salt in effect; therefore, solubility increased slightly for CP 12.

**CAMELINA PROTEIN COMPOSITION**

Non-reducing and reducing SDS-PAGE was performed to study subunit distribution in each camelina protein sample as affected by extraction pH. For the non-reducing condition, predominant components in CP 8, CP 9, and CP 10 were distributed at approximately 50 and 15 kDa of molecule weight (MW). Two faint bands appeared at 35 and 25 kDa of MW. However, bands at 50, 35, and 15 kDa disappeared under the reducing condition, as shown in figure 2, indicating the presence of disulfide bonds in 50, 35, and 25 kDa camelina proteins. Intensified bonds observed at around 30, 25, and 17 kDa resulted from reduced disulfide-linked protein complexes. At extreme alkaline pH 11 and pH 12, attenuated intensity of the band at 50 kDa and an intensified band at 15 kDa were observed in non-reducing SDS-PAGE profiles, which was attributed to NaOH effects on camelina protein that can destroy cystine and cleave disulfide bonds (Tecson et al., 1971). In addition, one new band with low molecular weight around 20 KDa appeared in CP 11 and CP 12 under non-reducing and reducing conditions, indicating severe molecular dissociation by extreme alkalinization. Extreme extraction pH such as pH 12 generated more low-molecule weight camelina subunits, potentially affecting adhesion properties of camelina proteins, as discussed in the following sections.

In addition, small amounts of high MW protein aggregates dwelling in the well of the gel were observed for all samples, but those aggregates disappeared under a reducing agent (fig. 2), suggesting that disulfide bonds primarily contribute to protein aggregates. Results showed that those bands intensified as extraction pH increased. Higher pH treatment generated more exposure of S-H bond and thiolate (S-), which were able to form intermolecular S-S bonds.

**THERMAL PROPERTIES**

Figure 3 shows two endothermic denaturation peaks at low extraction pH (pH 8 to 10) in the thermograms of camelina protein, indicating that two main protein fractions differed by denaturation temperature in the camelina protein extracts. As shown in table 3, higher extraction pH resulted in an increased degree of protein denaturation, as reflected by the reduced total denaturation enthalpy (ΔHd) from 0.76 J g⁻¹ for CP 8 to 0.36 J g⁻¹ for CP 10. Extreme alkaline conditions (pH 11 and 12) led to completely denatured camelina protein, with ΔHd of 0 J g⁻¹. Increasing the extraction pH from 8 to 10 caused irreversible disruptions of intramolecular ionic bonds in the camelina protein but only partial unfolding, thereby retaining partial native protein structure and conformation. Jiang et al. (2010) found that, after pH-shifting treatments (pH 12), soy proteins (pH 4.5) had a molten globule-like conformation that largely maintained the original secondary structure and overall compactness but partially lost the tertiary protein structure. For denaturation temperature (Td), when extraction pH increased from 8 to 9, Td increased slightly from 77.2 and 88.3 to 78.0 and 89.6 for peaks 1 and 2, respectively (table 3). Less stable protein structures were possibly unfolded at a low degree of denaturation, causing more stable structures to remain native and resulting in a slightly higher Td at pH 9. When extraction pH

![Figure 1. Turbidity of camelina protein as affected by extraction pH and NaCl treatment.](image-url)
was increased to 10, more protein denaturation was induced; protein with decreased stable structure was obtained with lower \( T_d \), and no phase transition of protein was observed at pH higher than 10.

**MORPHOLOGICAL PROPERTIES**

TEM images of camelina protein as affected by pH extraction and NaCl treatment are presented in figure 4. At extraction pH of 8 and 12, typical spherical globular (approx. 10 to 30 nm diameter) and rod-shaped protein aggregates were observed. These aggregates were connected to each other to form protein clusters. However, CP 8 demonstrated a chain and weblike network assembled from the small protein aggregates. As mentioned, extreme alkaline caused severe molecular dissociation and complete denaturation and generated increased amounts of proteins with low molecular weights, resulting in less-aggregated protein chains for CP 12.

NaCl treatment induced significant change in camelina protein morphology. With 3\% NaCl, protein clusters disappeared and only spherical globular proteins with diameters of approximately 10 nm were observed, indicating that NaCl reduced protein-protein associations, as shown by the reduced turbidity of CP 12 with NaCl treatment (fig. 1). However, at 9\% NaCl, a chainlike network composed of spherical globular protein was formed. As NaCl concentration increased, greater ionic strength was introduced to the camelina protein. Thus, increased salt-screening effects prompted the electrostatic interaction and strengthened the hydrophobic interaction between protein molecules, potentially favoring protein aggregation.

**RHEOLOGICAL PROPERTIES**

Rheological properties are important physical properties related directly to the flowability and wetting properties of adhesives. As shown in figure 5, apparent viscosities of CP 8, CP 9, and CP 10 were similarly lower than 150 cp. Camelina protein adhesive showed significantly increased viscosity, from 300 cp with extraction pH 11 to 650 cp with pH 12. At mild alkaline condition, low protein hydration capacity reduced the hydrodynamic volume of protein mi-
celles; therefore, the enhanced distance and decreased friction between proteins molecules led to low viscosity. However, extreme alkaline pH allowed full protein hydration and increased viscosity. With NaCl treatment of CP 12, salt-screening effects reduced the protein water-hydration capacity, resulting in decreased protein viscosity. At 9% NaCl, apparent viscosity decreased to 300 cp, compared to 650 cp for CP 12.

ADHESION PROPERTIES

Extraction pH

High extraction pH caused improved adhesion performance in terms of dry strength and wet strength (table 4). At lower extraction pH (pH 8 to 9), the wood samples were delaminated during soaking; however, CP 12 had wet strength of 1.05 MPa, indicating improved water resistance. Extreme alkaline treatment (pH 11 to 12) completely denatured the camelina protein by irreversibly disrupting intramolecular ionic bonds and certain disulfide bonds (fig. 2). Thermograms of camelina protein (fig. 3) further proved that the secondary and tertiary structures of camelina proteins were completely destroyed at extraction pH 11 to 12. This extensively unfolded protein structure with exposed functional groups can form more hydrogen bonds between protein and hydroxyl groups in wood, resulting in improved bonding strength. The hydrolyzed camelina protein had much smaller molecular weight polypeptide subunits (fig. 2), enabling the protein to easily penetrate the wood surface and improve adhesion performance. At mild alkaline extraction pH, protein remained in a native

Figure 4. TEM images of camelina protein as affected by extraction pH and NaCl treatment.

Figure 5. Apparent viscosity of camelina protein adhesives as affected by extraction pH and NaCl treatment.
state. The large protein aggregates from the strong protein-protein association may have had poor interfacial interactions and less entanglement with the wood surface, leading to decreased adhesion strength.

**NaCl Treatment**

NaCl treatment significantly improved the adhesion performance of CP 12. With 9% NaCl, the dry adhesion strength of control CP 12 increased to 4.361 MPa, with partial wood failure above 3.777 MPa. In general, NaCl ions interacted with charged groups on the protein via shielding effects, potentially preventing protein adhesives from curing, thereby reducing the effective interfacial area between the protein and wood surface and leading to reduced adhesive bonding strength (Mo and Sun, 2013). However, as mentioned, the reduced viscosity of NaCl-treated CP 12 allowed better adhesive wetting on the wood surface, benefitting camelina protein adhesive performance. Therefore, the increased flowability of CP 12 with NaCl treatment may outweigh the negative effects of NaCl on camelina protein adhesion properties. In addition, salt-soluble protein (camelina globulin) was also isolated with NaOH-soluble protein (glutelin) during acid precipitation (pH 4.5). Li et al. (2015) showed that globulin had better adhesion performance than glutelin protein due to the native loose and porous morphology of globulin. Therefore, incorporation of globulin fractions into camelina glutelin protein also contributed to improved adhesion strength, as shown in table 4.

**CONCLUSIONS**

Extraction and a combination of NaCl treatments could regulate camelina protein extraction yield, composition, and unfolding degree and then influence the adhesion properties of camelina protein as a veneer glue. Results showed that extreme alkaline pH 12 with 9% NaCl treatment was essential for high camelina protein solubility and yield, low viscosity, and increased adhesion performance. Results of this study provide fundamental information for future studies on camelina protein, such as protein yield and hydrophobicity of camelina protein affected by extracting conditions.

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**REFERENCE**


