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Structural, Thermal and Film forming properties of Gelatin from Pink Perch Surimi Refiner Discharge

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Abstract

The objective of the present study was to characterize the gelatin obtained from pink perch (*Nemipterus japonicus*) surimi refiner discharge [referred as refiner discharge gelatin (RDG)] with reference to its structural, thermal and film forming ability. Fourier transform infrared (FTIR) and Differential Scanning Calorimetry (DSC) techniques were used to characterize the gelatin. An edible film was developed with different concentrations of RDG (2-6%) by casting method. FTIR analysis showed that the gelatin from refiner discharge has similar spectra with porcine gelatin (PG). The amide I and amide II bands of RDG occurred at around 1654 and 1578 cm^{-1} respectively. RDG has a broad endothermic peak at 85.64ºC with transition enthalpy (ΔH) of 208.02 J g^{-1} as revealed by thermal analysis indicating the thermal transition temperature and the energy required for the transition respectively. RDG showed good film forming property. The gelatin concentration had strong significant ($p<0.05$) effect on the viscosity of film forming solution. Films were more opaque at higher concentration of gelatin compared to the films prepared at lower concentration. Higher L* values were observed for all the films prepared and there were no significant differences among the gelatin concentrations. Tensile strength of the film increased with increasing gelatin concentration whereas the film swelling was found to be decreased. The study clearly demonstrated that the waste from the surimi processing industries like refiner discharge can be an alternative raw material for gelatin production and can be used for edible packaging films development.

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Introduction

Gelatin is one of the well-known biopolymers extracted from waste generated in slaughter house such as skins, bones and connective tissue of animals. Land animals like bovine and porcine are the major sources of gelatin production. However, due to religious constraints and the outbreak of Bovine Spongiform Encephalopathy and its possible zoonosis, gelatin from aquatic animals has gained increasing attention (Karim & Bhat, 2009). Gelatin has wide applications in the field of food, pharmaceutical and cosmetic industries. In pharmaceutical industry gelatin is used in the manufacture of hard and soft capsules, wound dressing and adsorbent pad (Digenis et al., 1994). In the food industry, gelatin is mainly used as gelling, foaming and stabilizing agents (Nhari et al., 2012). In the recent past, gelatin has been explored for the production of edible film/coatings. Edible films (EF) are used as a wrappers or separators which are kept in between food components (McHugh, 2000). There is a growing interest in developing EFs from biodegradable natural biopolymers derived from proteins, carbohydrates and lipids, due to the environmental problems created by the films from the petroleum products. These natural biopolymers are not only biodegradable but also biocompatible and non-toxic materials (Weng et al., 2014; Kaewprachu et al., 2016). Proteins based EFs are most common because of its film forming properties as well as excellent barrier properties. Attempt has been made to develop films from fish gelatins in recent years (Carvalho et al., 2008; Kim & Min, 2012; Weng et al., 2014). Fish gelatin films having superior tensile and puncture strength than bovine gelatin films have also been reported (Sobral et al., 2001; Hanani et al., 2012). Gelatin films have been used in the food

industry as sausage casing (Johnston-Banks, 1990) and edible wrappers (Torres, 1994). Surimi processing industries generate waste in the form of head, viscera, skin and bone, which constitute 50 to 70% of whole fish (Morrissey et al. 2000). There is a step in surimi processing which is called refining. Refining step produces 15-22% of raw material as refiner discharge and comprises of scale, bones, skins and connective tissues (Wendel, 1999). Such waste can be an ideal raw material for gelatin production. Characteristics and properties of gelatin vary with the raw material. Kim & Park (2005) reported that collagen extracted from refiner discharge of pacific whiting showed better properties than the collagen from skin of the same species. The authors have highlighted the use of refiner discharge as a new raw material for collagen/gelatin extraction. Therefore, the aim of the present study was to extract the gelatin from pink perch surimi refiner discharge and to characterize with reference to its structural, thermal and film forming ability.

Materials and Methods

Pink perch surimi refiner discharge was collected from a commercial surimi processing plant (Ulka Seafoods Pvt Ltd., Maharastra, India). Porcine gelatin was obtained from sigma, Mumbai.

Gelatin was extracted from pink perch (*Nemipterus japonicus*) surimi refiner discharge following the methods described by Wang & Regenstein (2009) with slight modifications. To remove non-collagenous proteins and pigments, refiner discharge was soaked in 0.20 M NaOH with a waste to solution ratio of 1:10 (w/v) for 2 h at room temperature and the alkaline solution was changed at every 1 h interval. The treated waste was then washed with potable water until neutral pH of wash water was obtained. The waste was then soaked in 0.20 M EDTA with a waste to solution ratio of 1:10 (w/v) for 2 h to swell the collagenous material. Again the acid pre-treated waste was washed thoroughly with potable water to bring down the pH to neutral. The swollen waste was extracted in distilled water with a waste to water ratio of 1:3 (w/v) at 70°C for 3 h. The filtrate was concentrated in flash evaporator (BUCHI, India) at 45°C until the volume become half, and later the viscous solution was dried in a vacuum dryer (Heraeus vacutherm, Germany) for 12 h.

FTIR was used to characterize the presence of amide bonds by scanning over the range of 4000-400 cm^{-1} by using a Thermo Fisher Scientific FT-IR spectrometer (Model Nicolet^{TM} iS^{TM} 10, Thermo Fisher Scientific, Waltham, MA). A background spectrum was collected by keeping the resolution at 4 cm^{-1}.

Thermal property of the gelatin was determined by DSC (Mettler Toledo DSC 822e). About 10 mg of the sample was placed in DSC aluminium pans of 40 µL and hermetically sealed. An empty sealed pan was used as reference. The temperature range used was 20-200°C in inert atmosphere at a gas flow rate of 80 ml min^{-1}.

Minerals content of the gelatin were determined by using inductively coupled plasma optical emission spectroscopy (iCAP 6300 Duo, Thermo fisher Scientific, Cambridge, England). The spectroscopy was supported by dual configuration (axial and radial) and iTEVA (version 2.8.0.97) operational software. Known amount of the gelatin powder was digested with 8 ml of concentrated nitric acid (Trace-MetalTM Grade, Fisher Scientific) and 2 ml of hydrogen peroxide (30–32%, Optima, Fisher Scientific) in microwave (Milestone START D, Italy) digestion chamber for 40 min. The digested residue was then diluted up to 50 ml solution by Milli-Q water to determine the metal concentrations. Calibration curve was made by using Multielement standard solution (CertiPUR,Merck) and Yttrium was used as internal standard.

Film forming solution (FFS) was prepared with the RDG concentration of 2-6 % (w/v). Gelatin powder was first allowed to swell in distilled water for 30 min at room temperature. Then the solution was incubated at 60°C for 30 min. After complete solubilization of gelatin powder, glycerol was added as plasticizer at 0.25 g g^{-1} of gelatin and incubated for another 30 min with occasional stirring. The solution was cast in acrylic plate (40 ml in 12 x 12 cm dimension) and dried in a vacuum dryer for 24 h at room temperature.

The viscosity of the gelatin film forming solution was measured by Brookfield digital viscometer (model DV-E, Brookfield Engineering, Middleboro, MA, USA) using spindle No. 1 at 100 rpm at room temperature. The measured values were obtained directly in centipoises (cP) from the instrument.

The color and opacity measurements of the films were made using a Hunter Lab (Hunter Associates Laboratory Inc., Reston, VA, USA) based on three color co-ordinates, namely L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness). The

equipment was standardized using a white tile and black tile.

The mechanical properties of the films such as Tensile Strength (TS) and Elongation at Break (EAB) were determined as per ASTM D 882-97 (ASTM, 1999) standard using the Lloyds Texture Analyzer (Lloyd Instruments, Model LRX Plus, U.K).

Water solubility of the films was measured according to the method of Wang et al. (2007). The swelling index of films was determined using the method as described by Cao et al. (2007).

One-way analysis of variance (ANOVA) was used to determine descriptive statistics and DUNCAN'S multiple mean comparison test at significance level of 0.05 was performed using IBM SPSS 20 statistical software package.

Result and Discussion

FTIR spectroscopy is a useful tool to study the secondary structure and functional groups of proteins. The structure of gelatin from pink perch surimi refiner discharge (RDG) was compared with the gelatin from porcine skin gelatin (PG) (Fig. 1 and 2). FTIR spectroscopy is based on the vibrational excitation of molecular bonds by absorption of infrared light. Amide bands namely amide A, amide B, amide I, amide II, amide III represent different vibrational modes of the peptide bond and are related to the degree of molecular order involved with the triple helical structure of collagen/gelatin (Nikoo et al., 2014). The FTIR of RDG has similar spectra with PG. The corresponding wavenumbers (cm^{-1}) of the characteristics infrared bands of RDG and the PG are given in Table 1. The amide A band

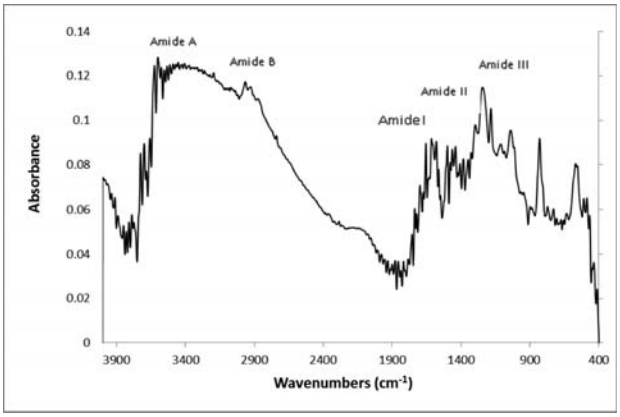

Fig. 1. FTIR spectra of Porcine gelatin

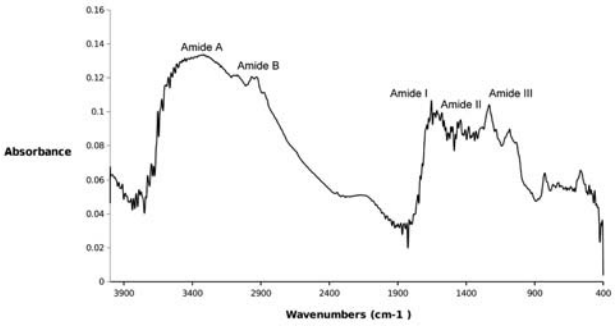

Fig. 2. FTIR spectra of refiner discharge gelatin

of RDG and PG was detected at 3315.95 and 3352.14 cm^{-1} respectively. Amide A band is associated with the N-H stretching vibration. It is established that a free N-H stretching vibration occurs in the range of 3400-3440 cm^{-1} and when the N-H group of a peptide is involved in a hydrogen bond, the peak position is shifted to the lower frequencies (Doyle et al., 1975). Thus the occurrence of amide A peak at 3315.95 cm^{-1} in RDG indicates that more N-H groups of refiner discharge gelatin were involved in hydrogen bonding. The amide B band of RDG and PG was detected at 2967.03 and 2965.86 cm^{-1} respectively. The amide B band is related to the asymmetrical stretch of CH_2. The amide I band which is associated with the C=O stretching vibration of the polypeptide backbone was found at 1654.47 (cm^{-1}) in RDG which is in agreement with data reported by Liu et al (2009) on gelatin from channel catfish (*Ictalurus Punctatus*) head bones. However, amide I band of gelatin extracted from the skin of giant squid (*Dosidicus gigas*) was observed at 1635 cm^{-1} (Uriarte-Montoya et al., 2011). The differences in the bands may be due to the fact that the amide I band shows several components or shoulders due to mixed secondary structures of protein. The extent of these changes are also influenced by the gelatin extraction conditions, the number of native crosslinks in the collagen structure, and the amount of collagenous tissues from which gelatin is extracted (Muyonga et al., 2004; Benjakul et al., 2009; Uriarte-Montoya et al., 2011). Nagarajan et al. (2012) reported that secondary structure and functional group of gelatins obtained from the skin of splendid squid was affected by extraction temperature. The gelatin obtained from pink perch surimi Refiner discharge (RDG) exhibited the amide II band at wavenumbers of 1578.53 cm^{-1}. The result was in accordance with the report of Kittiphattanabawon et al. (2016). The

amide III band of the RDG was observed at wavenumbers of 1232.79 cm^{-1}. The amide III helps in identifying the helix to coil structure of gelatin. So Amide III generally indicates disorder in the gelatin molecules due to loss of triple helix state of collagen. The amide III band indicates the combination peaks between CN stretching vibrations and NH deformation from the amide linkages as well as the absorptions arising from wagging vibrations of CH_2 groups in the glycine backbone and proline side-chains (Sinthusamran et al., 2014).

The result indicated that the primary structure, secondary structure and functional group of gelatin from pink perch surumi refiner discharge were similar to the porcine skin gelatin.

DSC measures the amount of heat energy absorbed or released by a sample, as it is heated, cooled or held at a constant temperature. Fig. 3 and 4 shows the DSC heating thermographs of RDG and PG. There is a broad endothermic peak at 85.64°C with onset temperature at 45.37°C and endset temperature at 144.10°C having transition enthalpy (ΔH) of

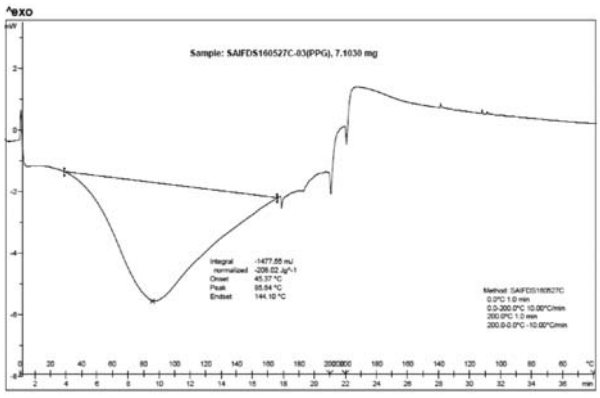

Fig. 3. DSC graph of refiner discharge gelatin

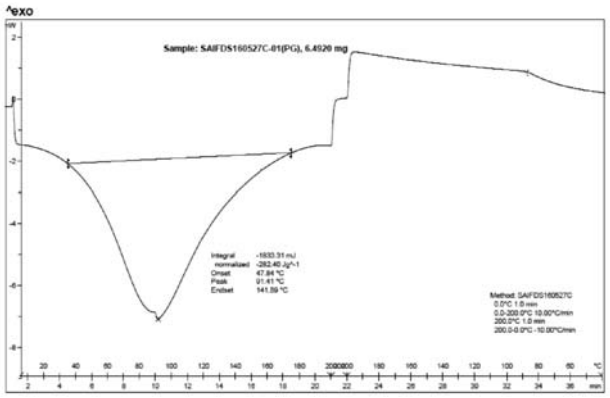

Fig. 4. DSC graph of porcine gelatin

Table 1. Infrared band of porcine gelatin (PG) and refiner discharge gelatin (RDG) given in wavenumbers (cm^{-1})

Designation	Approximate wavenumbers (cm^{-1})	PG	RDG
Amide A	3300	3452.14	3315.95
Amide B	3100	2965.86	2967.03
Amide I	1600-1690	1654.72	1654.47
Amide II	1480-1575	1579.00	1578.53
Amide III	1229-1301	1243.39	1232.79

208.02 J g^{-1} in RDG (Table 2). Similar transitions were also found in case of commercial porcine gelatin (Table 2). RDG showed lower melting/transition temperature than PG. Thus RDG is thermally less stable than PG. Beside this endothermic peak, there is no evidence of other major thermal events. The maximum of the endothermic temperature peak in DSC thermogram is taken as the melting/transition temperature of the gelatin. This transition is due to helix to coil transition and is a first order transition of kinetic character. Rahman et al. (2008) reported that the thermal transitions of gelatin differ with different sources and moisture content or water activity of the gelatin. Gelatin solution in water shows lower melting/transition temperature as water enhanced the mobility of gelatin polymer chains (Norziah et al., 2009; Uriarte-Montoya et al., 2011). DSC studies give an indication of the thermal stability of the triple helix in gelatin. The heat flow detected by DSC corresponds to the energy necessary to melt the junction zones and to achieve the helix-to-coil conformation (Cheow et al., 2007).

The Mineral composition of refiner discharge and extracted gelatin (in ppm) is given in Table 3. The mineral content of the extracted gelatin was lower than the gelatin obtained from the skins of bigeye snapper (Benjakul et al., 2009). This may be due to the demineralizing effect of EDTA, which was used as pretreatment agent in extraction process. The mineral in the refiner discharge could be leached out into the extraction medium during gelatin extraction to varying degrees and this have contributed to the lower mineral content in the final gelatin powder. Gelatin extracted from the skins of bigeye snapper were reported to be rich in minerals like calcium, sodium, potassium, and magnesium

Table 2. Transition temperatures and respective enthalpies of the porcine gelatin (PG) and refiner discharge gelatin (RDG)

Designation	PG	RDG
Onset temperature (°C)	47.84°C	45.37 °C
Peak temperature (°C)	91.41°C	85.64 °C
Endset temperature (°C)	141.89°C	144.10 °C
Enthalpy (ΔH)	282.80 J g^{-1}	208.02 J g^{-1}

Table 3. Mineral composition of refiner discharge and refiner discharge gelatin (ppm)

Minerals	Refiner discharge	Refiner discharge gelatin
Calcium	79327.14±393.25	1577.00±37.37
Sodium	950.81±9.45	708.58±16.96
Potassium	84.22±1.53	72.10±0.88
Magnesium	1775.97±36.61	94.23±2.01

Values are given as mean±standard deviation of triplicate. Same letters indicate insignificant difference ($p<0.05$)

and phosphorous (Benjakul et al., 2009). The available minerals in the gelatin could give added advantage in enhancing the functional properties or could be of health benefits.

Table 5 shows the viscosity of film forming solution (FFS) prepared with different concentrations of RDG. The concentrations of gelatin of the film forming solutions influenced solution viscosity. Viscosity of FFS increased from 2.26 to 7.38 cP with the increase in gelatin concentrations from 2 to 6%. Viscosity is partially controlled by molecular weight and molecular size distribution. Also viscosity of the FFS depends on the pH of the solution and the protein content (Cho et al., 2006) and presence of other chemical moieties like minerals. In this study the pH of the film forming solution was kept constant at neutral pH. Viscosity of the FFS is an important parameter because it affects the drying process. A lower viscous solution accelerates coacervation of the film forming solution from the film casting surface causing uneven coating to the surface. Higher viscosity reduces the phase separation between film forming solution and the surface and makes the film peeling process easy. However, highly viscous film forming solution may create uncontrollable film thickness (Han & Gennadios, 2005).

Color and opacity are important optical properties to evaluate the physical quality of the films for use in packaging. Color is related to the raw material used to make the products (de Rocha et al., 2013). In the present study, higher L* values were observed in all the films and there were no significant differences ($p>0.05$) among the gelatin concentrations in the FFS. However, the films show slight yellowness as seen from the b * values (Table 4). Also the films become more opaque at higher concentration of gelatin (Table 4). Hanani et al. (2012) reported that the colour of gelatin films made with commercial tilapia skin was affected by concentrations of gelatin in the film forming solution (4-8 %).

The mechanical properties of RDG films at different gelatin concentrations were expressed in terms of tensile strength (TS) and elongation at break (EAB) (Table 5). The films obtained with different concentration of gelatin were homogeneous, flexible and easy to handle except the one prepared with 2% gelatin concentration. The film from 2% gelatin content in FFS was too thin to peel off and rapidly shrunken after peeling off.

Table 4. Colour and opacity of the films as affected by gelatin concentration

Gelatin (%)	Colour parameters			Opacity
	L*	a*	b*	
3	87.92±1.50^{a}	-1.30±0.02^{b}	10.91±0.57^{b}	1.25±0.10^{a}
4	87.30±1.52^{a}	-1.29±0.02^{b}	10.67±0.52^{b}	1.20±0.10^{a}
5	87.83±1.50^{a}	-1.13±0.01^{a}	9.59±0.25^{a}	1.67±0.20^{c}
6	88.94±0.70^{a}	-1.14±0.02^{a}	9.63±0.50^{a}	1.63±0.75^{b}

Values are given as mean ± standard deviation of triplicate. Same alphabets in superscript indicate that the reulst are insignificant ($p>0.05$) within the parameter.

Table 5. Viscosity of film forming solutions, mechanical properties, solubility in water and swelling index

Gelatin (%)	Viscosity (Cp)	Tensile strength (kg/cm^2)	EAB (%)	Solubility in water (%)	Swelling index
2	2.268	-	-	-	-
3	3.624	66.69 ±4.05^a	129.1± 7.87 ^{a}	54.97±1.70^c	451.310± 2.05^c
4	4.152	73.50±7.05^a	190.8±8.40^b	52.69±1.20^b	451.069±2.10^c
5	5.09	69.50±5.38^a	186.6±7.26^b	51.89±2.00^b	172.030±3.00^b
6	7.83	71.33±7.27^b	203.6±9.90^c	47.12±2.70^a	160.890±2.80^a

Values are given as mean± standard deviation of triplicate. Same letters indicate insignificant difference ($p<0.05$) within a parameter.

TS of the film increased with the increase in gelatin concentration, but the increase was not significant ($p<0.05$). Similar observation was also reported by Kaewprachu et al. (2016) in myofibrillar protein based film. The result was also in accordance with Hanani et al. (2012) who found no significant difference in TS of gelatin films made with 4 and 6% of gelatin in film forming solutions. In general, TS of the film increased with increasing protein concentration because as the protein increases the chances of number of potential intermolecular interactions also increases. TS of the films also depend on the gel strength of the gelatin used in FFS. Ninan et al. (2010) compared the films properties of carp skin gelatin with porcine skin gelatin and bovine skin gelatin and found that the higher the gel strength of the gelatin used, the better is the TS.

There is a significant difference ($p<0.05$) between EAB of films with different gelation concentrations. EAB increases from 129.1 to 203.6% with increasing gelatin concentration from 3 to 6% in the FFS, respectively. The result was in accordance with those reported by Jongjareonrak et al. (2006) in which the EAB increased with increasing protein concentrations. Another possibility for higher EAB is that higher protein content might result in a higher intermolecular aggregation of protein, compared with the lower amount, resulting in improved flexibility of the films (Kaewprachu et al. (2016). Ironically, Ku et al. (2008) reported that the higher the polymer contents in the film forming solution the higher the resistance of the films.

Ultimately, the intended applications of the films will be the deciding factor for optimum values of TS and EAB of the films. Film with high extensibility is require for food wrap application whereas high TS and low EAB values of film is required to tolerate normal stress and maintain its integrity during handling and transport (Kaewprachu et al. 2016).

Solubility in water is an important property of edible films. Water solubility measure the film´s tolerance to water, and higher solubility of a film indicates lower resistance of the film in water (Handa et al. 1999).The solubility of the developed RDG films ranges from 54.97- 41.12% (Table 5) which is lower than film made with cod-skin gelatin which has high water solubility values of 90–100% (Perez-Mateos et al., 2009). However, it is higher than the tuna skin gelatin film solubility reported to be 39.90% (Gómez-Estaca et al., 2009). Different intended applications of films required different solubility index. Some applications may require water insolubility to enhance product integrity and water resistance. While, in some cases water solubility before consumption of the product might be beneficial. The solubility of the film depends on presence of very high molecular weight peptide fractions and heat stable crosslinked fractions after drying of the film (Carvalho et al., 2008, Gomez-Estaca et al., 2009).

Swelling index indicates the amount of water or liquid that can be absorbed by the film. The results reported in Table 5 indicated an inverse relation with the swelling index of gelatin film and the concentration of gelatin in FFS. The swelling index values decreased from 451.31 to 160.89 as the gelatin in FFS increases from 3 to 6% respectively. Generally, biopolymer films produced from carbohydrates or proteins initially swell when they absorb water, which results in changes of their structure. Hence, details relevant to swelling characteristics are essential for successful application of biopolymer films. As the film gets in contact with the

solvent, the solvent diffuses into the gel phase and mobilize the peptide chains. As a result, the probability that one peptide chain meets another chain increases, so that the rate of crosslinking reactions to form a strong network also increases (Durmaz et al., 2002).

The utilization of surimi processing waste (refiner discharge) as an alternative source of gelatin production was investigated in this study. The FTIR and DSC results show that the gelatin obtained from such processing waste has more or less similar properties with that of commercial porcine gelatin. The refiner discharge gelatin also shows good film forming properties with higher lightness value. Hence, refiner discharge of surimi processing industries can be explored as a gelatin source and for edible film development.

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