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Research Article

The Effect of Different Factors on the Emulsibility and Emulsifying Stability of Collagen

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Abstract: The objective was to study the effects of different concentrations, pH, temperature and electrolytic on Emulsifying Activity (EA) and Emulsifying Stability (ES) of collagen extracted from *Amiurus nebulosus* skin. Method: centrifugation. The results showed that EA of Acid-Soluble Collagen (ASC) and Pepsin-Soluble Collagen (PSC) increase quickly with the increasing collagens concentration and became stable when collagen concentration was up to 0.3%. The EA was also affected by pH, EA of ASC reached the minimum at isoelectric point pH 4.0 and EA of PSC reached the minimum at isoelectric point pH 5.0; EA of collagen increased with addition of NaCl within lower concentration; EA of ASC reached the maximum 51.11% at 55°C and EA of PSC reached the maximum 55.56% at 60°C. Conclusion: it is closely related to the collagen concentration, pH, temperature and electrolyte of collagen from *Amiurus nebulosus* skin. The change of ES was complicated, sometimes emulsibility was good, emulsifying stability was poor and vice versa.

Keywords: Collagen, emulsifying activity, emulsifying stability, influence factors

INTRODUCTION

Collagen of aquatic products which have the advantages of green, health, environmental protection and good quality. Collagen of fish as a green additives, it has been widely used in dairy products, clarifying agent, stabilizer and other food additives. Collagen will generate a low molecular weight collagen peptide that is also used for functional food additives in the process of hydrolysis (Friess, 1998; Willoughby *et al*., 2002; Takami *et al*., 1998; Shanmugasundaram *et al*., 2001; Chen *et al*., 2002).

It is a kind of important functional properties of collagen emulsification; it mainly includes the emulsifying activity and emulsifying stability. Oil and water can form a uniform mixture by the emulsification of the collagen, which can resist external force broken and keep the oil-water mixture is not isolated in a certain extent (Shen *et al*., 2008). In the process of food processing, it is an important quality control index that the emulsification and emulsion stability of the protein. Wang *et al*. (2003) and Zhang (2004) studied the effect of different factors on the emulsification and the emulsion stability of collagen. Lin and Chen (2006) studied the emulsification and emulsion stability of modification collagen. It can provide theoretical foundation for the research of collagen features.

It is far less than other countries in varieties and content of food emulsifier in China. Therefore, in order to enrich the role of food emulsifier in China, it will become the concept of the industry to develop new products which is the development of new varieties of multi-function emulsifier.

This paper studied the effect of different concentration, pH, temperature and electrolyte on *Amiurus nebulosus* skin ASC and PSC (Chen *et al*., 2010, 2011) emulsification and emulsion stability, it can provide certain theoretical basis for *Amiurus nebulosus* skin efficient, value-added utilization of resources and the application of collagen in the field of food. It is looking forward to *Amiurus nebulosus* skin collagen have good emulsifying properties in the applications of meat food.

MATERIALS AND METHODS

Raw material and equipment: Collagen (ASC and PSC) extracted from Amiurus nebulosus skin; Salad Oil.

76-1A digital constant temperature water-bath; The DELTA-320 pH meter; TDL-5A centrifuge; TP electronic balance; IKA-T25 dispersion machine.

The determination methods of emulsibility and emulsifying stability (Guo *et al*., 2007): A certain volume collagen solution and the same volume of salad oil mixed which 10000 r/min scatter 2 min in the high speed homogenizer, then 1500 r/min centrifuge 5 min.

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The total height of liquid and the height of emulsion layer were recorded after centrifuge. Emulsifying Activity was calculated as (1):

$$\text{Emulsifying activity (\%)} = (H1/H0) \times 100 \quad (1)$$

Centrifuge tube heated 40 min at 70°C water bath, cooling, then 1500 r/min centrifuge 5 min, height of emulsion layer was recorded once again. Emulsifying Stability was calculated as (2):

$$\text{Emulsifying stability (\%)} = (H2/H1) \times 100 \quad (2)$$

where,
H0 = The total height of liquid
H1 = The original height of emulsion layer
H2 = The second height of emulsion layer

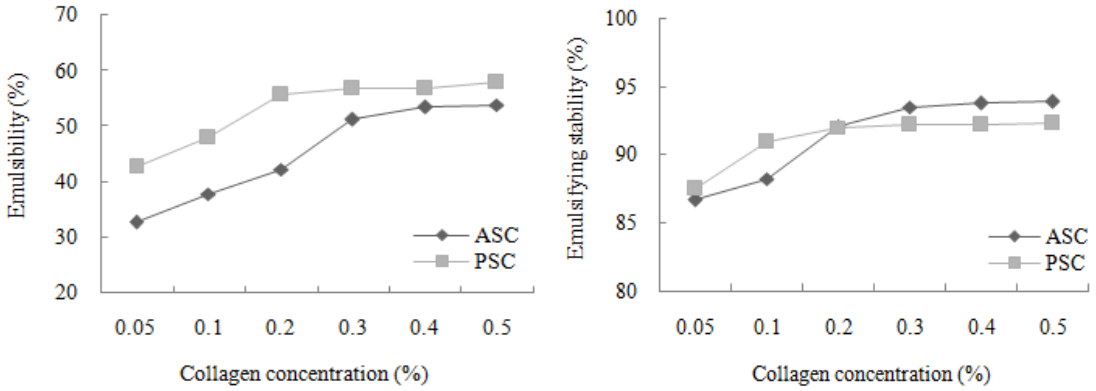
RESULTS AND DISCUSSION

The effect of collagen concentration on the emulsibility and emulsifying stability: Prepared Amiurus nebulosus skin collagen concentration were 0.1, 0.2, 0.3, 0.4 and 0.5% (W/V) at pH7.0, respectively, the influence of Amiurus nebulosus skin collagen concentration on the emulsibility and emulsion stability was shown in Fig 1. It could be seen that the emulsion of collagen increased as the increase of collagen concentration at pH 7.0 (Fig. 1a). Emulsibility of ASC and PSC increases faster that collagen concentration ranging from 0.1 to 0.3% (ASC) and ranging from 0.1 to 0.2% (PSC); collagen emulsibility of ASC and PSC stabilized gradually when collagen concentration continue to increase; collagen emulsibility of ASC and PSC reached the maximum when the concentration of ASC and PSC were 0.3 and 0.2%, respectively. This phenomenon may be attributed to the surface adsorption during the increasing of collagen concentration process.

Emulsifying stability stabilized gradually as the increase of collagen concentration (Fig. 1b), which was the same change trend with the emulsibility. Adsorption trend of collagen molecules was gradually increasing as the increase of collagen concentration that Amiurus nebulosus skin collagen concentration was in low range. Amiurus nebulosus skin collagen concentration continue to increase, adsorption layer could gradually form a certain thickness and strength, even become the relatively tight interfacial film, at this moment, it was not obvious change that collagen molecules was combination with salad oil; that was to say, the thickness and strength of interfacial film increased very slowly, then emulsifying stability become relatively stable (Gao *et al*., 2011).

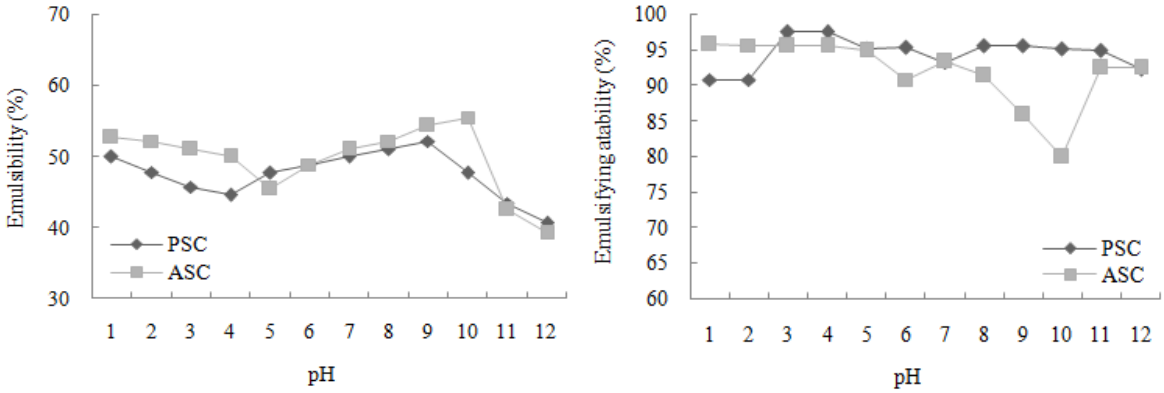
The effect of pH on the emulsibility and emulsifying stability: pH is one of the most important factors for determination of protein, In this section, pH was used to determine the effect of emulsibility and emulsion stability on Amiurus nebulosus skin collagen. It had obvious influence of pH on the emulsibility and emulsion stability for Amiurus nebulosus skin collagen (Fig. 2). pH range from 1 to 4 or 5, the emulsibility of Amiurus nebulosus skin collagen reduced gradually and the emulsibility of ASC and PSC achieved minimum respectively (Fig. 2a); then pH continued to increase, the emulsibility of ASC and PSC also increased. When the pH at 9 and 10, the emulsibility of ASC and PSC achieved maximum, respectively. It might be due to the collagen is typical of amphoteric polyelectrolytes, it had acidic and alkaline groups on its side chain which would gradually ionization to macromolecular ion and counter ion in the solution. Eventually, collagen net charge changed with the change of pH, so the emulsibility of ASC and PSC presented a different state at different pH.

When pH is far away from the isoelectric point of collagen, collagen static charges is the higher, the more repel of the intermolecular charges each other, which could form the better dispersion of intermolecular, then the higher the emulsibility of collagen solution. It was



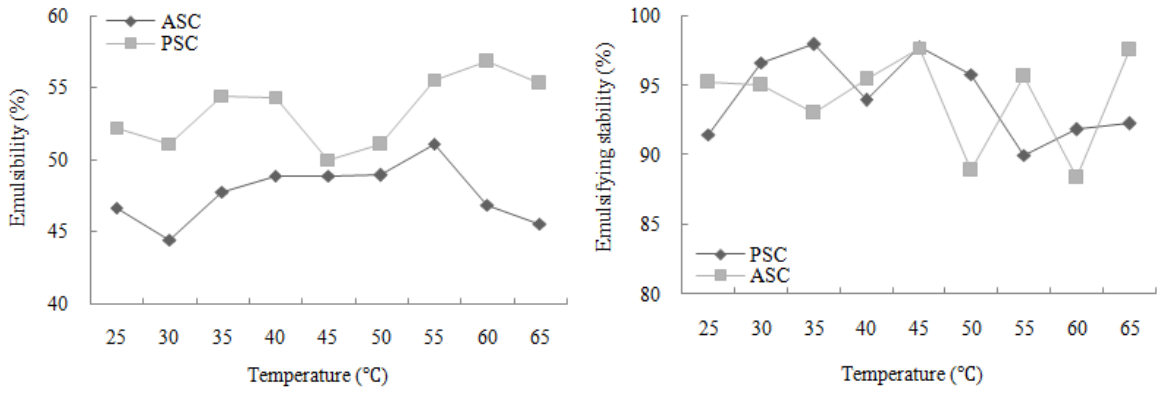
(a) The emulsibility (b) Emulsifying stability

Fig. 1: The effect of collagen concertration on the emulsibility and emulsifying stability of ASC and PSC



(a) The emulsibility (b) Emulsifying stability

Fig. 2: The effect of pH on the emulsibility and emulsifying stability of ASC and PSC



(a) The emulsibility (b) Emulsifying stability

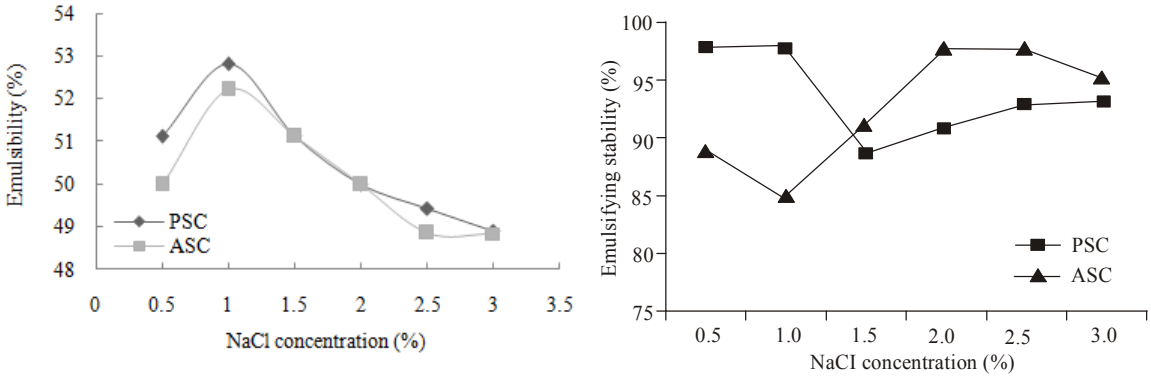
Fig. 3: The effect of temperature on the emulsibility and emulsifying stability of ASC and PSC

existed mainly in amphoteric ion and the electric charge was zero of collagen which pH closed to the isoelectric point of collagen, then the solubility was the minimum and the emulsibility was the lowest of collagen, this result was similar to the result (Wang *et al*., 2003) report. Moreover, as shown in Fig. 2b, emulsifying stability reduced, although emulsibility was improved which changed pH in a certain range. This might be due to be the structure of collagen was damaged by strong acid and alkaline.

The effect of temperature on the emulsibility and emulsion stability: It could be seen that the effect of temperature on the emulsibility and emulsion stability for Amiurus nebulosus skin collagen (Fig. 3). The emulsibility of ASC and PSC exhibited a similar change trend at different temperature (Fig. 3a), but the emulsibility of PSC was higher than ASC. It had obvious downtrend from 25 to 30°C and appear rising trend from 30 to 40°C for the emulsibility of ASC and PSC. The maximum of emulsibility of ASC (51.11%) and PSC (56.82%) was observed at 55 and 60°C, respectively. It indicated that the higher the temperature, the better the emulsibility at a certain temperature range, it was because that the conformation of collagen molecules was gradually broken, which was accompanied by denaturation, a large number of hydrophobic groups was exposed, eventually it was converted into the orderly configuration of collagen, so that it was easy for the salad oil to be adsorbed on the interface of collagen and the emulsibility of collagen was to be improved (Cui and Ge, 2000).

Figure 3b showed the emulsion stability of collagen from Amiurus nebulosus skin at different temperature. It showed a trend of wave changes for the emulsion stability of ASC and PSC and the changing trends of ASC and PSC were completely opposite. It might be due to be the change of external environment.

The effect of NaCl concentration on the emulsibility and emulsion stability: Figure 4a depicts the effect of NaCl concentration on the emulsibility of collagen from Amiurus nebulosus skin. The maximum of ASC (52.22%) and PSC (52.81%) were observed at NaCl concentration 1.0%. With the future increasing NaCl concentration, a sharp decrease in emulsibility of ASC



(a) The emulsibility (b) Emulsifying stability

Fig. 4: The effect of NaCl concertration on the emulsibility and emulsifying stability of ASC and PSC

and PSC was observed; which reached the lowest emulsibility of ASC and PSC at NaCl concentration 3.0%. It was possible that had two different effects adding electrolyte in emulsion system; on the one hand, it could promote collagen molecules repulsive force at low NaCl concentration range of 0.5-1.0%, which was to the benefits of dissolution of collagen, thus, NaCl fully play the role of the surfactant, which was easy to combine salad oil and collagen, eventually the emulsibility of collagen was enhanced significantly; on the other hand, NaCl at higher concentration range of (1.0~3.0%), might result in decreased the emulsibility of collagen via a 'salting out' effect by increasing hydrophobic interaction and aggregation and competing with the protein for water. collagen precipitation can be induced especially at high salt concentration (Jiang, 2006).

The effect of NaCl concentration on emulsion stability of ASC and PSC was shown in Fig. 4b. It was just the opposite to the emulsion stability of ASC and PSC, NaCl concentration was from 0.5 to 1.5%, the emulsion stability of PSC was better than ASC, the emulsion stability of PSC was the highest and the emulsion stability of ASC was the lowest when NaCl concentration was at 1.0%, but with the further increasing NaCl concentration, the emulsion stability of ASC had an advantage over PSC.

CONCLUSION

It was closely related to collagen concentration, pH, temperature and electrolyte of ASC and PSC emulsibility and emulsion stability, it had complicated changes of emulsion stability. Therefore, Comprehensive consideration the change of the collagen emulsibility and emulsion stability to get the best effect.

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