Improving Physicochemical Properties of Catfish Gelatin Films through the Maillard Reaction Assisted by Radio Frequency

by

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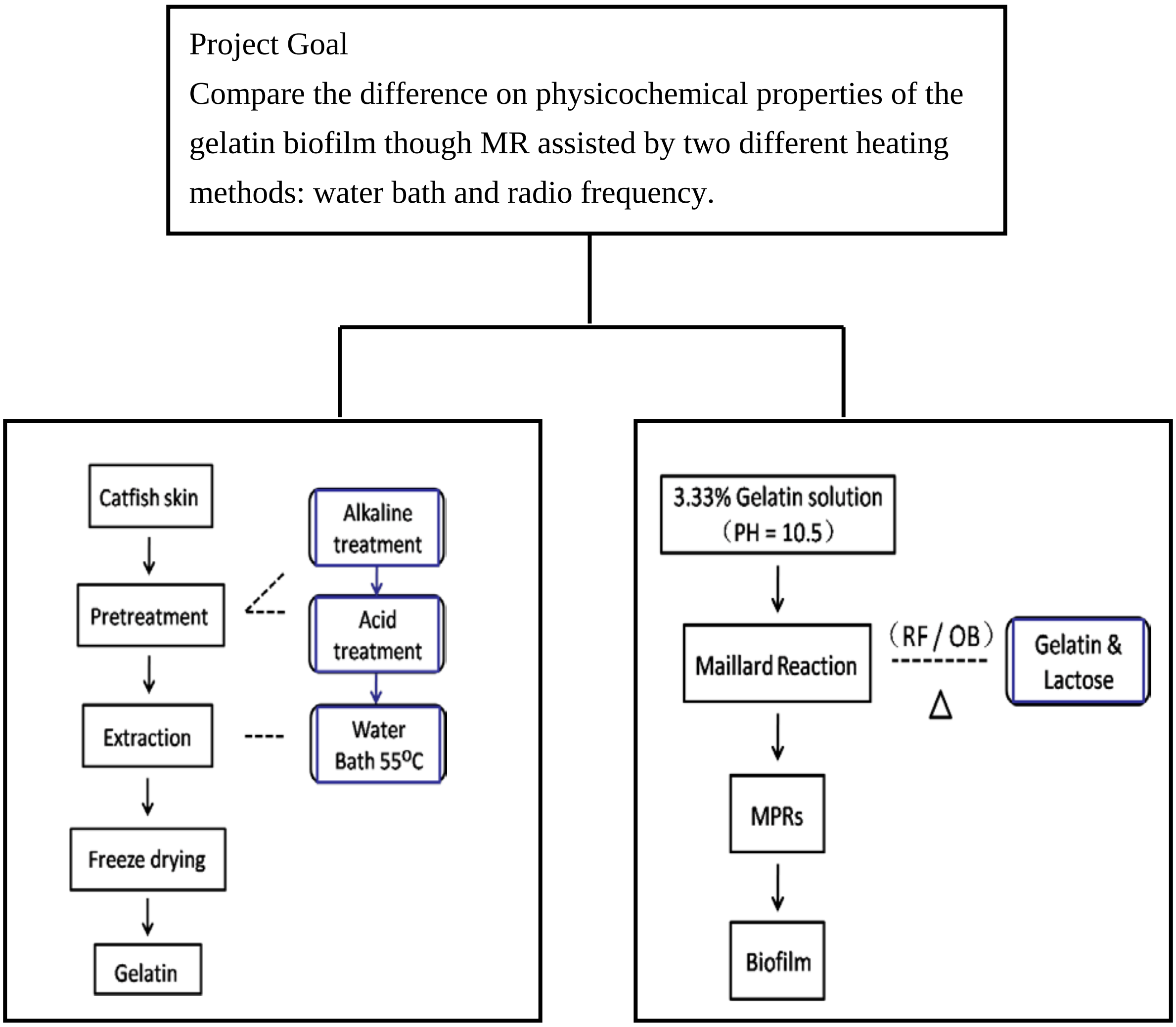
Abstract

Encapsulation is a promising technology in food industries and biotechnology field. Native gelatin extracted from the catfish skins to be made as the wall materials of encapsulation have potential applications. High qualities of the wall materials are mainly dependent on the low permeability, high thermostability, and good mechanical properties. In this study, lactose was applied to have the Maillard reaction (MR) with 3.33% gelatin solution, finally, the Maillard reaction products (MRPs) were formed to change the physicochemical properties of gelatin biofilm. Additionally, radio frequency (RF) system instead of the water bath (WB) method was used to heat MR because of its volumetric heat transfer, huge heat energy, deep heat penetration, and homogeneous heating mode. The viscosity, pH value, and the absorbance (294nm & 420nm) were important solution indicators to measure to extent of MR. Meanwhile, water vapor permeability (WVP), oxygen transmission rate (ORT) and mechanical properties were crucial factors to the gelatin films. Other film properties, such as light transmission (LT) and glass transition temperature (T_g) were also evaluated. Among the different reactant ratio (1:2, 1:1, 2:1) and heating time (2 h, 4 h, 6 h) used, the 1:1 MR system treated by RF heating at 2 h obtained the optimal yield of the MRPs, subsequently rendered the preferred tensile strength (TS), elongation (E), T_g and OTR,

while increased WVP. Furthermore, MR treated by RF heating could greatly reduce the heating time and save the energy consumption.

Visual Abstract



Project Goal

Compare the difference on physicochemical properties of the gelatin biofilm though MR assisted by two different heating methods: water bath and radio frequency.

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Chapter 1 Problem statement and Research Objectives

1.1 Problem statement

The development of encapsulation technology is promising in biotechnology field. With encapsulation technology, it is possible to allow the incorporation of sensitive functional ingredients into food, medicine and pharmaceutical without affecting their taste, aroma, texture, and nutrition. One significant reason to apply encapsulation technology is to greatly improve the stability and bioactivity of the final products. The other is to protect the core materials from external condition, especially the moisture and oxygen.

In encapsulation products, film (wall) materials play an important role in protecting the core ingredients, controlling the release rate, and maintaining the balance on two sides. The characteristics of film materials determine the quality of final outcome. As an alternative source, the native gelatin extracted from the fish skin to be made as the biofilm of the encapsulant is a good choice. Compared with the synthetic materials, natural gelatin is biodegradable and edible with high nutrition. Meanwhile, gelatin film is able to form a barrier between the internal phase and the surroundings (Viktor Nedovic et al., 2011). Among fish species, catfish is preferred owing to religious sentiments, low production cost, and easy availability.

In order to improve the physicochemical properties of the gelatin films, a chemical modification on the structure of gelatin is performed by Maillard reaction (MR). MR is an amino-carbonyl reaction between reducing sugars and proteinaceous compounds resulting in Maillard reaction products (MRPs), including melanoidins, reductions and nitrogenous or sulfurated heterocyclic compounds (Liu et al., 2009). In this study, lactose has been chosen as the reducing sugar because of its high thermal stability, which can modify the gelatin to form the encapsulant more efficiently. MR occurs spontaneously during food processing and storage. The final products MRPs have special odor with brown color and exhibit the strong antioxidant activities. Therefore, MRPs of protein-sugar have been investigated as suitable encapsulant to protect sensitive core materials against oxidation (Drusch et al., 2009 & Kosaraju et al., 2009). Additionally, the antioxidative activity of MRPs is affected by some factors, including reactant concentration, initial pH, and water activity, especially the heating condition (temperature and time).

In this research, radio frequency (RF) has been utilized to improve the MR process. RF heating is a promising kind of dielectric heating technology, which has advantages of volumetric heat transfer, huge heat energy, deep heat penetration and homogeneous heating mode. Compared with the traditional heating method (conduction/convection heating), RF heating is more uniform because of the direct interaction between the object being heated and electromagnetic waves (Datta and Hu, 1992). RF has been widely used in food processing. However, up to now, none research has been reported of RF heating on MR to gelatin biofilm.

1.2 Research objectives

This research focused on improving the physicochemical properties of catfish gelatin biofilms through MR assisted by RF heating. During gelatin extraction process, a combined alkaline-acid method was conducted to obtain good quality gelatin with high yield. In the following MR process, the ratio of gelatin and lactose, as well as the reaction time were optimized to get the highest yield of MRPs. Moreover, RF heating method was utilized for MR so as to obtain more MRPs, which may furtherly improve the physicochemical properties of the gelatin films. Limited research has been carried out on MR to improve gelatin films. While RF is the first time applied to heat MR. This work aims to gain a better understanding of RF and judge its feasibility for MR and the improvement on physicochemical properties of final biofilm, which can be summarized as follows:

- Study the effect of MR on the physicochemical properties of catfish gelatin films.
- Optimize the reactant ratio and reaction time to get the highest yield of MRPs.
- Compare the difference on physicochemical properties of the gelatin biofilm though MR assisted by two different heating methods: water bath and radio frequency.

Chapter 2 Literature review

2.1 Encapsulation technology

In food industry, encapsulation technology has caught abundant attention. The unique functions make it quite popular in food packaging and processing fields. Encapsulation technology can protect the core materials from degradation by reducing its reactivity to the outside environment, control the release of the core materials at slow speed over time, or at a particular time, and separate the components of the mixture that would otherwise react with one another. Furthermore, encapsulation technology is able to mask an unwanted flavor or taste of the core materials and modify the physical characteristics of the raw material to allow easier handling (Fang & Bhandari., 2010). As to the encapsulation technology, encapsulant is the key factor to the quality of final products. Encapsulant is also known as the wall materials or film materials, which has a strong protection on the core materials. Nowadays, the edible biofilm to replace those synthetic polymer films has become the tendency on the food industries.

2.2 Biofilm

Film (wall) materials, as the encapsulant, are considered a thin layer of given material formed on a food as a coating or placed as a preformed self-standing film on or between food components (Krochta, J.M. et al., 1997), as shown in Figure 1. It is an environmental friendly technology that is applied on many products to control moisture transfer, gas exchange or oxidation processes (R.K.DHALL, 2013). Film (wall) materials are a key factor of encapsulation products. The major advantage of utilizing film (wall) materials is to allow several active ingredients incorporated into the polymer matrix, and then to form a barrier between the core material and external surroundings, so as to improve safety, stability, even nutritional and sensory attributes.

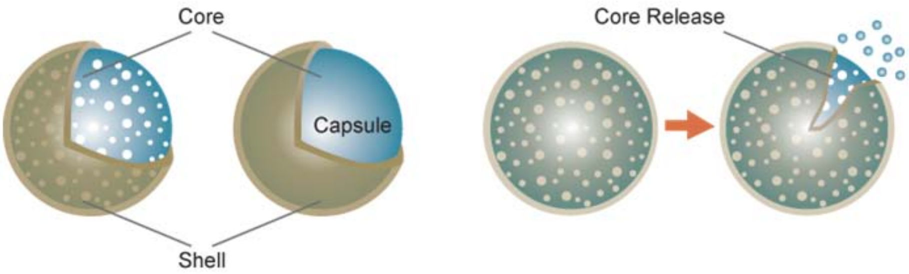


Fig. 1 The wall material of the encapsulation

Among all materials, the most widely used in food applications are polysaccharides. Proteins and lipids are also appropriate for encapsulation (Viktor Nedovic er al., 2011), which are reported to be good for the physical properties of the film (wall) materials. In food and pharmaceutical industries, the criteria for the film (wall) materials should be natural, biodegradable and food-grade, with good rheological characteristics. These explain why the majority encapsulant tended to be biotic, such as the gelatin, caseins, gluten, etc. Besides the functional properties, the physicochemical properties are of vital importance to indicate the quality of the film materials, mainly including the mechanical properties, thermal properties and permeability.

As a film (wall) material, the mechanical properties are very important, mainly including tensile strength (TS) and elongation at break (EAB). TS is the ability of a material to withstand a longitudinal stress, expressed as the greatest stress that the material can stand without breaking, while EAB is defined as the length at breaking point expressed as a percentage of its original length, which is also known as the moment of rupture of the material. High TS is generally necessary for edible films in order to withstand the normal stress encountered during their application, subsequent shipping, and food handling. However, the flexibility of edible films, for instance the EAB should be adjusted according to the intended application of edible films (Munehiko Tanaka et al., 2001). As TS and E have opposite function for film materials, it is crucial to control the balance between these two mechanical properties to obtain suitable biofilm.

As to chemical property, the glass transition temperature (T_g) is considered as a good indicator on thermostability as well as compatibility of the film material, at which the material undergoes a structural transition from an amorphous solid state (glassy state) to a more viscous “rubbery” state (L. Yang and A.T. Paulson, 2000). The films would be flexible and pliable over T_g, compared to rigid and brittle at normal state. Moreover, glass transition is proved to have a great effect on molecular mobility of the film structure, which will reduce the barrier properties of the biofilm. Therefore, the film material with high T_g is preferred.

The permeability also has quite important position on film (wall) materials, especially the water vapor permeability (WVP) and oxygen transition rate (OTR). WVP is a measure of the mass of water vapor transmitted through a unit area in a unit time under specified conditions of temperature and humidity, while OTR is a measure of the quantity of oxygen gas passing through a unit area of the parallel surfaces of a film per unit time under the conditions of test (ASTM D3985). Low WVP and OTR of the film material can ensure the core material in good protection during the food application.

There has been a variety of methods to be attempted in order to improve the physicochemical properties of biofilm on better protection of the core materials. In this study, an innovative Maillard reaction is applied to promote the quality of biofilm.

2.3 Maillard Reaction

The Maillard reaction, also famous as amino-carbonyl reaction, is a non-enzymatic interaction between reducing sugar and proteinaceous compound at high temperature, shown in Figure 2. The MR occurs spontaneously during food processing and storage with unfathomed details and complex processes. MR is not one reaction pathway but a whole network of various reactions in three major stages. It is known that the final Maillard reaction products (MRPs) possess strong antioxidant activities, which are composed of melanoidins, reductones, heterocyclic nitrogenous compounds and some other highly colored products with special aroma.

MRPs play an important role in improving the food quality and nutritional values, such as flavor, appearance, and texture. Meanwhile, MRPs have caught much attention on their antibiotic, antimicrobial, and antiallergenic effects. Above all, it has been reported that MRPs are most likely to be the suitable encapsulant to protect the core materials by the external condition.

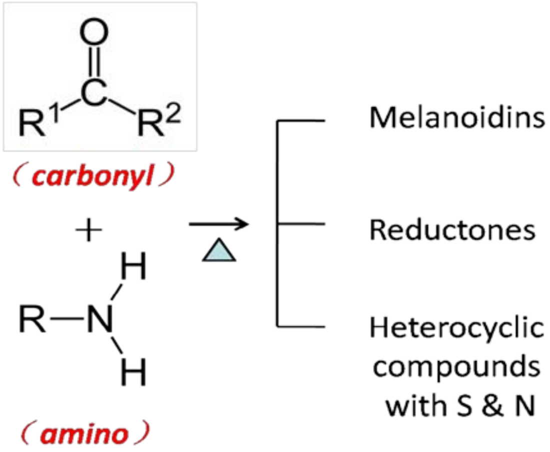


Fig. 2 Amino-carbonyl reaction

2.3.1 MR principle

MR is notoriously difficult to control. As a matter of fact, the details of MR are by no means understood nowadays. For the initial stage, the reactions have different paths for different sugars. Usually, the reducing aldose condenses with the proteinaceous compound, considered as the aldehyde group reacting with the ε-amino groups or α-amino groups of the terminal amino acids, to give a condensation product named Schiff′s base. It shortly rings to the N-substituted glycosilamine, which is still active and easy to rearrange to the Amadori rearrangement product (ARP) (1-amino-1-deoxy-2 ketose). Otherwise, the Heyenes rearrangement product (HRP)

(2-amino-2-deoxy-glucose) will be formed from N-substituted fructosamine, when ketone groups are used to react with the amino groups. ARP and HRP are primary products, the precursors of MR without special aroma. Subsequently, degradation, fragmentation, and dehydration of the primary products will occur in the intermediate stage, which are largely dependent on the pH of the system. Deamination and dehydration processes (1,2 -enolization) will happen under the acid condition, forming the furfural or hydroxymethylfurfural (HMF). HMF has a close relationship with the browning degree of MR, and the browning reaction will undergo as soon as the HMF are accumulated. Conversely, when it comes to alkaline condition, the primary products will continue on degradation and rearrangement (2,3 enolization) and form the unstable dicarbonyl compound, from which the reductones and some short-chain hydrolytic fission products can be obtained by cleavage reaction. Reductones have good antioxidant abilities by providing the electron to break free radical chain reaction. Under the high temperature, partial reductones will sequentially dissociate into a variety of small molecular products (acetic acid, acetol, and diacetyl) or furtherly condense with the amino substances. Additionally, the highly reactive dicarbonyl compound also takes part in another reaction, famous as the Strecker degradation. When dicarbonyl compound and amino acid exist together in the system, decarboxylation and deamination will happen on the amino acid, and amino groups will transfer to the dicarbonyl compound molecule, forming the Strecker aldehyde and carbon dioxide. So far, the intermediate products are already very complicated, but they will still take place a series of final reactions in the advanced stage, generally including two parts: aldehyde-self-condensation with the further dehydration to obtain more stable unsaturated aldehyde; and the random compounds polymerization with further rearrangement, cyclisation, isomerisation, and retroaldolisation to ultimately form the complex polymeric pigments, named melanoidins. Melanoidins, with reductones and heterocyclic nitrogenous compounds, are famous as the MRPs, possessing strong antioxidant abilities. The total process to form the final MRPs is shown in Figure 3.

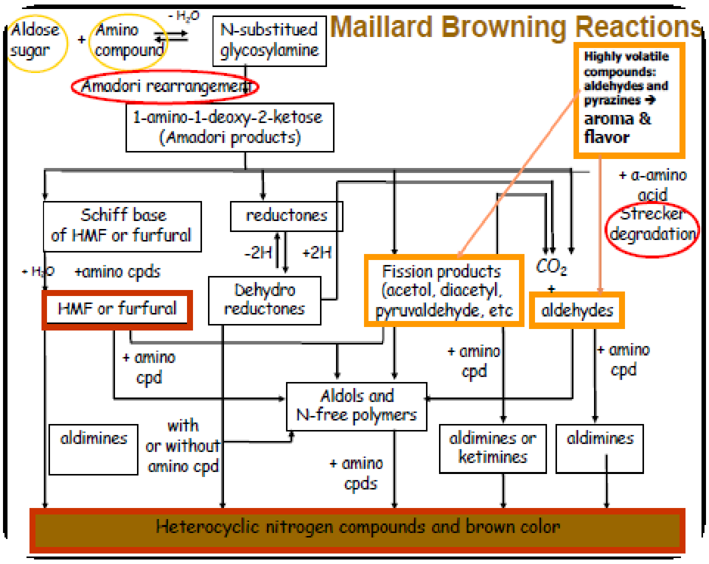


Fig.3 Maillard reaction process flow chart

2.3.2 MR influence factor

The complexity of MR is influenced by several factors, including the ratio and type of involved amino compounds and sugars, temperature-time combination, pH value, water activity as well as a variety of random chance. In reality, MR occurs at a wide range of temperatures, and the lower limit can be around the room temperature.

However, it may take long time to form the MRPs at low temperature. On the contrary, it is noticeable to see the browning phenomenon in a short time at high temperature. High temperature catalyzes the combination of carbonyl groups and amino groups, accelerates the transformation of molecular structure, and also advances the molecular rearrangement with the formation of MRPs rapidly. However, when the temperature reaches too high, caramelization (over 140^{o}C) and subsequently pyrolysis (over 180 ^{o}C) become more pronounced. The formation of those final products directly reduces the MRPs yield. In order to obtain more accessible MRPs, the optimal temperature is usually controlled between 100^{o}C and 140^{o}C. Table 1 explains the different temperatures on above reactions between proteinaceous materials and reducing sugars.

Table.1 The influence of temperature on Amino-carbonyl reaction

Above 400F	Mostly pyrolysis, with the possibility of carbonization. Complicated compounds and gas mixture will be formed.
~ 300 - 400F	Increasing caramelization with higher temps, which uses up sugars and thus inhibits Maillard at the high end of this
~ 250 - 300F	Maillard progresses at a fast pace, probably causing browning noticeably within minutes.
~ 212 - 250F	Maillard gets mildly as temperature goes up, generally requiring many hours to finish the whole process.
~ 130 - 212F	Maillard requires water, high protein, sugar, and alkaline conditions to advance noticeably in a matter of hours.
Below 130F	Enzymatic browning is often more significant in many foods than Maillard, but Maillard will still occur over periods from days or months to years, with progressively longer times at lower temperatures

Beyond that, heating time is regarded as another crucial factor to the MR. Generally, under the same condition, short time probably make the MR incompletely, resulting in low MRPs yield, while long time may also cause only a handful of the MRPs, owing to the formation of other products known as the advanced glycation endproducts (AGEs), which are harmful to human. Extended heating will increase the possibility of AGEs as the final products, but not the MRPs. The optimal heating time mainly depends on the kinds of proteinaceous compounds and reducing sugars.

Apart from the heating temperature and time, pH range is significant during the whole MR process. Generally, under acid condition, there will be lots of free H^+ floating around the negatively charged part of the amino acid (COO^-), which makes the amino group protonated and yield it less nucleophilic. As the negative oxygen bonds protonate with the free H^+ to form the stable –COOH, and it is hard for –COOH reacting with the amino groups. Additionally, the MR is a reversible process. Acid condition will cause the condensation products easily to be hydrolyzed, resulting in the hydrolysate (free amino groups) obstruction in the final stage, which will hinder the MR in a large extent. In contrast, alkaline condition can assist the MR to occur advantageously. Firstly, high pH value helps to loosen the protons and will possibly even deprotonate, which will give the reducing sugars a greater chance to react with the amino acids, shown in Figure 4. Secondly, it should be noted that alkaline condition can also increase electron density on the Amadori products making the occurrence of 2,3 enolization more easily than1,2 enolization, which contributes to favour the reductone formation as well as the aldehydes over furfural production, leading to the browning. However, alkalinity should not be too strong since excessive alkaline would probably make partial proteinaceous compound denatured; even hydrolyzed, which will cause the loss of the reactant. Therefore, the optimal pH should be adjusted between 9 and 12 initially.

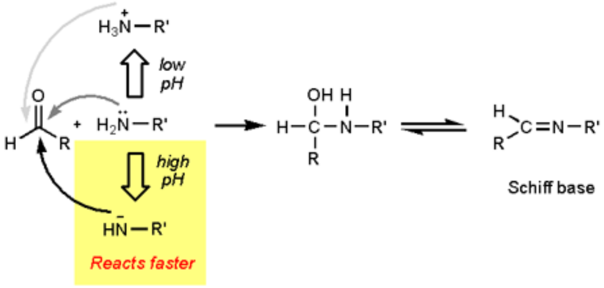


Fig.4 The influence of pH on Maillard Reaction

Besides the external conditions, the reactants themselves have great effects on MR, mainly including reactant category and reactant ratio. There are various kinds of reducing sugars and proteinaceous substance. Each of the reducing sugar has its own structure and characteristic, meanwhile, the number and the location of the carbonyl group in each reducing sugar are also different, same as the proteinaceous substance. This explains why different amino-carbonyl combination can produce hundreds of the MRPs. In addition, different reactant ratios will result in the different MRPs too. Commonly, more carbonyl groups can speed up the MR rate and obtain MRPs more rapidly. It is because the free amino groups (-NH_2), with relatively low Mw (16), are weakness parts during the MR process, which will be ‘attacked’ continuously by the carbonyl groups (-C=O), with relatively large Mw (28). Therefore, more carbonyl groups will accelerate the reaction to the positive direction at a fast rate, whereas excessive carbonyl groups will break the balance of system and result in caramelization, even the AGEs after the amino groups are totally exhausted. The reactant ratio (carbonyl/amino) for the optimum MR rate should be controlled from 1/1 to 2/1. Summary on the effect of reactant category and ratio on the MR rate are reported in Table 2. As to the biofilm, the edible catfish gelatin is a preferred choice, while lactose, as the reducing sugar, is selected to improve the quality of gelatin biofilm by MR.

Table.2 The effect of reactants on MR rate

Reducing sugars	
Unsaturated aldehyde > Dicarbonyl compound > ketose	Pentose: ribose > arabinose > xylose
Monosaccharide > Disaccharide (Polysaccharide is usually unreactive)	Hexose: galactose > mannose > glucose > fructose
	Carbonyl position: lactose > maltose
Proteinaceous	
Amino substance > amino acid > protein	Basic amino acid > acid amino acid
Benzpyrole & benzene ring contribute to MR	S-S bond & S-H bond slow down MR
ε-amino acid & terminal amino acid > α-amino acid	

2.4 Catfish gelatin

2.4.1 Gelatin

Gelatin is derived by an irreversibly controlled hydrolysis of the insoluble protein and collagen, which can be widely found in nature as the major constituent of skin, bones and connective tissue (Park et al., 2008). As a proteinaceous compound, gelatin is formed by the collagen triple-helix structure unfolding to single chains under special conditions. It is reported that the molecular weight (Mw) of collagen is around 300KDa. However, the Mw of gelatin is hard to define, mainly from 10KDa to 100KDa. During the hydrolysis process, the breakage of the interchain chemical bonds and intrachain polypeptide bonds occurs, resulting in the gelatin of a wide range of Mw (Zhou et al., 2006). According to the SDS-PAGE method, α- ($α_1$-, $α_2$-), and β-chain of the collagen have been measured around the120KDa and 250KDa respectively (Zhang et al., 2007; Jun-Hyun Oh, 2012). The functional properties of the gelatin, such as gel strength, viscosity, and thermostability, are greatly influenced by the amino acid composition and the molecular weight distribution (Muyonga, Cole, & Duodu, 2004; Simon et al., 2003), which is mainly depended on the protein spices and the extraction conditions.

Gelatin has high nutrition while low calories. Its other unique advantages as edible films and coatings for food packing and food application have also attracted much attention recently. Because of the biodegradability, environmental safety, low economic cost, and excellent film forming ability, gelatin has been widely applied in food, medicine, pharmaceutical, photographic and cosmetics industries as a multifunctional resource.

In order to reduce the possible outbreak of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD), also the religious problems, alternative non-mammalian species are regarded as the superior resource to obtain the gelatin. Gelatin from marine source (fish skin, bones and fins) seems to be a better choice with several advantages: 1) pretty safe with good quality, 2) better releasing aroma with less inherent off-flavor than mammalian, 3) the acquisition of fish skin and fins, as byproducts during process, can reduce the waste and pollution. In this study, catfish is applied as the raw materials. As the warm-fish, catfish owns good protein resources.

It is known that warm-water fish have quite similar properties to most mammalian. The warm-water fish species are able to yield gelatin with better thermostability (melting temperature & gelling temperature) and rheological properties than that from cold-water fish species (Karim,A.A et al., 2009). Additionally, collagenous material from fish skin is characterized by a low degree of intra and interchain covalent cross-linking (Gómez-Guillén et al. 2009), causing the gelatin can be easily extracted.

It is expected that gelatin, as other protein films, have sufficiently low oxygen permeability to serves as effective barriers, especially at low water activities (Perez-Gago and Krochta, 2001; Carvalho and Grosso, 2004). Conversely, the water vapor permeability of gelatin is unsatisfying, owing to the hydrophilic groups. Hydrophilic edible films are very prone to plasticization from water, which tends to cluster within the polymer matrix (R.J.Avena-Bustillos et al. 2006). However, compared with mammalian gelatin film, the WVP of fish gelatin film is relatively lower, which is also a significant reason why catfish skin is chosen as the suitable raw materials of the gelatin film.

2.4.2 Pretreatment and gelatin extraction

Converting insoluble native collagen into gelatin requires to breakdown the non-covalent bonds and disorganize the protein structure, allowing swelling and cleavage of intra and intermolecular bonds to solubilize the collagen (Gómez-Guillén et al. 2009).Subsequent heat treatment cleaves the hydrogen and covalent bonds to destabilize the triple helix, resulting in helix-to-coil transition and conversion into soluble gelatin (Djabourov et al. 1993). Gelatin extracted from the fish skin with desired physical properties is generally achieved by alkaline or acid pretreatment firstly followed by water bath (WB) extraction. During the whole process, pH, concentration, temperature, and time are the major factors.

For alkaline pretreatment, it is reported that alkaline can attack the telopeptide region of the collagen molecule predominantly then unfold the tissue structure. Furthermore, alkaline solution, especially the sodium hydroxide, with strong saponification function, can hydrolyze fish fat (oil) effectively. During the alkaline pretreatment, fishy smell would also be cleared away to a large extent. Simultaneously, several unnecessary soluble proteins (elastin, albumin, globulin, etc.) and the impurities of the skin can be removed. However, it is worth mentioning that longtime and high alkaline concentration would reduce the yield of gelatin, as partial gelatin may be already denatured.

For acid pretreatment, it is reported that acid can provide a great swelling power for the disruption of crosslinking bonds. Once water molecules penetrate into the skin matrix, the structure swells gradually and the tissue loosens slowly. Moreover, acid makes the collagen main chain degraded, reduces the intra and intermolecular binding force, unfolds the ionic crosslinking and hydrogen bond crosslinking, removes the black pigment of the fish skin, and neutralizes the alkaline-treated skin. Additionally, controlling the acid concentration and pretreatment time is also important.

Either alkaline or acid has its unique function. It has been found that only alkaline pretreatment results in a dark-colored gelatin, whereas only acid pretreatment results in partial fish oil left. Therefore, single pretreatment proved to be not enough, which will cause some insufficient problems. The combined method of alkaline pretreatment followed by acid pretreatment is indispensible for catfish skin gelatin extraction (Yang et al., 2007). Catfish skin pretreated by such comprehensive method is more transparent, swelling and loose, which is helpful to obtain the gelatin and increase the final yield in the WB extraction process.

As to the WB extraction process, temperature is the key factor, which is close related with the gel strength and gelatin yield. Some peptide bonds will continuously be destroyed to small single chain over the applicable temperature, called protein secondary hydrolysis, resulting in the disappearance of gelatin structure and formation of soluble collagen hydrolysate with low gelatin yield and weak gel strength, shown in Figure 5. The optimum extraction temperature was between 45^{o}C and 55^{o}C.

Hence, both alkaline-acid pretreatment and water bath extraction process are necessary to convert collagen to gelatin. Yang & Wang (2007) applied response surface methodology by 2-step optimization to determine the effects of multiple variables, including alkaline concentration, alkaline pretreatment time, acid concentration, and extraction temperature. Finally, the optimal condition can be designed to gain the highest yield of gelatin.

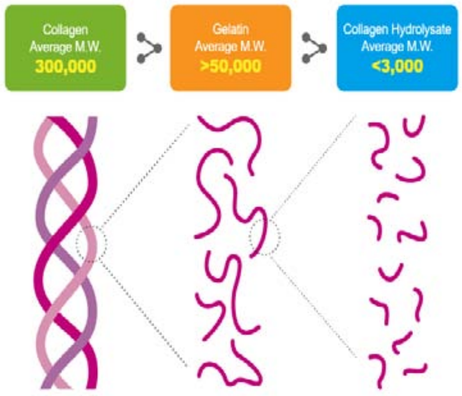


Fig. 5 The hydrolysis process of collagen

2.5 Lactose

Lactose is one important carbohydrate found in milk products, which makes up around 5% by weight of the milk. It is known as the reducing disaccharide, and is derived from the condensation of two other monosaccharides (glucose and galactose) through the β-1,4 glycosidic linkage. The structure of lactose is depicted in Figure 6, while the properties of lactose are shown in Table 3.

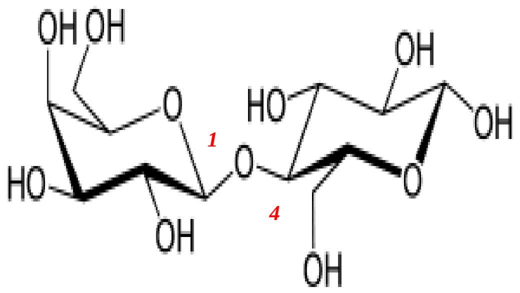


Fig.6 The structure of the lactose

Table.3 The properties of lactose

Chemical formula	$C_{12}H_{22}O_{11}$
Molar Mass	342.30 g/mol
Density	1.525 g/cm^3
Melting point	202.8°C
Boiling point	668.9 °C
Solubility (water)	21.6 g/100 ml

Lactose is quite stable and inert to react with other substance because of the low hygroscopicity. Most chemical reactions of lactose occur only in aqueous environment. However, lactose has excellent thermal property, especially the glass transition temperature (T_g), shown in Table 4, which is crucial for the spray drying method to improve the quality and the yield of the encapsulation product. As mentioned above, in order to improve the physicochemical properties of the film materials (gelatin biofilm), reducing sugar has been applied to the MR. In this research, it is noteworthy that spray drying requires the reducing sugar with high thermostability, primarily the high T_g. During the spray drying process under high temperature, the amino-carbonyl forming solution is dried very quickly with the occurrence of MR simultaneously, soon afterward, the MRPs mixed with the core materials are sprayed from the nozzle to the chamber to get tiny brown encapsulation powder. If T_g of the reducing sugar is low, the MRPs would be amorphous after the drying and MR process, owing to the sugar itself already changed to “rubbery” state, which will greatly affect the yield of the spray dried powder, as part of them will stick on the wall of the chamber with very high viscosity. This is the main reason lactose has been chosen to provide the carbonyl group; in spite it has higher Mw and lower reactivity compared with other reducing sugars.

Table.4 The T_g of the reducing sugars

Reducing sugar	M(Da)	Tg (℃)
xylose	150	10
Arabinose	150	4
glucose	180	23
fructose	180	8
galactose	180	32
maltose	342	70
lactose	342	101

2.6 Advanced glycation endproducts (AGEs)

Advanced glycation endproducts, known as AGEs, are complex compounds in the final products of the MR. AGEs mainly includes carboxymethyllysine (CML), carboxymethyllysine (CEL), pentosidine (Pento-s) and pyrraline (Pyr), which are quite harmful if absorbed by human being. The accumulation of the AGEs has a close relationship with many degenerative diseases, such as diabetes, atherosclerosis, chronic renal failure, and Alzheimer's disease.

The occurrence of AGEs is primarily dependent on the excessive heating time and overmuch carbonyl group. Protein glycation is a spontaneous reaction depending in vivo on the degree and duration of hyperglycemia (J.Peyroux & M.Sternberg, 2006).When the heating time is excessive, the reversible Schiff-base adducts rearrange to a more stable ketoamine or Amadori product (Soottawat, Benjakul, 2005). Through subsequent oxidations and dehydrations, including free radical intermediates, the Amadori product can then form cross-link between adjacent amino groups and transform into reactive dicarbonyl products. After that, a broad range of heterogeneous fluorescent and yellow-brown products with nitrogen- and oxygen-containing heterocycles forms (Gerald Műnch et al., 1997), resulting in polymeric aggregates, AGEs. However, not all AGEs have been identified and the mechanisms underlying their formation remain unclear up to now.

In order to prevent the formation of the AGEs, a variety of inhibitors and drugs have been studied. The nature water-soluble vitamin, named pyridoxamine, is considered as the most effective weapon to have the nucleophilic reaction with Amadori product and dicarbonyl compounds, so as to block the excessive carbonyl groups in the system. Ultimately, the production of AGEs can be controlled. However, extracting the pyridoxamine from the vitamin B_6 is a tough work with high cost, while other inhibitors have been found some disadvantages. For example, aminoguanidine, once considered as the best inhibitor, has some adverse reactions in the clinical trial.

In this research, radio frequency (RF) has been applied to heat the MR as a novel heating mode. Since RF heating is considered with the huge energy efficiency, it may achieve the high yield of the MRPs with enough short heating time, and probably prevent the occurrence of the AGEs in a large extent.

2.7 Radio frequency heating

RF heating, like other capacitative dielectric heating, is an innovative technique based on electro-heating. Dielectric heating can be more uniform than conventional heating because of the direct interaction between food materials and electromagnetic waves (Datta and Hu, 1992). The heat energy of the conventional heating system is transferred from a hot medium to a cooler product based on superficial heat transfer, resulting in large temperature gradients and slow heating rate. In contrast, dielectric heating utilizes the ability of some compounds to transform electromagnetic energy into heat that is volumetric and fast through a direct interaction between RF electromagnetic field and the object being heated (Wang, 2002). It should be noted that temperature, water content, and salt content of the products, especially the frequency of the electromagnetic waves, can affect the related dielectric properties and the resultant dielectric heating (Engelder and Buffler, 1991; Galema, 1997). According to different frequency (or wavelength), dielectric heating can be subdivided into microwave (MW) and radio frequency (RF) heating. The electromagnetic spectrum of RF ranges from 1 to 300MHz, while MW ranges from 300 to 30000MHz, shown in Figure 7. The permitted frequencies of RF for industrial, scientific and medical application are 13.56 ± 0.00678, 27.12 ± 0.16272 and 40.68 ± 0.02034 MHz, respectively (Rowley, 2001).

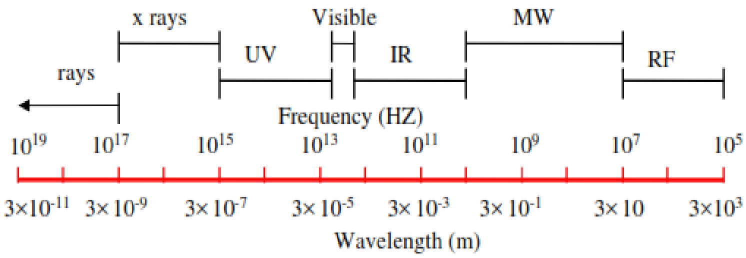


Fig. 7 The electromagnetic spectrum (Francesco Marra et al., 2009)

RF energy is generated by a triode valve, involving the high voltage alternating electric field to the medium between two parallel electrodes. One of the parallel should be grounded for setting up a capacitor to store the electric energy. Heating occurs because this field is not static, with polarity continually changing at high frequencies (27.12 MHz for RF or 2450 MHz for MW) (Francesco Marra et al., 2009). The continued reversal of polarity in the electrical field leads to the oscillation of ions forwards and backwards in the product with the net effect of this being the internal generation of heat within the product by friction (Buffler, 1993). The interaction between RF waves and the ions of the samples results the volumetric heat generation inside the product, as shown in the Figure 8.

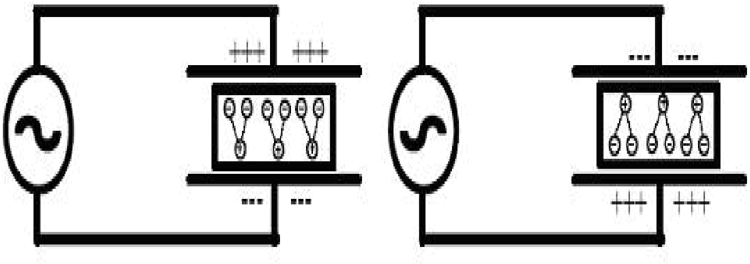
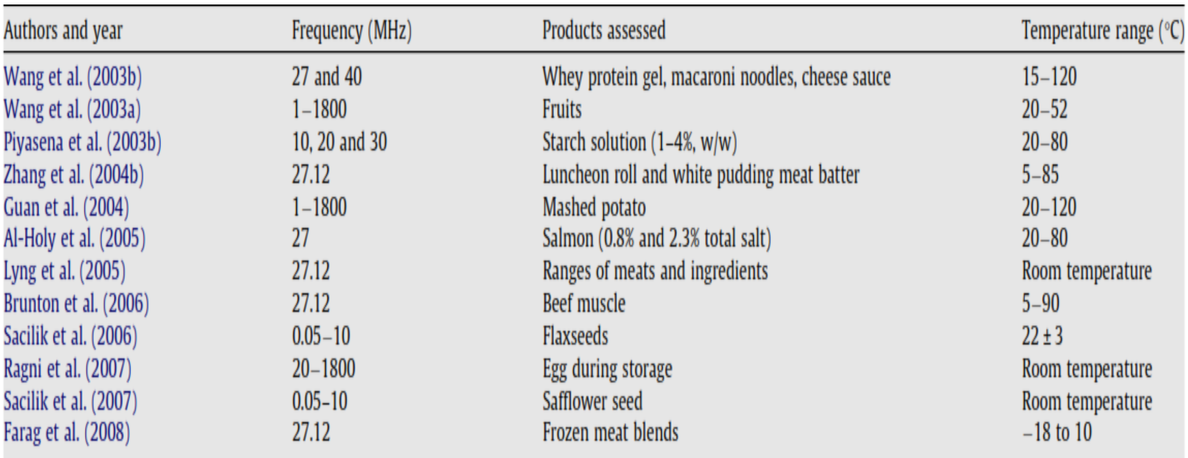


Fig. 8 Reversal of the polarity causes the sample to heat

Between two dielectric heating mode, RF heating has several advantages compared to MW heating. Above all, RF heating possesses stronger penetrating power than MW heating. Here, a concept, penetration depth (d_p) is introduced, which is defined as the depth into the material where the energy is reduced to 1/e of the surface energy value. The d_p is proportional to the wavelength. As to RF heating, the electromagnetic power can penetrate much deeper into the products with less surface overheating, hot/cold spots or other common problems compared to MW heating, which also explains RF heating can offer more uniform field patterns compared to MW heating. In addition, when it refers to the device, the uniform electric field strength of RF heating is inside the application chambers, which is much safer as well as easier to control. Therefore, RF heating method is considered as a good choice for food process, including the meat processing, blanching vegetables, fruit disinfestations, drying, thawing, pretreatment, etc. The application of RF heating can reduce the overall processing time while retain the quality and flavor of the products, which can greatly enhance its industrial and commercial prospects. The RF main contribution on food application and processing are exhibited in Table 5.

Table.5 Summary of successful applications of RF heating for food industry (Francesco Marra et al., 2009)



Authors and year	Frequency (MHz)	Products assessed	Temperature range (°C)
Wang et al. (2003b)	27 and 40	Whey protein gel, macaroni noodles, cheese sauce	15–120
Wang et al. (2003a)	1–1800	Fruits	20–52
Piyasena et al. (2003b)	10, 20 and 30	Starch solution (1–4%, w/w)	20–80
Zhang et al. (2004b)	27.12	Luncheon roll and white pudding meat batter	5–85
Guan et al. (2004)	1–1800	Mashed potato	20–120
Al-Holy et al. (2005)	27	Salmon (0.8% and 2.3% total salt)	20–80
Lyng et al. (2005)	27.12	Ranges of meats and ingredients	Room temperature
Brunton et al. (2006)	27.12	Beef muscle	5–90
Sacilik et al. (2006)	0.05–10	Flaxseeds	22 ± 3
Ragni et al. (2007)	20–1800	Egg during storage	Room temperature
Sacilik et al. (2007)	0.05–10	Safflower seed	Room temperature
Farag et al. (2008)	27.12	Frozen meat blends	−18 to 10

Wang et al applied RF heating to treat whey protein gel and Piyasena et al employed RF heating to process starch solution. Up to now, there has been no report on RF heating used in MR. In this research, RF heating was explored to heat the mixed solution with catfish gelatin and lactose so as to get highest MRPs yield and reduce AGEs production as low as possible in short time. Additionally, RF can heat the reactants more volumetrically, resulting in uniform solution. Last but not least, RF may be controlled at lower temperatures for MR (less than 100°C) depended on high energy efficiency, with safe environment, economical cost and low energy-consuming. Overall, it is expected that RF heating may bring a breakthrough to MR.

Chapter 3 Improve the Physicochemical Properties through Maillard Reaction (MR) by Water bath heating

Abstract

The catfish gelatin film served as the film (wall) materials of the encapsulation products requires good quality. In this study, Maillard reaction (MR) was applied to modify the physicochemical properties of the gelatin film. Lactose was chosen to react with 3.33% gelatin by different ratio of 1:2, 1:1, and 2:1 under different water bath (WB) heating time at 2 h, 4 h, and 6 h. The solution properties, including pH value, viscosity, and absorbance were examined to measure the MR extent, while the film properties, such as mechanical properties, thermal property and permeability, were tested to investigate the modification extent on gelatin films. MR had great influence on the above properties. The tensile strength (TS) decreased remarkably while elongation (EAB) increased dramatically after the MR process. The brittle and rigid raw gelatin film became quite flexible and ductile. The glass transition temperature (T_g) increased significantly. Thus the thermal stability of gelatin film became very high. The light transmission (LT) and oxygen transition rate (OTR) reduced obviously, while the water vapor permeability increased largely due to the changes of gelatin molecular structure. The optimal MR modification on gelatin film was treated in 1:1 MR system at 4 h with WB heating.

Key words: catfish gelatin film, encapsulation products, physicochemical properties, Maillard reaction, lactose

3.1 Introduction

Edible film (wall) material constitutes an environmental friendly technology that may enhance food quality, safety, stability, and the mechanical handling properties by providing a permeable barrier to water vapor, oxygen and carbon dioxide between the food and the surrounding atmosphere (Greener-Donhowe, I.K., 1994). Gelatin is one of the natural edible film (wall) materials in encapsulant, which is a potential and competitive biopolymer with a broad range of functional properties and applications in food, pharmaceuticals, photographic, and cosmetic industries. In this research, the gelatin encapsulation powder after spray drying is too tiny to study accessibly. In order to analyze the encapsulant accurately as well as expediently, the film (wall) material (gelatin biofilm) formed at macroscopical size is reasonable.

Gelatin is mostly extracted from the skins, bones, and connective tissue by hot water with the pretreatment of acid or alkaline hydrolysis. The collagen triple-helix structure can be unfolded then transformed into soluble collagen. The gelatin molecule weight (Mw) is indefinite depending on the corresponding raw materials and extraction condition. Meanwhile, the gelatin properties are closely related to the amino acids composition as well as the relative hydrolyzed α- and β-chains. It is worth noting that fish skins are easy to hydrolyze for its high content of soluble collagen. An increasing demand of fish gelatin has been brought to replace the traditional mammalian gelatin in recent years in terms of religious needs and the outbreaks of sanitary (BSE & FMD). Moreover, fish skins have low gelling temperature which provides many new potential applications, known as the microencapsulation (A.A. Karim and Rajeev Bhat, 2009). Other than that, fish gelatin can offer lower water vapor permeability (WVP) of biofilm than mammalian, as well as its excellent film-forming properties with potential high barrier efficiency that has promising applications for edible films and coatings (Avena-Bustillos et al., 2006). Good protein resource, fast growth speed, and appropriate breeding condition allow catfish to be the potential choice of film (wall) materials of the encapsulation. An optimized alkaline-acid extraction method has been applied in this research to get catfish gelatin.

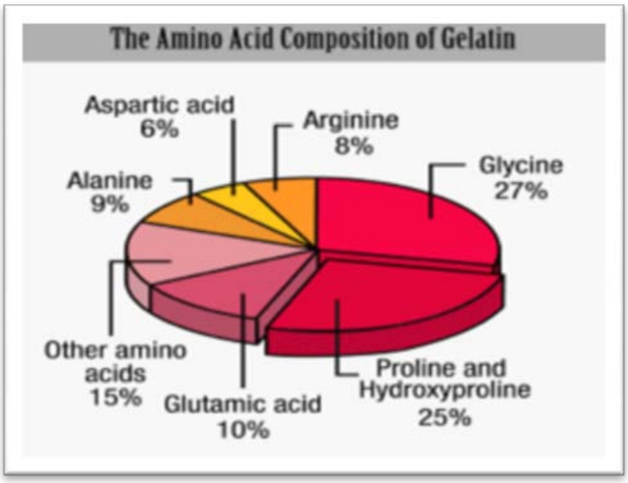


Fig.9 Amino acid composition of the gelatin

The edible gelatin film from natural catfish skins is an ideal alternative to the film formed by the synthetic polymer (Piotrowska et al., 2008). It is due to not only the biodegradable characteristics, ideal mechanical properties, and effectiveness as a barrier to gas, but also the reduction of environmental pollution, serious ecological problems and plastic wastes caused by the synthetic films with petrochemical based and non-biodegradable disadvantages. The structural and physicochemical properties of catfish gelatin are mainly influenced by the Mw distribution and amino acid composition that plays an important role in the rheological and barrier properties of the resulting films (Gómez-Guillén et al., 2009).

Gelatin has been reported well suited for encapsulation of volatile systems dependent on their unique characteristics and typical functions with some improvements to be more ideal resource. Therefore, the chemical treatments can be applied to modify the polymer network through cross-linking of the polymer chains to improve the hydrocolloid film functionality (Arvanitoyannis et al., 1997c and Cao et al., 2007b). In this research, Maillard reaction (MR) has been employed to improve the quality of pure gelatin biofilm, mainly on permeability with modification on other physicochemical properties. MR plays an important role in improving the appearance and taste of foods and it has been a central and major challenge in food industry (Sara I.F.S Martins et al., 2000). MR is well-known because of the Maillard reaction products (MRPs) processing strong antioxidative activities with other special abilities (antibiotic, antimicrobial, and antiallergenic), which might greatly enhance the physicochemical properties of the gelatin biofilm attaching some other new characteristics.

The major focus of this work is to study the feasibility and tendency to improve the physicochemical properties of the catfish gelatin biofilm through MR by water bath (WB) heating. Owing to the unknown gelatin Mw, different reactant ratios

(1:1, 1:2, 2:1) and heating time (2 h, 4 h, 6 h) have been compared to obtain the optimal conditions for highest yield of MRPs. The amount of intermediate products and final MRPs can be approximately measured by the spectrophotometer at 294 nm and 420 nm, respectively; the changes of pH value and viscosity of film-forming solution are also important to analyze the extent of MR. Furthermore, mechanical properties, thermal properties, and permeability of the gelatin biofilm have been evaluated in order to figure out whether the MR modification effects on properties is proportional to the MRPs yield. Therefore, the objectives of this study can be summarized as: 1. studying the effect of MR on the physicochemical properties of gelatin biofilms. 2. optimizing the MR conditions in order to get the highest yield of MRPs with most suitable physicochemical properties of the gelatin biofilm.

To the best of our knowledge, there were limited researches employing MR to modify the gelatin biofilm. Drusch et al (2009) and Kosaraju et al (2009) reported MRPs of protein-sugar have been investigated as suitable encapsulant to protect sensitive core materials against oxidation.

3.2 Material and Methods

3.2.1 Raw materials

Fresh catfish skins were obtained from the Harvest Select Inc. (Uniontown, AL. USA). The skins were kept at -18^{o}C with the maximum storage of less than 2 months before use. Anhydrous lactose was purchased from VWR (VWR Scientific, Pennsylvania, USA). All reagents were analytical grade.

3.2.2 Gelatin extraction

An optimized procedure of gelatin extraction was applied for gelatin preparation with a slight modification (Yang, Wang, et al., 2007). The frozen skins were thawed at 4 ^{o}C for 12 h followed by cut into small pieces (about 2-3 cm squares), then washed with the tap water (1:6 w/v) at 4 ^{o}C for 10 min. The cleaned fish skins were drained using cheesecloth for 5 min to remove the liquid as much as possible. A combined alkaline-acid pretreatment was chosen for this research. Prepared skins were put into the flask and treated with 0.20 M NaOH (1:6 w/v) at 4 ^{o}C for 84 min with hand shaking every 10 min. Then the samples were rinsed and drained for three times. After the alkaline step, the swelled skins were put into flask again to treat with 0.115 M acetic acid (1:6 w/v) at 4 ^{o}C for 60 min with the same following steps of the alkaline pretreatment. As to the extraction process, the cleaned skins were added into the deionized water (dd H_2O) (1:4 w/v) and heated in 55 ^{o}C water bath (WB) (Model 86; Precision Scientific Co., Chicago, IL, USA) for 240 min. Finally, the gelatin solution was filtered through the cheesecloth to remove the undissolved impurities. The pure spongy gelatin can be obtained by lyophilization for 96 h (Labconco, Corp., Kansas City, MO, USA).

3.2.3 Preparation of gelatin film

The gelatin film forming procedures are dependent on Jiang, Wang et al (2010) with slight modification. The 3.33%film-forming solution was prepared by dissolving 6.66 g lyophilized gelatin in 200 ml dd H_2O agitated with magnetic stirrer (Fisher

Scientific, Pittsburgh, PA, USA) for 12 h in room temperature. Then, a 200 ml portion of the film-forming solution was cast onto a right square (25 cm × 25 cm), leveled plate covered by Teflon tape (Bytac®, Saint-Gobain Performance Plastics, Poestenkill, NY, USA). Plates were put inside an environmental chamber (Model AA-5460A, Espec Corp., Hudsonville, MI. USA) at 25 ^{o}C and 50% relative humidity (RH) for 48 h. The final gelatin film was peeled off the plate after drying. The margins were trimmed off and the middle part was kept for the following tests. All the other processes to modify the gelatin were done before film-forming solution drying.

3.2.4 Preparation of Maillard reaction products (MRPs) by water bath (WB) heating

Lactose was dissolved in 200 ml 3.33% gelatin solution at three different ratios (1:2, 1:1, and 2:1). The pH of the solution was adjusted by buffers (0.05 M NaOH – 0.1 M $NaHCO_3$, pH 11). The solution was then transferred to 250 ml flask, tightly capped by aluminum foil and heated in 100 ^{o}C water bath for 0, 2, 4, and 6 h, respectively. After the heating period, the MRP solution was immediately cooled down in ice bath then kept at 4 ^{o}C prior to analysis. All model systems were prepared in triplicate.

3.2.5 Characterization of MRP solution

3.2.5.1 Measurement of pH

The pH value was measured using ATI Orion PerphecT® meter line Model 370 (Thermo Fisher Scientific, MA, USA) calibrated with buffer solution of pH 7.0 and 10.0, respectively.

3.2.5.2 Viscosity

The Cannon-Fenske routine viscometer No.100 R698 (Cannon Instrument Co., State College, PA, USA) was used to test the viscosity (V, cP) of the solution at 40^{o}C. The efflux time was recorded using a stopwatch. The viscosity can be calculated from the equation: viscosity (cP) = efflux time (s) × viscometer constant (cSt/s) × density of the measured solution (g/ml); the density (ρ) of the solution was determined by weight/volume. Viscometer constant = C_0 (1 – B [T_T-T_F]) = 0.01434 × (1-78×10^{-6}[40-23]) = 0.01432 cSt/s; where C_0 is a constant, 0.01434 cSt/s (mm^2/S^2); B is the coefficient of the thermal expansion of the viscometer, 78×10^{-6} / ^{o}C; T_T is test temperature, 40^{o}C; T_F is filling temperature for calibration of the instrument, 23^{o}C.

3.2.5.3 Measurement of absorbance

The UV-absorbance and browning intensity of the MRP solution was recorded using a Helios Omega UV-VIS spectrophotometer (Thermo Fisher Scientific Inc. MA, USA). Appropriate dilution (50 fold) was made so as to obtain an optical density of less than 1.5. The absorbance measured at 294 nm and 420 nm in room temperature, were as the indication of intermediate products and final MRPs, respectively.

3.2.6 Characterization of gelatin films

3.2.6.1 Film thickness

Film thickness was measured at three different locations using a hand-held electronic micrometer (Model IP54, Fowler Electronic Micrometer, Fred V. Fowler Co., Inc. Newton, MA, USA).

3.2.6.2 Mechanical properties

Tensile strength (TS) and elongation at break (EAB) were evaluated at room temperature based on ASTM D882-97 (ASTM., 1998), taking an average of three determinations in each specimen. The film strip was cut into 10 × 50 mm and mounted between the grips attached to the TA. XTPlus Texture Analyzer (50kg load cell, Texture Technologies Corp., Scarsdale, NY, USA). The initial grip separation and cross-head speed were set at 20 mm and 50 mm/min, respectively. The TS and EAB can be measured until film broken. TS (pa) and EAB (%) were calculated using the following relationships:

$TS = F_{max} / A$

Where F_{max} is the maximum load for breaking film (N) and A is the cross-sectional area of the sample (thickness × width);

$E\ (\%) = (L/L_o) \times 100$

Where L_o is the original length of the sample and L is increased length at the moment of the rapture.

3.2.6.3 Thermal properties

The thermal properties of the film were accomplished by differential scanning calorimeter (DSC) (Model Q100, TA Instrument-Waters L.L.C., New Castle, DE, USA) with the cooling system assessor. Samples around 6 mg (± 0.5 mg) were weighed in a precision balance (Model WAX110, RADWAG USA L.L.C. Ventura, CA, USA). Then, they were encapsulated in hermetic aluminum pans with another reference empty pan. Both sample and reference pans were heated at 5 ^{o}C/min over the range 0 - 180 ^{o}C in inert nitrogen atmosphere. The glass transition temperature (T_g) was calculated as the inflexion point of the base line, caused by the discontinuity of the specific heat of the sample.

3.2.6.4 Water vapor permeability

Water vapor permeability (WVP) of the films was measured by ASTM E96-95 standard method (ASTM., 1999). The film was sealed on an aluminum permeation cup containing 15 ml distilled water (100% RH) with vacuum grease. The cup was placed in the environmental chamber at 25 ^{o}C and 50% RH for 7 h. The weight of the sealed cup was measured initially and at 1 h intervals using a digital balance (Model GP5202, Sartorius North America Inc. Edgewood, NY, USA). All tests were carried out triplicately. The WVP equation was as follows:

$$\text{WVP (g/Pa/S/m)} = \frac{w}{tA}\,\frac{x}{\Delta P} = \text{WVTR}\,\frac{x}{\Delta P}$$

Where w is the weight change of the cup (g); x is the film thickness (mm), t is the elapsed time (h); A is the area of exposed film (m^2), ΔP is the vapor pressure difference across the film (3169Pa, 25 ^{o}C), WVTR is the water vapor transmission rate ($g{\cdot}m^{-2}{\cdot}h^{-1}$) through a film, defined by linear regression ($r^2 > 0.99$) from the points of weight reduce and time. Results were expressed as $g{\cdot}mm{\cdot}h^{-1}{\cdot}cm^{-2}{\cdot}Pa^{-1}$.

3.2.6.5 Light transmission and transparency

The ultraviolet and visible light transmissions of the films were conducted by the UV-VIS spectrophotometer with the method according to Fang et al. (2002). Film portion of 10 × 20 mm was tested at selected wavelengths between 200 and 800 nm. The film transparency equation (Han & Floros, 1997) was calculated as follows:

Transparency (A/mm) = -log T_{600}/X

Where T_{600} is the fractional transmittance at 600 nm and X is the film thickness (mm). The greater transparency value represents lower transparency of the films.

3.2.6.6 Oxygen transition rate

The film oxygen transition rate (OTR) was analyzed by ASTM -D1434 standard method using the Oxygen Permeability Tester (BTY-B, LanGuang JiDian Company, JiNan, China). Each film was mounted on a stainless steel mask with an open testing area of 5 cm^2. Nitrogen gas (98% N_2 + 2% H_2) was blown into the chamber through one pipe, while the other pipe is opened to evacuate the chamber until the nitrogen reading becomes stable. Both pipes were then shut. The sensor measured the increase in oxygen content (100%) over the time during the testing period. The system was programmed to have a 10 h waiting period to allow the films to achieve equilibrium with the controlled temperature (25 ^{o}C) and RH (50%). Oxygen permeability was calculated by dividing the oxygen transmission rate by oxygen partial pressure (101kPa) and multiplying by the film thickness. Results were expressed as (cm^3·μm/m^2·day·kPa). All tests were carried out triplicately.

3.2.7 Statistical analysis

Statistical tests were performed using the SAS program (V.9.1.3, Statistical Analysis Systems, Cary, NC, USA). One-way analysis of variant (ANOVA) was carried out; analysis of covariance (ANCOVA) was also applied to identify the effects of film thickness on the light transition results; the linear regressions ($r^2 > 0.99$) was used to calculate the WVTR. Level of significance was set for $p \leq 0.05$. The dataset were expressed as mean ± SD (standard deviation).

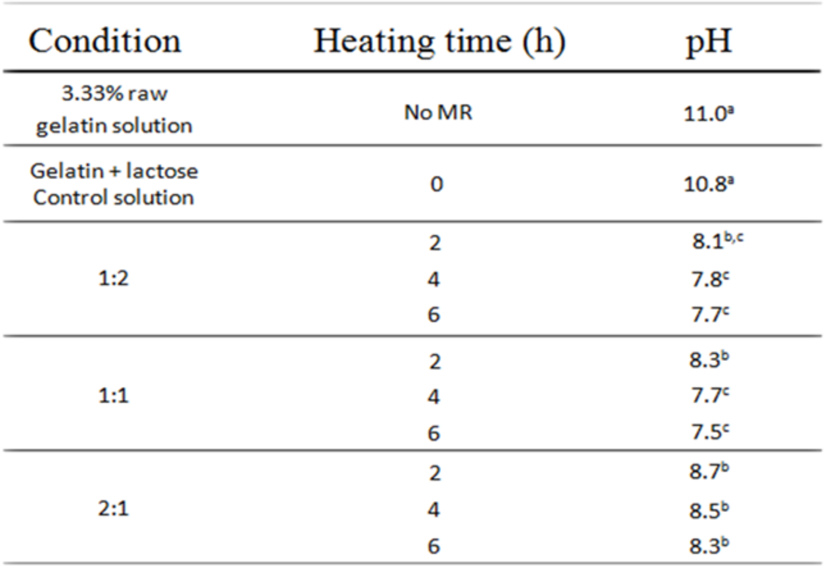
3.3 Results

3.3.1 Changes in pH

The pH of MR system decreased as the heating time prolonged ($p < 0.05$) (Table 6). It decreased remarkably from the initial value during the first 2 h. Thereafter, the decrease in pH was leveled off with the expended heating time. The phenomena were due to the formation of organic acids such as formic and acetic acid during the MR process (Brands and Van Boekel, 2002).The reductone, formed at the end of the intermediate stage, was quite unstable and could be dissociated into acetic acid under the high temperature. The results suggested that consumption of amino group, together with formation of acids, could be the mechanism of pH decreasing tendency in the MR system (Liu et al., 2008).

Gelatin solution and lactose mixed together without MR didn’t cause significant difference in pH value ($p < 0.05$) as time went. This proved that MR was the reason to make pH descending. During the MR process, the results obtained at first 2 h showed that more lactose in MR system could increase the reaction speed and gain the lower pH, because more carbonyl groups with relatively large Mw (28) were more powerful to attack the relatively low Mw (16) of amino groups, and then accelerate the reaction to the positive direction more rapidly. However, when the heating time increased to 4 h, it could be figured out the relatively lower pH value occurred in 1:1 MR system. The other two MR systems both had the same problem: one of the reactant was insufficient, which concretely meant the lactose was excessive in 1:2 MR system while lacking in 2:1 MR system, resulting in the MR speed gradually slowed down and less MRPs formed. Especially for the situation of limited reducing sugar, there was less impulse to make MR occur at a fast speed with the continuous consumption of lactose, causing the pH value decreased slightly. Consequently, when the time was up to 6 h, the lowest pH 7.5 was from 1:1 MR system with the most complete MR extent, while the pH 8.3 from 2:1 MR system was even no less than the results from other two MR systems obtained at first 2 h, which explained again that less organic acid were formed due to the inadequate lactose.

Table.6 Changes in pH at different reactant ratios (Gelatin/lactose) and heating time with WB heating



Condition	Heating time (h)	pH
3.33% raw gelatin solution	No MR	11.0^{a}
Gelatin + lactose Control solution	0	10.8^{a}
1:2	2	$8.1^{b,c}$
	4	7.8^{c}
	6	7.7^{c}
1:1	2	8.3^{b}
	4	7.7^{c}
	6	7.5^{c}
2:1	2	8.7^{b}
	4	8.5^{b}
	6	8.3^{b}

All standard deviation were less than 0.1; $^{a-c}$ different letter superscripts donated significant difference ($p < 0.05$).

3.3.2 Changes of viscosity

The viscosity of 3.33% gelatin solution was 3.72 cP in this study. Measuring temperature was set at 40 ^{o}C. High temperature 60 ^{o}C, as the standard temperature for viscosity, would supply good condition for MR to occur again, while low temperature 35 ^{o}C, as the gelling point for gelatin, would cause the gelatin lumped. The results from three MR systems had quite different tendency. The viscosity gradually decreased in 1:1 and 2:1 MR systems while largely increased in 1:2 MR system, shown in Table 7. Generally, sugars could be considered as a good plasticizer with the function of lowering the viscosity of the raw object. Here, the viscosity was reduced from 3.72 cP to 3.61 cP of the control solution, which was owing to the lactose plasticizing on gelatin. The small lactose molecules entered into the gelatin molecules easily, followed by occupying the free volume between peptide bonds, which caused the collision and friction between these two molecules and resulted in high hydrodynamic properties of solution system. During the MR process, the viscosity from 1:1 and 2:1 MR systems reduced continuously. When the extent of MR proceeded higher, the free amino groups in gelatin molecules became fewer so that the interactions between the main chains of gelatin became weak and resulted in the decrease in the activation energies of the MRPs solutions (Chuang et al., 2006). Each of the viscosities from 1:1 MR system were relatively lower than 2:1 MR system at the same time, which was probably related with more MRPs produced in former system. The abundant lactose in 1:1 MR system could advance MR to a higher extent, while the limited lactose in 2:1 MR systems had less impulse to promote MR.

Table.7 Changes in viscosity at different reactant ratios and heating time with WB heating

Condition	Heating time (h)	V/cP
3.33% raw gelatin solution	No MR	$3.72^{b} \pm 0.08$
Gelatin + lactose Control solution	0	$3.61^{b,c} \pm 0.06$
1:2	2	$5.62^{a} \pm 0.02$
	4	$5.76^{a} \pm 0.02$
	6	$6.21^{a} \pm 0.04$
1:1	2	$3.35^{c,d} \pm 0.01$
	4	$3.24^{c,d} \pm 0.02$
	6	$2.99^{d} \pm 0.06$
2:1	2	$3.57^{b,c} \pm 0.02$
	4	$3.46^{c} \pm 0.03$
	6	$3.06^{d} \pm 0.05$

SD: standard deviation from triplicate determinations; $^{a\text{-}c}$ different letter superscripts donated significant difference ($p < 0.05$).

On the contrary, the viscosities in 1:2 MR system were abnormally increased. It was assumed that new products might be formed to break the balance of MRPs solution. Apart from Maillard reaction, caramelization of sugar could occur, leading to browning of the mixture (Ajandouz et al., 2001; Benjakul et al., 2005), though the temperature for caramelization was tough to reach by WB heating. In addition, another possibility might be relevant to the formation of AGEs under the condition of overmuch lactose and overlong heating time to MR. The compounds of caramel products and AGEs with high viscosity could probably promote the viscosity of the whole system. On the other side, the free volume could be determined by molecular rearrangements and transport properties in polymer solutions (Chuang et al., 2006).

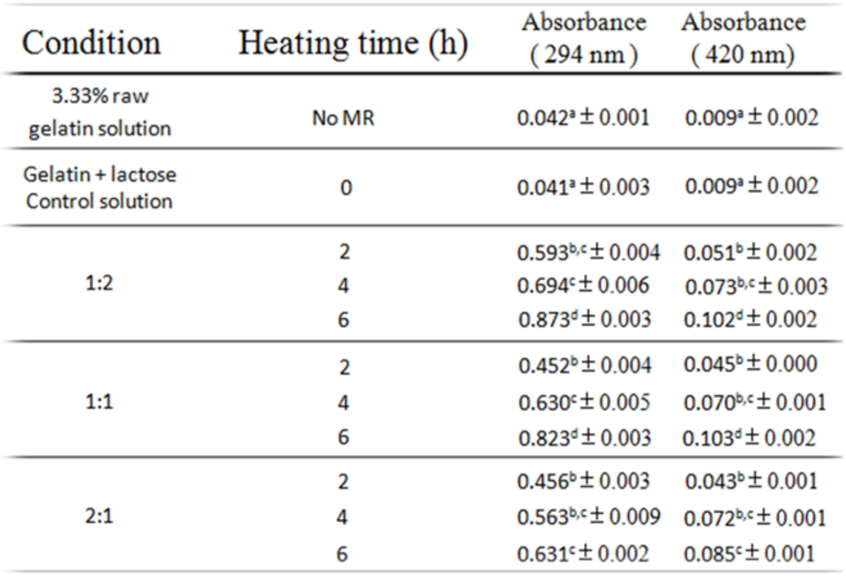
The rest heated lactose was more likely to full in these gaps between the polymer chains and even stick on the molecular surfaces of MRPs, which could greatly enhance the intermolecular force while reduce the fluidity of the whole solution, causing the viscosity increased. It could be supposed that more lactose left in the final MRPs solution with longer time would cause the higher viscosity.

3.3.3 Changes in absorbance

The intermediate compounds and browning intensity were the primary measurable consequences of MR. Absorbance at 294 nm was used to determine the intermediate compounds of MR (Ajandouz et al., 2001; Lerici et al., 1990), and absorbance at 420 nm was used as an indicator for browning development in the final stage of MR (Ajandouz et al., 2001; Morales et al., 2001). As shown in Table 8, the absorbance value of A_{294} and A_{420} increased sharply in the first 2 h ($p < 0.05$). The increase in A_{294} was considered as the formation of an uncolored pigment, which could be the precursor of MR. While the browning intensity A_{420} increased with the concomitant increase in A_{294}, owing that most intermediate products contributed to the polymerization process in the advanced stage to form the MRPs (Lertittikul et al., 2007). During extended heating, the increase rate of intermediate compounds and browning intensities were relatively slight. The results mainly owing to the consumption of the amino groups and formation of the acidic compounds by MR, shifting systems into more acidic condition and lowered the browning reaction (Gu et al., 2009), which was affected by the same reason as to the change of pH value.

It is worth mentioning that the increase rate in browning was greatly dependent on sugar concentration. Among the results during the first 2 h, the highest A_{294} and A_{420} both occurred in 1:2 MR system ($p < 0.05$), due to the abundant lactose, which accelerated the carbonyl-amino combination, promoted the molecule rearrangement, and caused the MRPs formed more rapidly. Meanwhile, the browning intensities caused by 1:1 and 2:1 MR systems had similar results, owing to the same proportion of the lactose involved in these two MR processes. It was also possible for those two MR systems to have the close MR speed and MRPs yield. When the heating time arrived at 4 h, the browning intensities from three MR systems were no significant difference ($p < 0.05$), while 1:2 MR system possessed higher intermediate compounds A_{294}, because of the faster MR rate at the initial stage. The whole MR systems gradually tended to relative equilibrium phase, and the MR speeds slowed down. The intermediate compounds were transformed to the final MRPs little by little. The yields of the MRPs were nearly equal, because the gelatin amount was fixed in three MR systems, as long as the lactose amount was not exhausted, the browning intensities A_{420} should be close enough with the heating time expanded. It was obviously that none of the three MR systems stopped at 4 h. As heating time went on, the A_{294} and A_{420} from 1:2 and 1:1 MR systems were still in same pace, but much higher than 2:1 MR system at 6 h. The redundant gelatin in 2:1 MR system was unable to form any more MRPs due to the small amount of lactose. Conversely, the excessive lactose in 1:2 MR caused no more strong browning intensity either, compared to 1:1 MR system, which indicated excessive lactose in MR system also wouldn’t increase the MRPs yield. The above results were in accordance with that of the studies conducted by Benjakul et al., (2007).

Table.8 Changes in Absorbance at different reactant ratios and heating time with WB heating



Condition	Heating time (h)	Absorbance (294 nm)	Absorbance (420 nm)
3.33% raw gelatin solution	No MR	$0.042^{a} \pm 0.001$	$0.009^{a} \pm 0.002$
Gelatin + lactose Control solution	0	$0.041^{a} \pm 0.003$	$0.009^{a} \pm 0.002$
1:2	2	$0.593^{b,c} \pm 0.004$	$0.051^{b} \pm 0.002$
	4	$0.694^{c} \pm 0.006$	$0.073^{b,c} \pm 0.003$
	6	$0.873^{d} \pm 0.003$	$0.102^{d} \pm 0.002$
1:1	2	$0.452^{b} \pm 0.004$	$0.045^{b} \pm 0.000$
	4	$0.630^{c} \pm 0.005$	$0.070^{b,c} \pm 0.001$
	6	$0.823^{d} \pm 0.003$	$0.103^{d} \pm 0.002$
2:1	2	$0.456^{b} \pm 0.003$	$0.043^{b} \pm 0.001$
	4	$0.563^{b,c} \pm 0.009$	$0.072^{b,c} \pm 0.001$
	6	$0.631^{c} \pm 0.002$	$0.085^{c} \pm 0.001$

SD: standard deviation from triplicate determinations; $^{a\text{-}c}$ different letter superscripts donated significant difference ($p < 0.05$).

3.3.4 Mechanical properties

The tensile strength (TS) decreased noticeably from 52.21 Mpa (raw 3.33% gelatin film) to lowest 2.04 Mpa (1:2 MR system, 6 h), while elongation (EAB) had a quite reversed trend, increasing from 4.08% to highest 330.36% (1:1 MR system, 6 h)($p < 0.05$), shown in Figure 10 and 11, respectively. The 3.33% raw catfish gelatin had high TS but low EAB with rigid and brittle nature. The fact might be attributed to the tight molecule interaction, which was determined by Van der Waals' force, hydrogen bonding, and/or electrostatic effect. The biggest drop of TS happened when the lactose was added into the solution without MR, resulting in less than half of the original. The control film became more stretchable and flexible due to the plasticization effect by lactose which increases the mobility of the gelatin polymer chains. Most sugars were cited as good plasticizers because of their ability to reduce intermolecular hydrogen bonding while increase intermolecular spacing (Vanin et al., 2005). The small size molecule of lactose could allow it to be more readily penetrated into the polymer chains of gelatin, and loosed the networks. Finally it resulted in the less brittle composite film. The results were approximate in accordance with the study by R. Bhat (2012), who explained lactose/ribose had great influences on the mechanical properties of fish gelatin films. Moreover, Jiang and Wang, (2009) also reported a significant decrease of TS in the catfish gelatin film incorporated with triacetin mixture plasticizer, but the greatly increased in EAB. During the MR process, the TS reduced sequentially with the expanded heating time. The lowest TS of the MRPs film reached 2.04 Mpa at 6 h in 1:2 MR systems, which was very flexible and extensile. The MRPs most likely possessed the weaker chain to chain interaction or less junction zones mainly via the transformation of the hydrogen bond and some functional groups during the MR process. It could be supposed that some intrinsic functional groups to form the cross linking structure of the gelatin molecules were altered or disappeared during the complicated MR processes. The original gelatin hydrogen bond might be replaced by the new intermolecular force. As a result, direct interactions between the gelatin molecular chains were reduced, and the chain segmental mobility was increased, causing the TS of the film to be reduced and the extensibility enhanced (L. Yang & A.T. Paulson, 2000). Additionally, the MRPs were composed of many macromolecular products. The reduced TS could be possibly caused by the incompatibility of each MRPs component molecule, resulting in the lessened integrity of film structure. Jongjareonrak et al. (2008) reported the incompatibility of antioxidant additives and gelatin molecules would probably decrease the TS of gelatin film.

At the same heating time, the lowest TS were always obtained from 1:2 MR system while highest from 2:1 MR system. The redundant gelatin in 2:1 MR system still probably kept the original structure with higher strength, but was not affected by MR, causing the reduced rate was relatively slow. Conversely, though the drop extent of TS was drastic at first 2 h in 1:2 MR system, it was almost no longer falling after that. Once the gelatin was close to exhaustion, the excessive lactose seemed not to have much influence on MRPs films to decrease the TS furtherly, mainly due to the high temperature affecting a few physical properties of the lactose structure. It was worth mentioning that the TS downtrend from 1:1 MR system seemed to be more moderate during the whole MR.

Generally, the TS and EAB were considered to be negatively correlated to each other. The largest increase of EAB occurred during the MR at first 2 h. The influence on EAB of gelatin film by lactose plasticization seemed to be much less than MR, especially from the 1:2 and 1:1 MR system. The formation of the MRPs had altered gelatin film structure, expanded the intermolecular spacing between the polymer chains, and increased the movement of the macromolecules in the film matrix, leading to the great enhance of EAB. Furthermore, high temperature could easily lead to the proteinaceous compounds denaturation. Brandenburg et al., (1993) reported that denaturation of protein was known to change the shape of the protein from globular to extended chain, thus more free volume would occur to reduce the cross-linking and lead to high EAB finally.

The increase trends of EAB were quite different among three MR systems. The EAB increased extremely rapidly during the first 2 h, and then nearly no significant difference was shown in next four hours in 1:2 MR system. It could be inferred that the plasticization by excessive lactose indeed didn’t have much influence on mechanical properties of the MRPs films. Meanwhile, the increase of EAB from 2:1 MR system was relatively gentle, which has the same reason to the slow decrease in TS. The lactose in 2:1 MR system didn’t have enough impulse to achieve greater MR any more, and then caused slight EAB changes. In addition, the EAB from 1:1 MR system increased gradually as well as continuously, and obtained the highest value at 6 h.

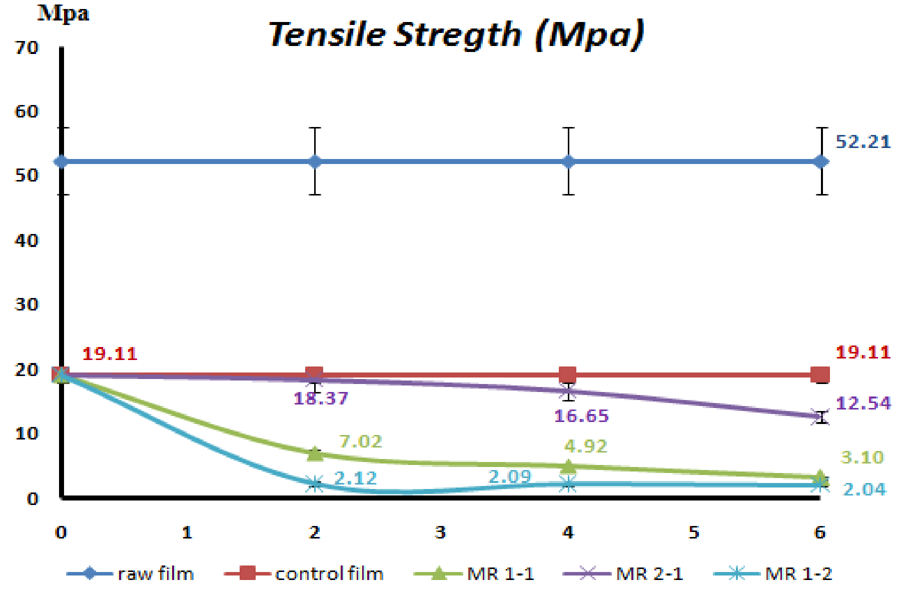


Fig.10 Effect of MR on the tensile strength of catfish gelatin films with WB heating

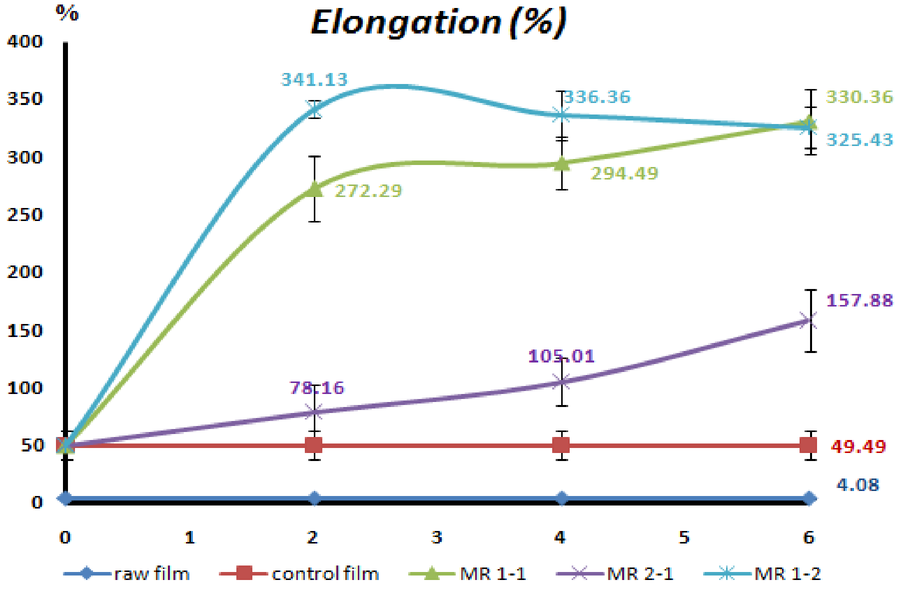
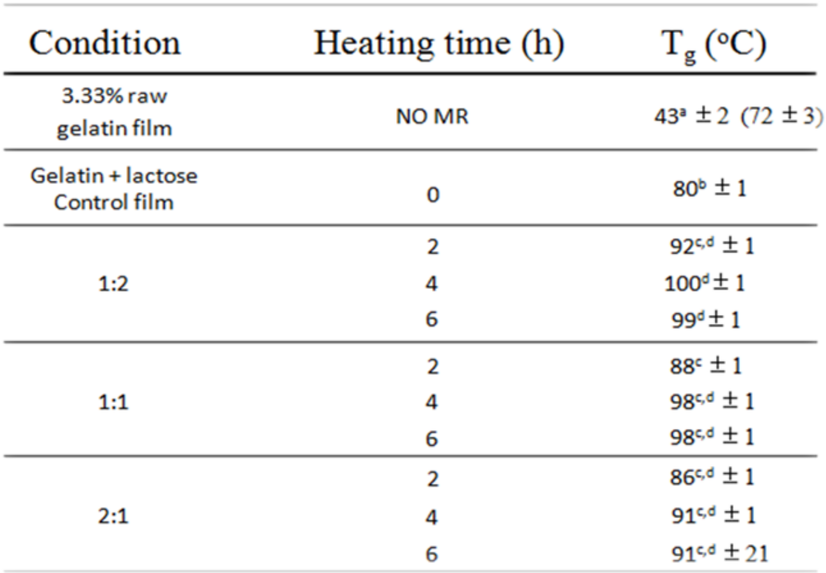


Fig.11 Effect of MR on the elongation at break of catfish gelatin films with WB heating

3.3.5 Thermal properties

The DSC thermograms of MRPs films are shown in Table 9. The lyophilized gelatin had T_g of 74 ^{o}C, which was similar to the anhydrous gelatin powder measured by Dai et al., (2006). Meanwhile, the raw catfish gelatin film had T_g of 43 ^oC, which was somewhat higher than the films made from tuna skin gelatin (T_g, 40.7 ^oC) (Gómez-Estaca et al., 2009). Generally, the moisture content in gelatin would greatly affect its T_g. In addition, the T_g of the anhydrous lactose was 101 ^oC, and it had the highest thermal stability among the reducing sugars. In this research, all the T_g of the blend films obeyed the Fox equation, which were intermediate between the raw value of each pure reactant, while the T_g of control film (80 ^oC) was coincidently around the middle position. Gómez-Estaca et al. (2011) also reported the glass transition temperatures of complex gelatin-chitosan films were intermediate between those for the respective single components, indicating some level of blending after interaction/association between components. The T_g of MRPs films were continuously increased with the expanded MR time, which indicated the thermal stabilities of MRPs films were enhanced. Usually, the increase of T_g was assigned to the improvement on molecular interaction as well as the reduction on mobility of polymer chains. However, in this research, the intermolecular force was inversely decreased while the free volume was increased based on the results of the mechanical properties. The raised T_g was probably due to an unknown crosslink between the unreacted (excessive) lactose and new formed MRPs molecules. Under the same condition, greater MR extent in the system obtained relatively higher T_g. De Carvalho and Grosso (2004) also reported that a reduction in the number of hydrogen bonds with a simultaneous increase in the extent of covalent cross-linking could increase the thermal stability. It could be envisaged that a partial components in the final MRPs compounds probably have high thermal stability. The reductone formed from cleavage reaction at intermediate stage as well as heterocyclic compounds with stable structures are most likely to promote the T_g of the whole system. Furthermore, T_g is an important criterion for the miscibility of the polymer. As to the MRPs films, each sample only had one discontinuity of the specific heat, which indicated the gelatin and lactose had good miscibility and formed a wide range of MRPs films.

Table.9 Changes in glass transition temperature at different reactant ratios and heating time with WB heating



Condition	Heating time (h)	T_g (°C)
3.33% raw gelatin film	NO MR	$43^{a} \pm 2$ (72 ± 3)
Gelatin + lactose Control film	0	$80^{b} \pm 1$
1:2	2	$92^{c,d} \pm 1$
	4	$100^{d} \pm 1$
	6	$99^{d} \pm 1$
1:1	2	$88^{c} \pm 1$
	4	$98^{c,d} \pm 1$
	6	$98^{c,d} \pm 1$
2:1	2	$86^{c,d} \pm 1$
	4	$91^{c,d} \pm 1$
	6	$91^{c,d} \pm 21$

SD: standard deviation from triplicate determinations; $^{a\text{-}c}$ different letter superscripts donated significant difference ($p < 0.05$).

3.3.6 Water vapor permeability (WVP)

The variation tendency of WVP was similar with that of mechanical properties, which were both related with the molecular structure. Figure 11 shows the film WVP increased in small extent without MR while increased significantly with the MR heating time expanded. Generally, WVP through a hydrophilic film depended on both diffusivity and solubility of water molecules in the film matrix (Gontard & Guilbert, 1994). The WVP of control film was slightly enhanced compared to the raw gelatin film because of the lactose plasticization. The increase in the interchain spacing, due to inclusion of lactose molecules between the gelatin polymer chains, might modify the molecular organization of the gelatin network and increase the free volume with less dense polymeric matrix, thus promote water vapor diffusivity through the film and hence accelerate the water vapor transmission. The WVP of gelatin films were significantly increased when MR started. This could be explained considering that gelatin possessed a wide range of hydrophilic amino acids. During the MR process, most hydrophilic groups (—OH, —CHO, —COOH, —NH_2) were stimulated and activated. After a series of complicated processes (rearrangement, fragmentation, dehydration, isomerisation, and retroaldolisation), they would have established more hydrophilic interaction with other reactive groups, enhanced the free volume of the polymeric matrix and absorbed more water molecules from the MR system. Finally, the hydrophilic groups of MRPs could normally promote water molecule sorption, which might induce easier water vapor migration through the film. Additionally, the thermal treatment during the MR process under alkaline conditions would cause the disruption of polymeric structure accompanied by a partial gelatin denaturation (Mauri and Anón, 2006) and hydrolysis. As it mentioned to the reason of high temperature reducing the TS while enhancing the EAB, the denaturation of gelatin would unfold the polymer chains and enlarge the molecular gap, which affected the intra- and intermolecular cross-linking of amino acid or residues, as well as the functional groups, resulting in the water barrier property decreased. Additionally, Giménez et al., (2009) observed that WVP of film significantly increased ($p < 0.05$) when gelatin hydrolysates was incorporated in the films. It could be presumed that a portion of gelatin would hydrolyze simultaneously around the beginning of MR because of high alkaline condition in this research.

From the results among three MR systems, the WVP increased more linearly from 2:1 MR system. To some extent, the independence of WVP on the degree of MR, the 2:1 MR system might be the suitable choice in applying MRPs film for food coating or packaging applications, as the film flexibility and ductility could be modified by MR, without great changes in the moisture battier properties. Choi & Han (2001) also reported the film with little variation on WVP, when modified to a better flexibility, was a preferred choice. The WVP of MRPs film formed from 1:1 MR system increased significantly from 2 h to 4 h. It indicated that MR occurred drastically and had great influence on WVP during that time. Whereas not much difference was found on WVP in the last two hours, which could possibly be explained from the investigation by Garcia et al., (2000a) and Arvanitoyannis et al., (1994), who considered slight differences in WVP values maybe related to the difference in water molecule diffusion and hydrophilic-hydrophobic ratio. The highest WVP could be obtained from the 1:2 MR system at 2 h, followed by gradual reduction at 4 h and 6 h, respectively. This strange phenomenon was most likely connected with the excessive lactose in system. The abundant lactose prompted MR at rapid speed during the first 2 h, producing more MRPs in a short time, thereupon WVP of the MRPs increased to a large extent. However, the rest heated lactose with high concentration would probably enter into MRPs macromolecules and stick on the surface of the polymeric chains. At first, it would increase the mobility of the MRPs polymer chains, resulting in the increased WVP continuously. However, with the lactose accumulation gradually increased, the molecular gaps and free volume would lessen, causing the WVP reduced, which was the similar reason to the increased viscosity in 1:2 MR system.

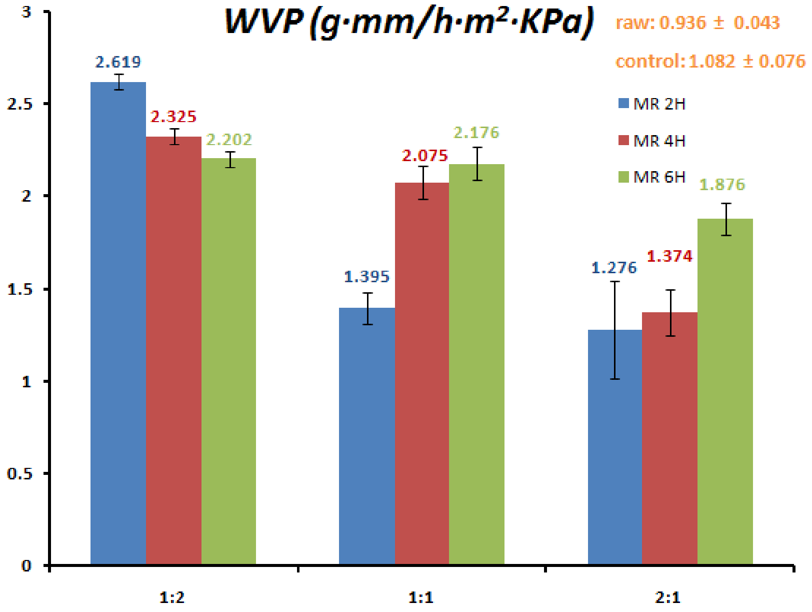


Fig.12 Effect of MR on the WVP of catfish gelatin films with WB heating

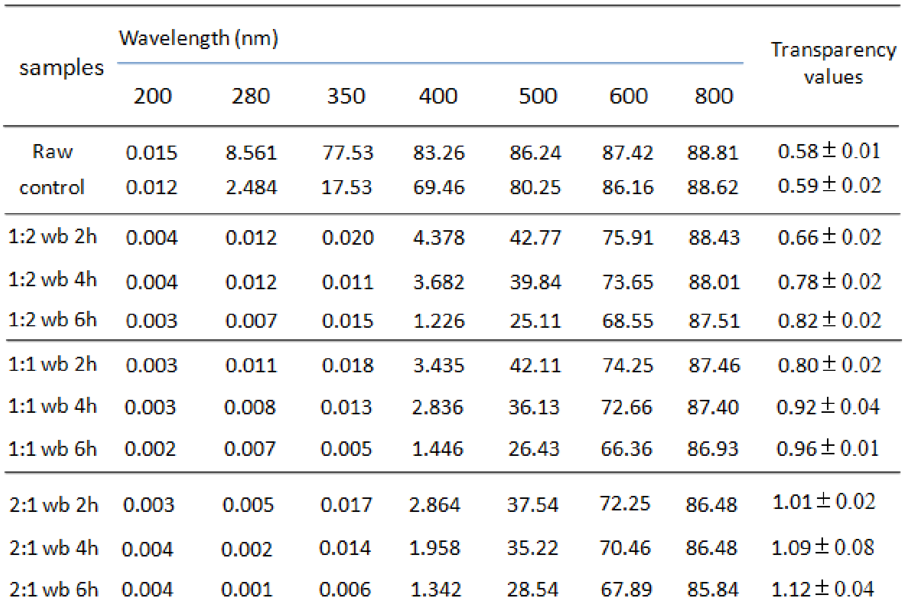
3.3.7 Light transmission (LT) and transparency

Transmission of UV and visible light at selected wavelengths in the range of 200 to 800 nm of the raw/control/MRPs gelatin films were presented in Table 10. LT of all tested films was negligible at 200 nm and also very low in 280 nm. Therefore, gelatin films were considered to possess excellent UV barrier properties. However, MRPs film showed slightly lower LT at 280 nm, compared with the raw and control films, which indicated the MRPs films could furtherly prevent the lipid oxidation induced by UV light in food system. Jiang et al. (2010) and Martins et al. (2012) also thought the low UV transmission of proteinaceous film could cause oxidative deterioration of packaged foods, mainly due to the high content of aromatic amino acids which absorb UV light. Coincidentally, the final compounds with a variety of aromatic functional groups would be formed via the MR process, causing MRPs film exhibiting very low light transmission.

MR had great effects on transmission of the gelatin film in visible wavelength (350-800 nm), resulting in all MRPs films with much lower LT, especially at violet range, from 77.53% to 0.05% in 350 nm, while from 83.26% to 1.34% in 400 nm. The raw gelatin film with high violet LT dropped dramatically and might become lightproof at the same wavelength after MR modification. The remarkable changes also occurred in green range at 500 nm, nearly reducing to one-third of the initial value, while slight changes of LT were observed in 800 nm of the infrared range. The reducing tendency and extent were not significantly different among the three MR systems. All the light transmission would reduce continuously with MR expanded. The reduced light transmission was mainly due to the light scattering effects of the lactose molecules spread in the gelatin film. In addition, the redundant lactose would fill out the intermediate gaps between the polymer chains while reduce the free volume of the MRPs structure, which might also effectively obstacle the light pass though the films.

As a result of the increased light blocking properties, MRPs films had higher transparency values compared to the raw gelatin film. The extension of MR to gelatin solution led to the increased transparency and therefore opacity of the final films. Wu et al. (2013) also got the similar results with the decreased LT and increased transparency when fish gelatin film was modified by the antioxidant additive. The greatly reduced LT as well as the slightly enhanced transparency is essential to the ability of the films, coatings, even the encapsulant, which would greatly affect the appearance of the core materials.

Table.10 Changes in light transmission and transparency at different reactant ratios and heating time with WB heating



samples	Wavelength (nm)							Transparency values
	200	280	350	400	500	600	800	
Raw control	0.015	8.561	77.53	83.26	86.24	87.42	88.81	0.58 ± 0.01
	0.012	2.484	17.53	69.46	80.25	86.16	88.62	0.59 ± 0.02
1:2 wb 2h	0.004	0.012	0.020	4.378	42.77	75.91	88.43	0.66 ± 0.02
1:2 wb 4h	0.004	0.012	0.011	3.682	39.84	73.65	88.01	0.78 ± 0.02
1:2 wb 6h	0.003	0.007	0.015	1.226	25.11	68.55	87.51	0.82 ± 0.02
1:1 wb 2h	0.003	0.011	0.018	3.435	42.11	74.25	87.46	0.80 ± 0.02
1:1 wb 4h	0.003	0.008	0.013	2.836	36.13	72.66	87.40	0.92 ± 0.04
1:1 wb 6h	0.002	0.007	0.005	1.446	26.43	66.36	86.93	0.96 ± 0.01
2:1 wb 2h	0.003	0.005	0.017	2.864	37.54	72.25	86.48	1.01 ± 0.02
2:1 wb 4h	0.004	0.002	0.014	1.958	35.22	70.46	86.48	1.09 ± 0.08
2:1 wb 6h	0.004	0.001	0.006	1.342	28.54	67.89	85.84	1.12 ± 0.04

SD: standard deviation from triplicate determinations

3.3.8 Oxygen transmission rate (OTR)

Generally, the oxygen transmission values of the gelatin films were lower than synthetic and other biopolymer films. In this study, the 3.33% raw catfish gelatin film got the OTR of 1.070 ± 0.028 ($cm^3 \cdot \mu m/m^2 \cdot d \cdot KPa$) (25 ^oC, 50% RH), shown in Table 13. Lim et al., (1999) reported the OTR of most gelatin films was highly dependent on relatively humidity (RH). They observed that great increases in OTR were evident at RH levels above 50%. Here, this OTR value of pure gelatin film was low enough and satisfying. As is well known, films or coatings containing hydroxyl, ester or other polar groups tend to present low oxygen permeability (Torres, 1994). The control film was increased obviously to 1.682 ± 0.166 ($cm^3 \cdot \mu m/m^2 \cdot d \cdot KPa$), mainly due to the lactose plasticization by increasing the intermolecular mobility and reducing the hydrogen bonding, the same reason to the increased WVP. As to the modified MRPs films, the OTR was largely increased at the first 2 h, followed by the gradually decreased in the next 4 h. The results could be considered as the formation of the MRPs with the reduction of lactose continually breaking the solution status. Compared to the OTR of raw gelatin film, all MRPs films derived from 1:2 MR system had much higher OTR due to the intense plasticization by excessive lactose, while only 6 h MRPs films obtained from 1:1 and 2:1 MR systems got relatively lower OTR owing to the greater modification by MR.

At the beginning of the MR process, the unreactive lactose had plasticization performance on gelatin molecule. The interaction between polymer chains were greatly affected by lactose, thus not only WVP, also the OTR of MRPs films were in the high degree. However, the formation of MRPs could reduce the OTR of the corresponding films. MRPs had strong antioxidative characteristics, which could obstruct the O atom effectively. As the composition of MRPs was quite complicated, and the components were not totally clear, the mechanism of MRPs molecules possessing the repulsive interaction on O atom was inconclusive yet. But Hong and Krochta, (2003) reported the low OTR was probably due to the ring structure of the products which prevented efficient disruption of hydrogen bonding between some of the protein chains. They demonstrated the sucrose could assist films to have the preferred oxygen barrier, which was also proved by Sothornvit and Krochat. (2000b). It was likely that the heterocyclic nitrogenous compounds, one sort of the MRPs components, had great influence on the restriction of oxygen. Additionally, the increase in crystallinity, orientation, and molecular mass will probably result in a decrease in oxygen permeability (Miller & Krochta, 1997). The MRPs polymeric compounds had intricate macromolecular structure with large molecular mass. The increased T_g also illustrated the crystallinity of MRPs film was improved to the higher degree. Last but not least, there was close relationship between WVP and OTR of the MRPs film. Srinivasa et al. (2007) reported low OTR in the blend film was attributed to the solute presumed to diffuse through the micro-channel or pore within the membrane structure, and then the water molecule thus fitted in the micro- channel may obstruct the movement of the oxygen molecule. Miller & Krochta (1997) also considered most edible films are highly sensible to moisture, but due to the high degree of hydrophilic properties, present an excellent barrier to oxygen and to some aromatic components. In some way, it could be regarded that the too much water molecular would affect the oxygen transmission through the MRPs films.

Usually, the modification by plasticizer on gelatin film would increase both WVP and OTR. However, the MRPs characteristics could overwhelm the effect of lactose plasticization and obtain the decreased OTR in this research, though the free volume were increased of the final MRPs structure. It was convinced that the lactose was a “double-edged sword” to the gelatin films.

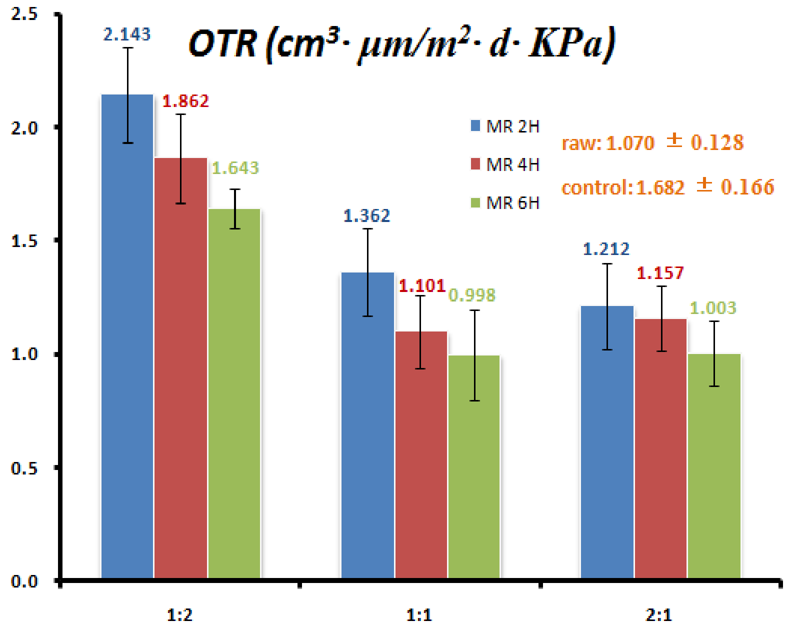


Fig.13 Effect of MR on the OTR of catfish gelatin films with WB heating

3.4 Conclusion

Maillard reaction plays an important role in improving the food quality, nutritional values and protective capability. In this study, MR was used to modify the physicochemical properties of the catfish gelatin films. Different reactant ratios of gelatin and lactose with different MR time were attempted to get an ideal condition for the optimal modification on the gelatin films. As a result, 1:1 MR system was preferred compared with other two MR systems based on the solution properties. Under the same circumstance, the 1:1 MR system got lowest pH value and viscosity while highest absorbance. The insufficient lactose in 2:1 MR system caused the lower yield of MRPs while the excessive lactose in 1:2 MR system broke the balance of the final MR solution. Besides that, 4 h MR time was prior, dependent on the film properties. The expanded heating time resulted in the continuous increase on EAB, WVP, and T_g, simultaneously the reduce on TS, LT, and OTR of the gelatin films. Usually, the equilibrium between TS and EAB are crucial to the edible film to withstand the normal stress as well as possess enough flexibility. Therefore, the mechanical properties controlled at intermediate value, with 4.92 Mpa TS and 294.49 % EAB of 4 h MRPs film, were relatively suitable. As to the thermal property, the 4 h and 6 h MRPs film both had the same highest T_g (98 ^{o}C) with much better thermal stability than that of the raw gelatin film. In addition, the difference on permeability between 4 h and 6 h MRPs film were not significantly different. In order to get the relatively low WVP, the MRPs film obtained at 4 h was considered as the dominant option. Thus, 4 h MR modification on gelatin biofilm in 1:1 MR system was proved to be the ideal condition for optimal improvement on their physicochemical properties.

Chapter 4 Comparing the difference on physicochemical properties of the catfish gelatin films through Maillard reaction (MR) by radio frequency (RF) heating and traditional water bath (WB) heating

Abstract

Radio frequency (RF) dielectric heating is a promising heating technology for food industries. In this study, RF heating was applied to take place of WB heating to assist the MR modification on the physicochemical properties of gelatin film. All the MR procedures between RF and WB heating were accordant. MR was performed at 100 ^{o}C for both RF and WB heating with different reactant ratios (1:2, 1:1 and 2:1) at different heating time (2 h, 4 h, and 6 h). Compared to the WB heating, RF system promoted the MR to a higher extent with greater modification on physicochemical properties of gelatin film, due to the unique heating mechanism. The RF MRPs film became far more extendable, with much higher thermal stability than the WB MRPs film. The barrier properties of light and oxygen were also much better in RF MRPs film. Interestingly, the WVP of RF MRPs film increased firstly then dropped down with the expanded MR time. The modification on 2 h RF MRPs film was already greater than that on 6 h WB MRPs film. Overall, the optimal modification on gelatin film was conducted under the condition of 1:1 MR system with RF heating at 2 h.

Key words: radio frequency heating, water bath heating, Maillard reaction, physicochemical properties, MRPs film

4.1 Introduction

Maillard reaction (MR), a chemical method, was attempted the first time to modify the physicochemical properties of the gelatin films in this research. MR is known as the formation of MRPs with strong antioxidant ability. Amino-carbonyl reaction is the nature of MR with many other complex processes and unfathomed details. The final MRPs are very complicated, including melanoidins, reductones, heterocyclic nitrogenous compounds and other highly colored polymeric mixture. MRPs have special aroma and exhibit antioxidant, antibiotic, antimicrobial, as well as antiallergenic functions, which could reduce the oxygen transition rate (OTR) and simultaneously modify other physicochemical properties of the gelatin biofilm. MR is largely influenced by several factors, such as temperature, time, initial pH, water activity (a_w), and reactant source and concentration (Lingnert, 1990 and Wijewickreme et al., 1997). Moreover, the heating time and reactant ratio are the keys to the advanced glycation endproducts, known as AGEs. AGEs are generated in the final stage of MR irreversibly and spontaneously. Prolonged incubation of proteins with reducing sugars results in a Schiff base and Amadori rearrangement products to form AGEs (Takemi Kimura et al., 1996). On the other side, excessive reducing sugar in the MR systems breaks the balance of the amino-carbonyl, thus leading the redundant carbonyl groups to accelerate and stimulate the Amadori products transforming into reactive dicarbonyl products to form AGEs finally. The formation of the AGEs is shown in the Figure 14. AGEs are quite harmful to human when absorbed into the body. It is reported that diseases such as atherosclerosis, cataract and diabetic nephropathy, retinopathy and neuropathy are suggested to be either caused or promoted by AGEs (Brownlee, M, 1995; Palinski, W et al., 1995). Furthermore, according to the aging tissue caused by AGEs, it has been postulated that AGEs may be also involved in the aging process affecting the central nervous system in human (Takemi Kimura et al., 1996). Therefore, controlling the occurrence of AGEs during the MR process is significant. In this study, in order to keep the high yield of MRPs with the reaction time as short as possible, radio frequency (RF) was applied to treat the MR as an innovative heating method.

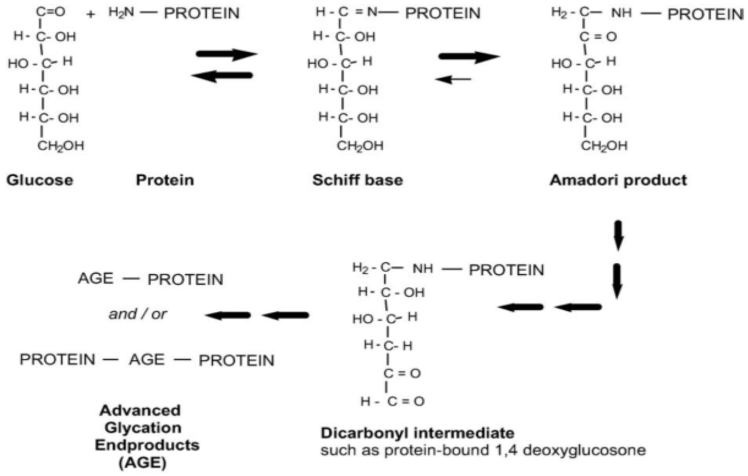


Fig.14 The AGEs formation process

RF dielectric heating is a promising heating technology for food industries owing to the homogeneous mode, volumetric transfer, uniform distribution, huge energy, deep penetration, and low energy consumption. RF heating involves the transfer of electromagnetic energy directly into the products instead of by slow heat conduction/convection as in conventional heating. During RF heating, heat is generated within the product due to molecular friction resulting from oscillating molecules and ions caused by the applied alternating electric field (Piyasena et al., 2003). This heating mode can cause the MR film forming solution to be heated rapidly throughout the entire mass and accelerate the combination of carbonyl and amino groups uniformly, which essentially means all space of the MR solution were more or less heated simultaneously at the same rate. RF heating can reduce reaction time as well as increase the quality of the MRPs solution.

As is concerned to all, RF heating has been used in food industries for many decades, mainly including meat, vegetable, fruit, aquatic, etc. Only a few researches reported the effects on the proteinaceous materials influenced by RF heating. Wang et al. (2003) reported dielectric properties of foods (whey protein gel) relevant to RF and microwave pasteurization and sterilization, they found RF treated samples to be much closer in color and flavor to the control; Ahmed et al. (2007) studied rheological and gelatin characteristics of RF heated egg white dispersions and found RF heated protein dispersions produced stronger gels, especially in the alkaline condition. However, none research has been reported on MR to improve the gelatin assisted by RF heating.

The major purpose of this work is to study the superiority on the physicochemical properties of the catfish gelatin biofilm through MR assisted by RF heating. The MRPs solution properties (pH, viscosity, absorbance) and the subsequent MRPs film properties (TS, EAB, Tg, WVP, OTR) are necessary to be measured to investigate whether the MR modification treated by RF heating has the same tendency as that of WB heating, as well as whether RF heating can offer higher yield of MRPs, and then obtain better physicochemical properties compared to WB heating at the same condition (reactant ratio & heating time). The overall goal of this research is to compare the difference of WB and RF heating assisting MR on physicochemical properties of the gelatin biofilm.

4.2 Material and Methods

The methods in Chapter 4 were the same as to those used in Chapter 3, expect the RF heating process.

During the preparation process, lactose was dissolved in 250 ml 3.33% gelatin solution at three different ratios (1:2, 1:1, and 2:1). The pH of the solution was adjusted by buffers (0.05 M NaOH – 0.1 M $NaHCO_3$, pH 11). The solution was then transferred to 250 ml flask, tightly capped by rubber stopper and aluminum foil. Since the RF energy is huge enough, it would more or less evaporate the top portion of the solution and finally cause the gelatin separated out on the flask wall. Therefore, in this study, the flask was placed into a glass container filled with 2 cm higher water height than the flask bottleneck, to keep the MR solution not affected by overheating problem. Before solution was treated by the RF systems, four fiber-optic sensors (UMI, FISO Technologies, Quebec, Canada) were inserted into different locations of the container to monitor the water temperature. The temperature was controlled at $100^{o}C$ with ± $3^{o}C$ fluctuation for 2 h, 4 h, and 6 h, respectively. The RF heater (SO6B;

Strayfield, Berkshine, England), with scientific frequency of 27.12 MHz and maximum power output of 6 kW, was employed in this study, shown in figure 15. Energy was created by a pair of parallel rectangular pate electrodes. The lower plate electrode was mounted fixedly, while the upper applicator was adjustable. During the RF heating process, the air column (the distance between upper plate and the water surface) was controlled at 2 cm firstly to make the temperature reach 100°C as fast as possible, and then the RF heater was paused for 1min to add the warm water (60°C) followed by another 5 min RF heating, meanwhile the air column was adjusted to 3.5 cm for the desired power of RF energy to keep the temperature at100°C. The pause/heat step was repeated until the predetermined RF heating time was completed. After the heating period, the MRP solution was immediately cooled down in ice bath then kept at 4 °C prior to analysis. All model systems were prepared in triplicate.

Fig 15 The RF heater with MRPs solution

4.3 Results

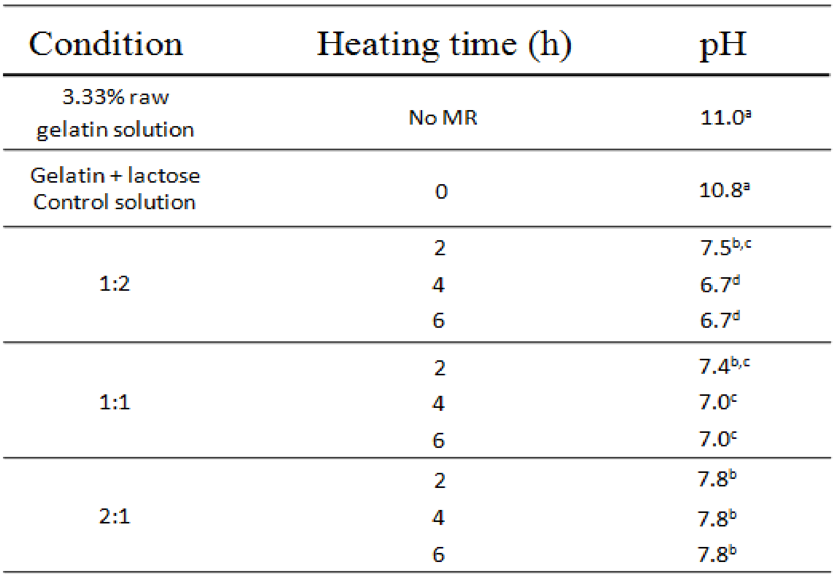
4.3.1 Comparison on the pH changes between RF and WB heating

The pH values of MRPs solution treated by RF heating are shown in Table 11. The tendency of pH variation was the same with WB and RF heating. The pH decreased remarkably after MR started, but the lowest value 6.7 occurred in 1:2 MR system. All pH values significantly decreased from RF system compared to WB method under the same condition (reactant ratio and heating time), which demonstrated the RF heating offered MR much faster speed and higher yield of organic acid. Actually, the downtrend of pH was quite obvious and clear with RF heating. From the results at 2 h, all pH values were below 8.0, which indicated MR reached very high extent and formed abundant organic acids. Furthermore, according to the results at the 4 h and 6 h in every MR system, the pH values were constant. The whole process was at extremely rapid speed and might already past the acidic formation process at the intermediate stage of MR between 2 h and 4 h. As to the 2:1 MR system, the pH values were equivalent with different time, the MR process might already entered into final stage even earlier before 2 h, while the pH was lower than 7.0 after 4 h in 1:2 MR system, it is mainly due to the occurrence of caramel reaction caused by the huge energy efficiency of RF system under the alkaline condition.

RF heating showed advantage on the MR speed, reaction time, and final pH versus WB heating. When the heating time arrived at 2 h, more lactose in 1:2 MR system didn’t arrive the lowest degree among three MR systems, like what happened with WB heating in Section 3.3.1. This phenomenon emphasized the above hypothesis that the MR process might had already entered into the final stage very early by RF heating. In other words, the order of pH value by WB heating at 2 h might be in line with that of RF heating around 1 h, even earlier. Lower pH obtained from RF system at 2 h than WB method at 6 h could also prove the MR assisted by RF heating was dominant.

The major reason caused the pH difference was the heating mechanism. During RF heating, heat was generated inside the MR solution due to molecular friction resulting from oscillating molecules and ions caused by the applied alternating electric field. Therefore, RF heating mode could cause the MR solution to be heated rapidly throughout the entire mass and accelerate the movement among the carbonyl-amino groups and alkaline buffer ions constantly, which essentially meant all space of the MR solution was more or less heated simultaneously at the same rate. Compared with the RF dielectric heating, the heat energy of WB heating system was transferred gradually from hot medium (boiling water) to the solution surface dependent on superficial heat transfer, resulting in large temperature gradients and moderate heating rate. RF system could provide more opportunity for the contact of gelatin and lactose molecules through the direct interaction between an electromagnetic field and MR solution, which promoted the carbonyl-amino combination and then formed more organic acids at a fast rate, leading to the pH reduced in a short time. As to the organic acids, most acetic acid was derived from the pyrolysis of dicarbonyl compound probably at the end of the intermediate stage, which required high energy to occur. RF system could provide huge energy and lead the cleavage reaction happened accessibly. The above explanations showed why RF technique had advantage over WB method and got lower pH value.

Table.11 Changes in pH at different reactant ratios and heating time with RF heating



Condition	Heating time (h)	pH
3.33% raw gelatin solution	No MR	11.0^{a}
Gelatin + lactose Control solution	0	10.8^{a}
1:2	2	7.5^{b,c}
	4	6.7^{d}
	6	6.7^{d}
1:1	2	7.4^{b,c}
	4	7.0^{c}
	6	7.0^{c}
2:1	2	7.8^{b}
	4	7.8^{b}
	6	7.8^{b}

All standard deviation were less than 0.1; $^{a-c}$ different letter superscripts donated significant difference ($p < 0.05$).

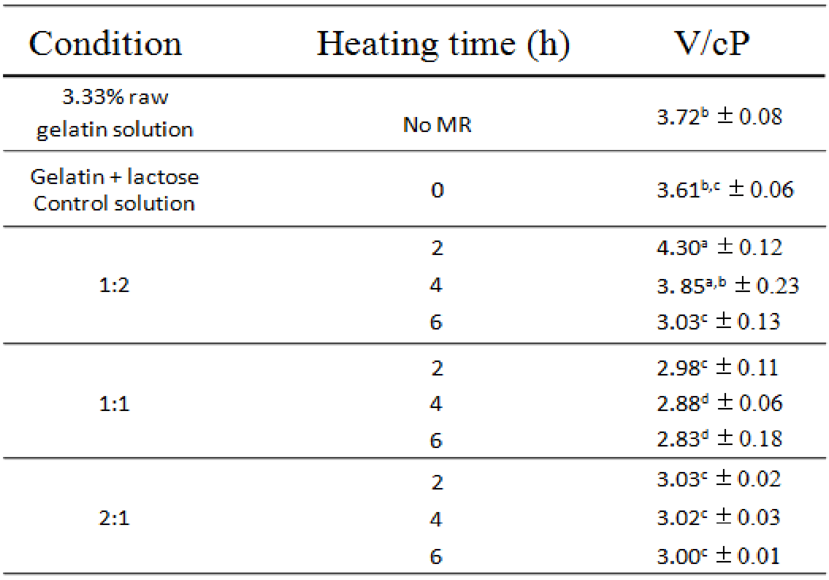
4.3.2 Comparison on the viscosity changes between RF and WB heating

The changes in viscosity of MRPs solution assisted by RF heating can be seen in Table 12. The viscosity decreased more significantly in 1:1 and 2:1 MR systems and increased firstly then decreased afterwards in 1:2 MR system, partial different to WB heating results. All the viscosities caused by RF heating were below 3.0 cP with a sequential decline trend in 1:1 MR system. It was convinced that such a remarkable drop in viscosity had close relationship with the big changes in MR solution. RF heating could accelerate the formation of MRPs in a short time. Even the lactose was insufficient in 2:1 MR system, resulting in relatively high viscosities by WB heating, the significant change in viscosity, reduced to 3.03 cP, was found after 2 h due to the huge assistance by RF technique. However, the viscosities nearly no longer reduced with the expanded heating time, it could be inferred most MR processes probably had already been to the terminal during the first 2 h, there were no more changes in the subsequent MR solution. As to viscosity variation in the 1:2 MR system, the first increase was related with lactose accumulation, same reason to the WB results, while the subsequent decrease was connected with more MRPs and caramel products formed, resulting less lactose occupying the intermolecular gaps between polymer chains. Thus the free volume was enlarged again with the increased fluidity.

Under the same condition, the viscosity of MRPs solution was lower with RF heating compared to WB heating. Furthermore, even the viscosity obtained at 2 h by RF system was lower than 6 h by WB method in both 1:1 and 2:1 MR systems, which explained MR assisted by RF heating was far more efficient than WB heating. From another perspective, it could be inferred the variation on MR compositions, mainly the formation of MRPs, were much more remarkable in RF system. RF technique could improve the MR efficiency via the special heating mechanism by oscillation. The continued reversal of polarity in the electromagnetic field led to the oscillation of ions forwards and backwards in the MR solution with the net effect of this being the internal generation of heat within the MR system by friction (Buffler, 1993). All the molecules were in constant movement at the accordant rate during the whole process, resulting in the energy efficiency enough high as well as MR solution adequately uniform. Liu et al. (2010) and Wang et al. (2015) reported the more polar part would absorb more energy in RF system. This could promote the combination as well as transformation of carbonyl and amino groups, and finally advance the MR extent. Meanwhile, the improved MR speed by RF system would expedite the consumption rate of the amino groups, which accelerated the weakening of molecules interaction and intermolecular forces between gelatin polymer chains, resulting in the rapid increase on molecule fluidity of the MRPs. That was why the viscosity of MRPs solution treated by RF heating decreased significantly in a short time.

Under normal circumstance, the increasing degree of viscosity in 1:2 MR system should be more significant with RF heating than WB heating, which was not occurred. The abnormal phenomenon might be related with the different component ratio in the final solution caused by dielectric and conventional heating method. The reason on the increasing trend of viscosity in 1:2 MR system with WB heating was mentioned in Section 3.3.2. Since RF system could assist MR to a greater extent, it could be concluded that more MRPs were formed with less excessive lactose left at the end. Additionally, the caramel products and/or AGEs were more likely to occur under the condition of abundant energy offered by RF heating, which would also consume the lactose. Thus there was relatively small amount lactose left eventually, causing the lactose accumulation extent on free volume of intermolecular gaps to be lower. Above all, with the MR time expanded, the excessive lactose in 1:2 MR system continuously accumulated between the polymer chains with WB heating, while hydrolyzed by themselves to form the caramel products with RF heating. That was primary reason on the different viscosity tendency with dielectric and conventional heating mode.

Table.12 Changes in viscosity at different reactant ratios and heating time with RF heating



Condition	Heating time (h)	V/cP
3.33% raw gelatin solution	No MR	$3.72^{b} \pm 0.08$
Gelatin + lactose Control solution	0	$3.61^{b,c} \pm 0.06$
1:2	2	$4.30^{a} \pm 0.12$
	4	$3.85^{a,b} \pm 0.23$
	6	$3.03^{c} \pm 0.13$
1:1	2	$2.98^{c} \pm 0.11$
	4	$2.88^{d} \pm 0.06$
	6	$2.83^{d} \pm 0.18$
2:1	2	$3.03^{c} \pm 0.02$
	4	$3.02^{c} \pm 0.03$
	6	$3.00^{c} \pm 0.01$

All standard deviation were less than 0.1; $^{a\text{-}c}$ different letter superscripts donated significant difference ($p < 0.05$).

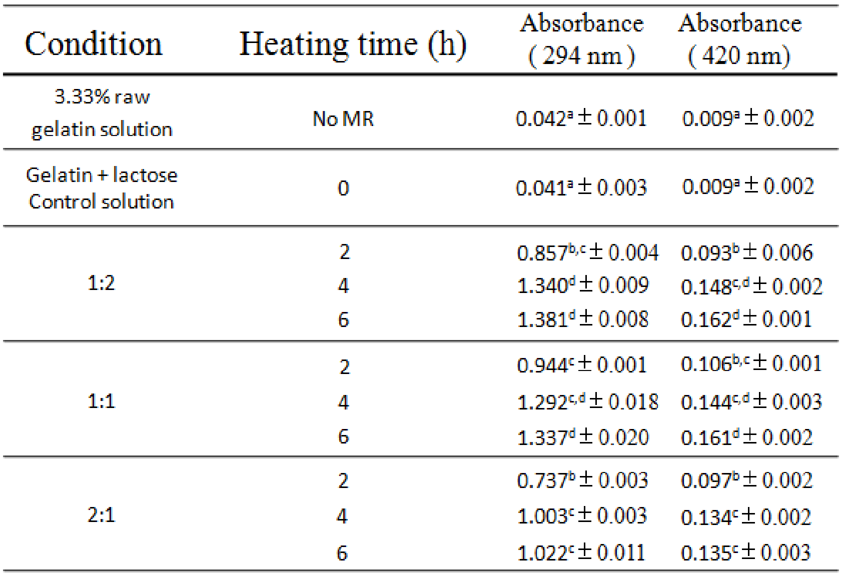
4.3.3 Comparison on the absorbance changes between RF and WB heating

Table 13 shows the absorbance on the intermediate products and browning intensity of the MRPs solution with RF heating. In some perspective, the A_{294} and A_{420} could, at least qualitatively, depict the MR speed as well as the MRPs yield. The overall tendency on absorbance with RF and WB heating were alike. Both A_{294} and A_{420} raised as the heating time expanded with most intense degree at the beginning. At first 2 h, the largest A_{294} and A_{420} happened in 1:1 MR system higher than those of the other two MR systems, which accorded with the pH variation tendency. MR had arrived at much greater extent with RF heating compared to WB method, especially at initial period. After that, 1:2 and 1:1 MR systems had similar results, same to the WB results, but also much higher than that of 2:1 MR systems. Actually, the A_{294} and A_{420} were nearly equivalent in 2:1 MR system at the last 4 h with RF heating, which demonstrated the MR process might stop around 4 h due to the insufficient lactose.

RF showed superiority on the final MRPs yield with much preferable MR speed. RF heating had remarkable advantages on both intermediate products value (A_{294}) and final browning intensity (A_{420}) over WB heating results. Compared with the WB heating, RF heating nearly got double values under the same condition. In fact, none MR was complete by WB heating because of the inadequate energy efficiency. The heating efficiency of RF system was too high for WB method to catch up. The final browning intensities (A_{420}) with 6 h WB heating were even lower than 2 h RF heating results. It can be boldly regarded the extent of 2 h MR with RF heating was greater than 6 h WB heating. The maximum A_{294} and A_{420} were both created in 1:2 MR system at the first 2 h with WB heating, due to the promotion of excessive lactose, shown in Section 3.3.3, but occurred in 1:1 MR system with RF heating. It was because that huge energy by RF system assisted MR already passed over the initial phase and entered into more advanced stage. Generally, the lactose effect on MR in middle-late stage was much less than initial stage. In addition, the relatively low intermediate products value (A_{294}), occurred in 2:1 MR system with RF heating, were overall higher than the optimum 1:1 MR system with WB heating, which also explained heating mode had far more influence on MR than the reactant ratio.

RF brought a qualitative change to MR. The peculiar advantages could impulse MR to greater extent within shorter time. A study by Hu and Wen (2008) reported the special heating property of RF heating resulted in an “explosion” effect among the particles. In this study, the carbonyl and amino groups were affected by such “explosion” and they would absorb huge electromagnetic energy for connection as soon as the RF system was on, instead of the slow heat conduction followed by WB heating. Thus, these unique heating mechanisms mentioned above explained why MR assisted by RF worked better on MRPs yield and reaction speed than WB.

Table.13 Changes in absorbance at different reactant ratios and heating time with RF heating



Condition	Heating time (h)	Absorbance (294 nm)	Absorbance (420 nm)
3.33% raw gelatin solution	No MR	$0.042^{a} \pm 0.001$	$0.009^{a} \pm 0.002$
Gelatin + lactose Control solution	0	$0.041^{a} \pm 0.003$	$0.009^{a} \pm 0.002$
1:2	2	$0.857^{b,c} \pm 0.004$	$0.093^{b} \pm 0.006$
	4	$1.340^{d} \pm 0.009$	$0.148^{c,d} \pm 0.002$
	6	$1.381^{d} \pm 0.008$	$0.162^{d} \pm 0.001$
1:1	2	$0.944^{c} \pm 0.001$	$0.106^{b,c} \pm 0.001$
	4	$1.292^{c,d} \pm 0.018$	$0.144^{c,d} \pm 0.003$
	6	$1.337^{d} \pm 0.020$	$0.161^{d} \pm 0.002$
2:1	2	$0.737^{b} \pm 0.003$	$0.097^{b} \pm 0.002$
	4	$1.003^{c} \pm 0.003$	$0.134^{c} \pm 0.002$
	6	$1.022^{c} \pm 0.011$	$0.135^{c} \pm 0.003$

All standard deviation were less than 0.1; $^{a\text{-}c}$ different letter superscripts donated significant difference ($p < 0.05$).

4.3.4 Comparison on the Tensile strength and elongation at break changes between RF and WB heating

The effects of RF system on the tensile strength (TS) of the MRPs films were revealed in Figure 16. As expected, TS decreased markedly with the increased MR time ($p < 0.05$). Comparing the RF and WB MRPs films, the most significant difference appeared in 2:1 MR system. Both of the TS decline rate and reduction extent with RF heating were superior to those of WB heating. At the same time, RF MRPs film had the TS value less than half of WB MRPs film, mainly due to the greater MR extent assisted by huge energy from RF system.. RF system could provide the enormous energy immediately to those important functional groups of the reactants, as carbonyl and amino groups, making the MR process always towards the positive direction at fast speed. This was the primary reason why the TS value of 6 h WB MRPs film was still higher than 2 h RF MRPs film. Low energy efficiency of WB method caused the MR in relatively low speed, especially when the lactose was lacking in the system without any impulse to accelerate the MR rate. As a result, the change of WB MRPs films on TS was not complete, at least lower than RF MRPs films. The significant difference on TS was also quite obvious in 1:1 MR system. The TS decreased drastically to lower degree at first 2 h followed by a slight variation in the rest time with RF heating, whereas the TS dropped moderately and continuously during the whole 6 h MR process with WB heating and finally reached a little bit higher degree. This phenomenon could prove again that RF heating prompted MR to the significant extent in a quite short time and obtain the lower TS of the MRPs films. There was no statistics difference on the TS between these two films in 1:2 MR system, both of which arrived at greatest extent in the first 2 h and kept in the same level subsequently, resulting in very flexible and extensible characteristics. In 1:2 MR system, the abundant lactose could impel the MR speed, as mentioned in Section 3.3.1, and accelerate the modification on TS. However, this result could not demonstrate the RF system didn’t take advantage over WB method on MR extent and the final TS of MRPs films. At least, the MRPs films treated by RF heating still got a little bit lower TS than that of WB heating. It could be hypothesized that the TS of MRPs film with RF heating might reached around 2.02 Mpa in less time than 2 h, and stayed at that level afterwards.

Compared to the tensile strength, RF system showed more significant difference on elongation (EAB) than WB method (Figure 17). The EAB modified by MR with RF heating increased to a larger extent than WB heating. Among all the MR system, the EAB of RF MRPs films were much higher than that of WB MRPs films at the same time, even the EAB of 2 h RF MRPs films was higher than that of 6 h WB MRPs films. These results revealed the intermolecular force were much weaker while the intermolecular spaces were more expanded in RF MRPs films. Actually, RF heating had a better performance in disrupting the hydrogen bonds compared to WB heating. The RF dielectric heating induces rapid rotation of the polarized dipoles and ions in the molecules, which generates tremendous heat inside the MR solution. The functional groups could absorb enough energy simultaneously in all space. Therefore, the molecule interactions during the MR process by RF were definitely more intense than WB. The greater MR extent by RF would generate more MRPs, therefore more original gelatin hydrogen bond would be replaced by new intermolecular force, and resulted in much lower TS while higher EAB. In addition, the huge energy by RF heating could assist 2:1 MR system to overcome the deficiency of low MR extent with WB heating caused by insufficient lactose, and finally got remarkable MR extent as well as high EAB even at first 2 h. Furthermore, the high energy efficiency by RF heating could also promote 1:2 MR system to reach greater MR extent with more modification on the EAB. The reaction condition would gradually become tough with the consumption of the gelatin. At the later stage of MR, there was not enough impulse to drive the reaction to the positive direction at a high speed if without the abundant huge energy. That was why the EAB of WB MRPs films did not increase in the last 4 h. The WB heating was too moderate to satisfy the reaction condition of MR. In contrast the RF heating was generated inside the MR solution and it was enormous and dramatical all the time, which could ensure the MR solution always at intense movement. As a result, the MR could be kept at the great extent from the beginning to the end. That was why the EAB of RF MRPs films had an extreme increase to 484.13% only after 2 h MR process, which was already higher than any EBA of the WB MRPs films. Subsequently, the EAB of RF MRPs films increased continuously with the expanded heating time. It is because the modification by MR was still on process.

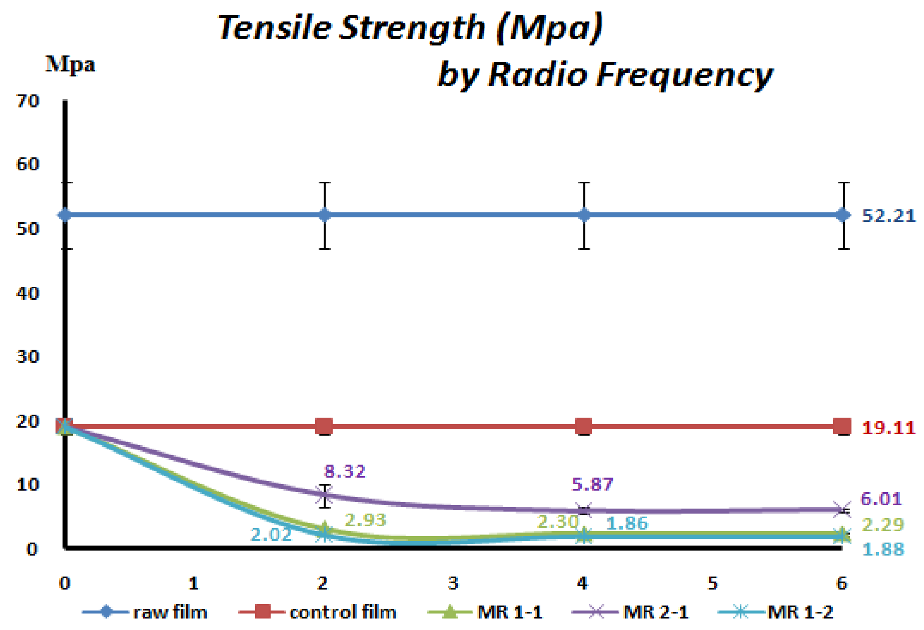


Fig.16 Effect of MR on the tensile strength of catfish gelatin films with RF heating

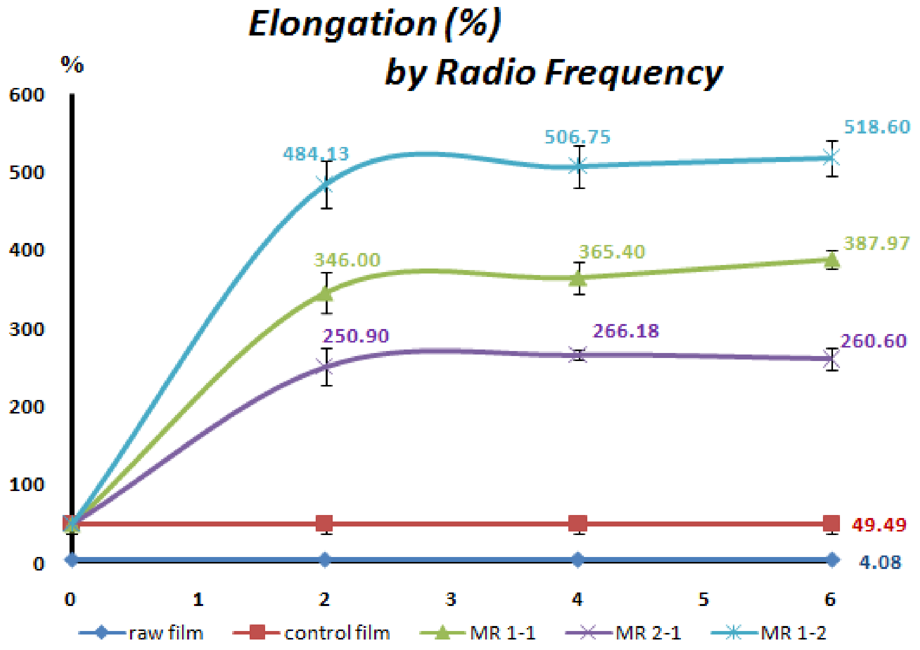
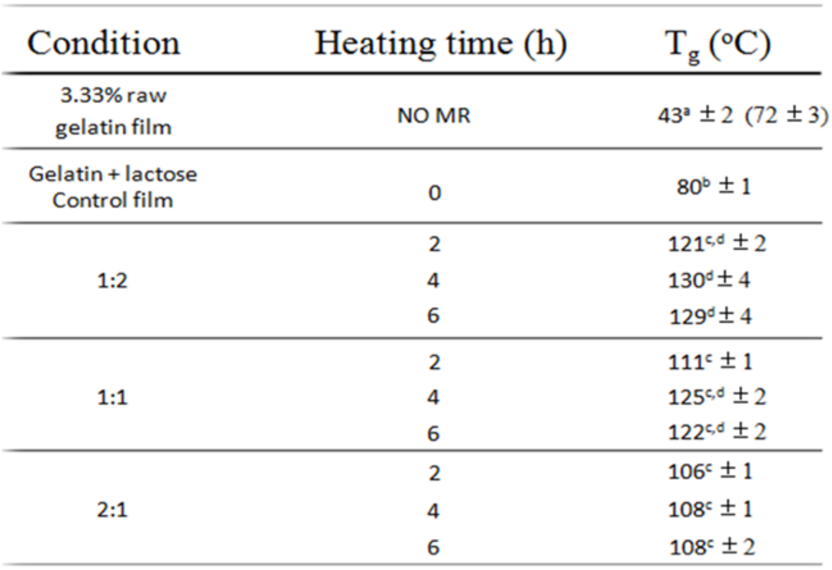


Fig.17 Effect of MR on the elongation at break of catfish gelatin films with RF heating

4.3.5 Comparison on the thermal properties changes between RF and WB heating

DSC results obtained from the RF MRPs films are presented in Table 14. The T_g of RF MRPs films were significantly increased compared to that of the WB MRPs films. All the results were over 101 ^{o}C, the T_g of anhydrous lactose. This phenomenon demonstrated there might be some different components mixed with MRPs in the final MR system. Moreover, the thermal stabilities of these new products were higher than that of MRPs. In other words, they had tighter molecular structure with closer molecular interaction and stronger intermolecular force. It was concluded the caramel products, even the AGEs were generated simultaneously during the MR process attributed to the unique heating mechanism by RF system. The caramel products were formed from lactose itself, without those high hydrophilic groups in gelatin molecule, while the AGEs were derived from another pathway, which was different from the MR process since the initial stage. In this case, the great increases on T_g of MRPs films seemed to be accessible. The formation of caramel products was mainly related to the lactose amount in the MR system on the premise of RF heating. The 2:1 MR system got lowest yield of caramel products since lactose was insufficient in the whole system. MR took precedence over the caramel reaction. When the lactose was close to exhaustion, the caramel reaction was quite tough to occur. That is why the T_g increase extent in 2:1 MR system was the lowest and the increase tendency was inactive firstly among these three MR systems. On the contrary, T_g increased to very high temperature in 1:2 MR system as more lactose were left after MR terminated. RF system would give the excessive lactose enough energy to conduct the caramel reaction. Furthermore, when the heating time was overlong, the formation of AGEs was not impossible. That might be the reason why T_g dropped back at 6 h. This phenomenon also happened in the 1:1 MR system. But it can be confirmed the amount of new products in 1:1 MR system was lower than 1:2 MR system based on the lower T_g value, due to the less initial lactose amount in the MR system. Additionally, most error limits of the T_g value were enlarged as well, especially the MRPs films obtained at 6 h. Suyatma et al., (2004) and Hosseini et al., (2013) thought if two components were only partially miscible, the T_g value of each component phase should be affected by the other one, and it was usually composition dependent. The fluctuant T_g value and enhanced error limits might be related with phase separation caused by partial immiscibility among the MRPs, caramel products, and AGEs.

Table.14 Changes in glass transition temperature at different reactant ratios and heating time with RF heating



Condition	Heating time (h)	T_g (°C)
3.33% raw gelatin film	NO MR	$43^{a} \pm 2$ (72 ± 3)
Gelatin + lactose Control film	0	$80^{b} \pm 1$
1:2	2	$121^{c,d} \pm 2$
	4	$130^{d} \pm 4$
	6	$129^{d} \pm 4$
1:1	2	$111^{c} \pm 1$
	4	$125^{c,d} \pm 2$
	6	$122^{c,d} \pm 2$
2:1	2	$106^{c} \pm 1$
	4	$108^{c} \pm 1$
	6	$108^{c} \pm 2$

SD: standard deviation from triplicate determinations; $^{a\text{-}c}$ different letter superscripts donated significant difference ($p < 0.05$).

4.3.6 Comparison on the WVP changes between RF and WB heating

The influence on the WVP of MRPs films with RF heating was examined in Figure 18. The tendency on the WVP variation of MRPs films with RF heating was significantly different from that of WB MRPs films. Generally, the WVP of MRPs films increased with the MR time expanded, just as the results of 1:1 and 2:1 MR systems with WB heating. The gelatin hydrophilic groups would combine with other reactive groups and establish more hydrophilic interaction during the MR process. Consequently, the intermolecular gap was enlarged, and the free volume of the polymeric matrix was enhanced as well as the water diffusion in the matrix of the film was increased. However, the WVP of RF MRPs films increased markedly at first 2 h then reduced abnormally in the following 4 h. Furthermore, the highest WVP of WB MRPs films (2.619 $g \cdot mm \cdot h^{-1} \cdot cm^{-2} \cdot Pa^{-1}$) occurred in 1:2 MR system, while the RF MRPs films got the highest WVP (2.623 $g \cdot mm \cdot h^{-1} \cdot cm^{-2} \cdot Pa^{-1}$) in 2:1 MR system. This strange phenomenon mentioned above might be related with the new products, such as caramel products and AGEs, which broke the original balance of the MRPs solution. It could be inferred the WVP increased with the formation of MRPs and afterwards decreased with the formation of caramel products and/or AGEs assisted by the high energy efficiency of the RF system.

In 2:1 MR system, RF MRPs films had extremely high WVP after the modification by MR, while the increase on WVP of WB MRPs film was relatively slight. It was because RF system did better works on MR compared to WB method, RF could promote the MR extent and obtained more MRPs, resulting in more changes on molecular structure. Thus the WVP increased much higher in RF MRPs films than WB MRPs films. After the WVP reached the highest degree at first 2 h, it began to reduce step by step in the next 4 h, which also happened in 1:1 and 1:2 MR systems with RF heating as well as in 1:2 MR system with WB heating. However, it was confirmed the reason for decline part in WVP for RF system and WB method was different. As mentioned in Section 3.3.5, the excessive lactose in the WB MRPs system would sequentially increase the WVP by enhancing the mobility of the intermolecular polymer chains, after that, the WVP reduced gradually due to the less molecular gaps and free volume existed by the continuous lactose accumulation. On the contrary, the rest lactose in RF MRPs system might be still involved in the advanced caramel reaction attributed by the special heating mechanism of RF technique. Usually, the temperature limit for caramel reaction was around140 ^{o}C (lactose: 129.5 ^{o}C), thus the possibility for the occurrence of caramel reaction in WB heating was very tough. Conversely, the RF dielectric heating transformed the electromagnetic energy into heat that is volumetric and fast through a direct interaction between RF electromagnetic field and the MR solution (Wang, et al., 2002). The unique “strength” inside RF heating techniques could lead to the “explosion” and “hot pot” effect on MR solution. Though the temperature might be still less than 129.5 ^{o}C, the tremendous heat efficiency was most likely to cause the caramel reaction happened in RF system. Furthermore, another theory was introduced in this study. It was reported that the electromagnetic field could generate a kind of athermal effect by microwave radiation (MWI). This special effect could provide additional alternative energy to the MR solution, although it was different from the normal heating and was not defined by the temperature variation. Furthermore, athermal effect was able to change the activation energy of the reaction condition, which might bring more change to the formation of caramel products, even the AGEs. Garcia et al. (2000a) and M.H. Norziah & A.A. Al-Hassan (2012) considered that WVP mainly depended on the ratio between crystalline and amorphous zone, polymeric chain mobility, and specific interaction between the functional groups of the polymers. The hydrophilic groups of the lactose molecule were not as many as that of gelatin, while lactose had high thermal stability with the highest glass transition temperature among the reducing sugars. The caramel products were derived from lactose, they were more likely to possess tight molecular structure with better water barrier properties compared to MRPs. That might be the reason why the MR speed and final extent in 1:1 and 1:2 MR systems were greater than in 2:1 MR system with RF heating, whereas the WVP variation of the MRPs films were relatively lower. Actually, the highest WVP in 1:1 and 1:2 MR systems appeared earlier before 2 h, not shown on the Figure 17. Afterwards they reduced gradually with the formation of the caramel products. In addition, more lactose in 1:2 MR system got lower WVP compared with 1:1 MR system, which also due to more caramel products formed.

Finally, it was worthy to mention the formation of AGEs could also occur in the final products, especially in the 1:2 MR system at 6 h assisted by RF heating, with overlong time and excessive lactose. AGEs might have much more influence on the WVP and other physicochemical properties either.

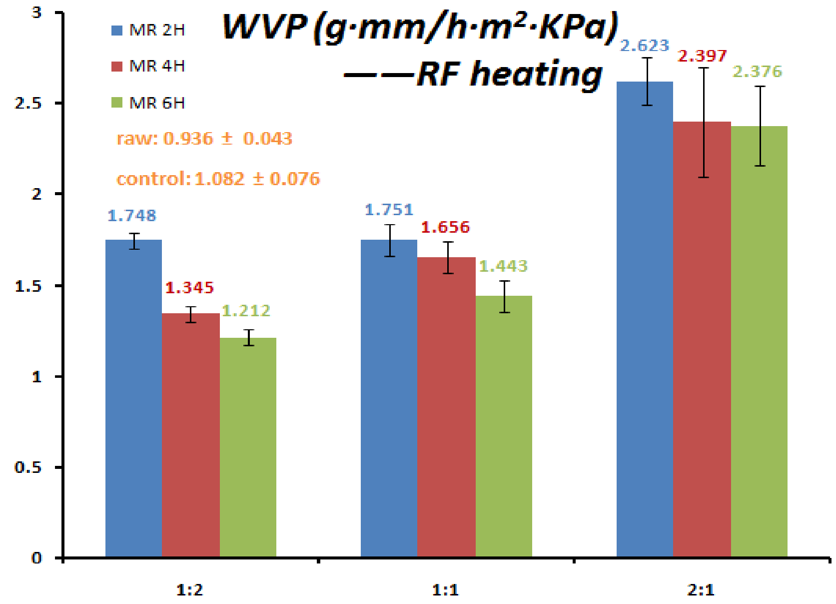


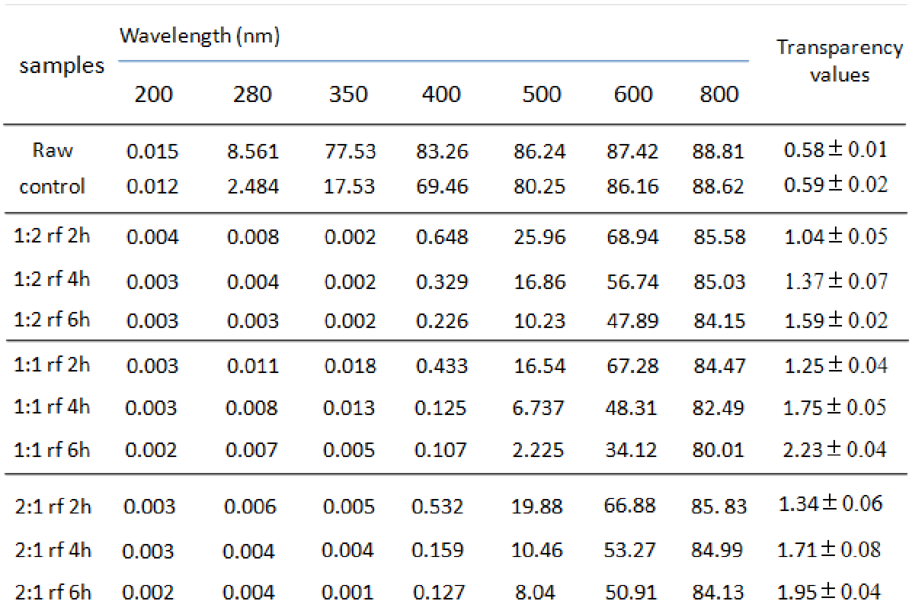
Fig.18 Effect of MR on the WVP of catfish gelatin films with RF heating

4.3.7 Comparison on the LT and transparency of the MRPs films between RF and WB heating

LT and transparency of RF MRPs films at the selected wavelengths are shown in Table 15. In the UV range of 200-280 nm, the RF MRPs films still presented low LT results. Thus, it was known the MR extent on gelatin films with RF system or WB method would not cause significant difference on UV transmission. However, the RF MRPs films exhibited less LT in visible range (400 – 800 nm) than the WB MRPs films. In the wavelength of 400 nm, the RF MRPs films could prevent almost all the violet light, while part of that still could pass though the WB MRPs film. After that, in the wavelength of 500 nm, the RF MRPs films showed faster transmission reduction and finally got much lower transmission with the expanded MR time. The LT of RF MRPs films were overall less than half of the WB MRPs films, even less than one over ten in 1:1 MR system at 6 h (26.43% versus 2.23%), which could indicate the RF MRPs films could prevent far more green light than WB MRPs films at the same time. This phenomenon could also be found in the following 600 and 800 nm, orange and red range, respectively. The RF system indeed promoted the MR effectiveness furtherly on the transmission of the MRPs films. Besides that, it could be found the descending range and reducing rate of transmission were much greater in 1:1 MR system compared with other two systems with RF heating, which was different from the WB MRPs films with similar tendency and close results among the three systems, shown in the Section 3.3.6. The browning MRPs with caramel products and AGEs, possessing very complicated structure, had greater influence on the constitution of gelatin molecules. It was inferred the nonuniform network could effectively obstruct most light, even the infrared light in wavelength at 800 nm.

The transparency of RF MRPs film had quite a reversed increase. The highest transparency value still appeared in 1:1 MR system and arrived at 2.23, which was nearly double as the highest value in the WB MRPs film. Gelatin film has been considered as highly transparent film (Paschoalick et al., 2003). The MR extent on the gelatin film didn’t make it remarkably increased, even if assisted by RF system. Vanin et al. (2005) and Hoque et al. (2011) also reported plasticizer type and concentration as well as the chain length of gelatin had no impact on the transparency of the gelatin films.

Table.15 Changes in light transmission and transparency at different reactant ratios and heating time with RF heating



samples	Wavelength (nm)							Transparency values
	200	280	350	400	500	600	800	
Raw	0.015	8.561	77.53	83.26	86.24	87.42	88.81	0.58±0.01
control	0.012	2.484	17.53	69.46	80.25	86.16	88.62	0.59±0.02
1:2 rf 2h	0.004	0.008	0.002	0.648	25.96	68.94	85.58	1.04±0.05
1:2 rf 4h	0.003	0.004	0.002	0.329	16.86	56.74	85.03	1.37±0.07
1:2 rf 6h	0.003	0.003	0.002	0.226	10.23	47.89	84.15	1.59±0.02
1:1 rf 2h	0.003	0.011	0.018	0.433	16.54	67.28	84.47	1.25±0.04
1:1 rf 4h	0.003	0.008	0.013	0.125	6.737	48.31	82.49	1.75±0.05
1:1 rf 6h	0.002	0.007	0.005	0.107	2.225	34.12	80.01	2.23±0.04
2:1 rf 2h	0.003	0.006	0.005	0.532	19.88	66.88	85. 83	1.34±0.06
2:1 rf 4h	0.003	0.004	0.004	0.159	10.46	53.27	84.99	1.71±0.08
2:1 rf 6h	0.002	0.004	0.001	0.127	8.04	50.91	84.13	1.95±0.04

SD: standard deviation from triplicate determinations

4.3.8 Comparison on the OTR changes between RF and WB heating

The modification extent on the OTR of MRPs films with RF heating could be observed in Figure 19. All the OTR values were less than that of the raw gelatin film, which was significantly different from the results by WB heating. The highest OTR of RF MRPs film was 1.011 ± 0.234 (cm^3· μm/m^2· d· KPa), and it was less than half of highest results by WB heating and occurred in the 1:2 MR system as well. Whereas the lowest OTR value of RF MRPs film was also obtained from the same MR system. This strange tendency might be related to the remarkable changes on the components of final products. It was known that the MR process was incomplete with WB heating based on the comparison among the results of other properties. RF heating could promote the MR to a larger extent with higher MRPs yield. The RF system could provide huge energy with high heat capacity to the MR solution, resulting in more MR modification on the gelatin films during much shorter time. The lactose amount in RF system would disappear more rapidly with the formation of MRPs. On the other side, the strong antioxidative characteristics of MRPs had been verified to obstruct the oxygen pass through the film structure successfully (Section 3.3.7). In conclusion, the MRPs films treated by RF heating could possess better oxygen barrier properties with lower OTR compared to the WB MRPs films.

Besides that, the new products formed in the final MRPs with better molecule structure could probably reduce the free volume and decrease the OTR of the corresponding films. The unique RF heating mechanism could make it possible for such production of the caramel products, even the AGEs during the MR process. Whatever the “hot pot” explosion or athermal effect created by RF heating could supply the abundant accumulated energy with huge heat capacity for the excessive lactose to degrade and polymerize by themselves, and eventually formed the caramel products mixed in the final MRPs. The caramel products were derived from the lactose with strong intermolecular force, at least much better than that of the MRPs, which could enhance the molecular interaction between the polymer chains and reduce the free volume of the intermolecular gaps. Moreover, the chemical nature of macromolecules, crystallinity, molecular mass, orientation, and the degree of crosslink could typically affect the permeability of film (De Carvalho and Grosso, 2004). The caramel products, as one macromolecular polymer, had large molecular mass with interlaced orientation and tight crosslink, which could effectively enhance the oxygen barrier properties. The greatest reduction extent on OTR of the RF MRPs films happened in 1: 2 MR system, because more caramel products were formed due to more lactose left in the MRPs solution, whereas the descending range was quite slight in 1:1 and 2:1 MR systems, as most lactose had already reacted with gelatin by the predominant MR. The residue lactose in MR solution was not much, especially in 2:1 MR system, it could be inferred that there were scarce caramel products formed during the whole process. In addition, the influence by the formation of the AGEs could not be neglected, especially when the MR time was over 4 h under the RF system. From the results in the 1:2 and 2:1 MR system, it could be found the OTR values were a little fluctuant between 4 h and 6 h MRPs films. This phenomenon could be probably connected with the formation of AGEs.

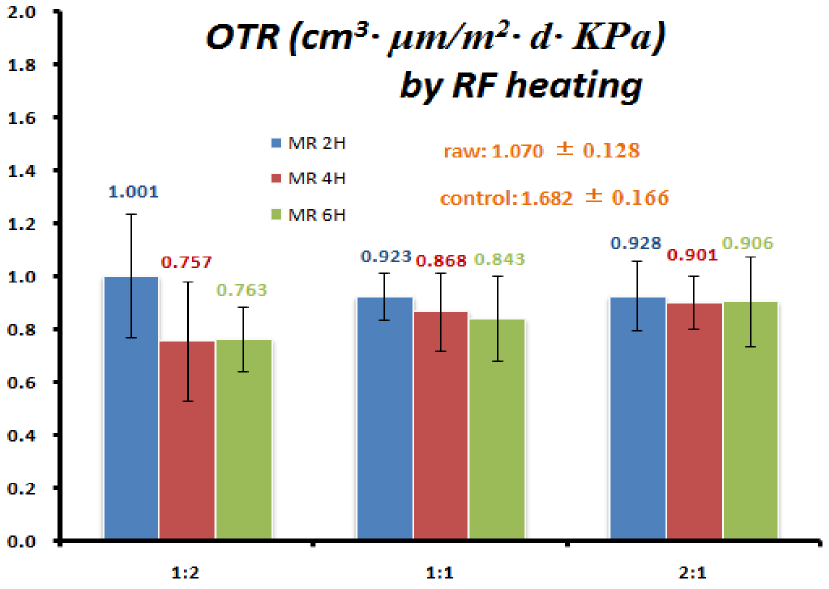


Fig.19 Effect of MR on the OTR of catfish gelatin films with RF heating

4.4 Conclusion

In this study, RF heating was first time applied to assist the MR process on gelatin structure. Comparing the properties of MRPs solution and final MRPs film, RF system had remarkable advantages over WB method on the MR modification extent to the gelatin biofilms. As to the solution results, the pH value and viscosity with RF heating was far lower than those of WB heating, while the absorbance with RF heating were much higher than that of WB heating. Furthermore, RF system promoted MR gain tremendous influence on the film properties. In the same MR system, the modification extent on mechanical properties with 2 h RF heating was already greater than that of 6 h WB heating. In addition, the lowest T_g in 2:1 MR system with RF heating was indeed higher than the top one in 1:2 MR system with WB heating. RF heating could advance the thermal stability of the MRPs film to a much higher extent. The RF system even altered the variation tendency on the WVP mainly due to the formation of caramel products and AGEs. Similarly, the improvement on the LT and OTR of MRPs films with RF heating were also more prominent. Compared to WB heating, RF heating already assisted MR to reach the great extent at 2 h and got sufficient improvement on the physicochemical properties of the gelatin film. In order to save energy as well as control the formation of caramel products and AGEs, the optimal modification on the gelatin films in this study was treated by 2 h MR with RF heating in 1:1 MR system.

Chapter 5 Conclusion and future work

5.1 The overall conclusion

In this study, a famous chemical reaction (MR) and an innovative heating method (RF) were employed to modify the physicochemical properties of the catfish gelatin film, which was used as the encapsulant of the spray dried powder. The outcome proved MR had great influence on gelatin films, as well as a remarkable difference between RF and WB heating assisted the MR modification on the film properties. The overall conclusions were drawn as follows:

1. MR could largely alter the mechanical properties, thermal stability as well as the permeability of the gelatin films. With the MR extent enhanced, the tensile strength decreased markedly while the elongation increased drastically. The glass transition temperature went up to quite high degree while both the light transmission and oxygen transmission rate declined to low extent. The increase on WVP was related to the changes on the gelatin molecule structure.
2. RF heating brought a qualitative change to MR, which provide huge energy to obtain a much higher MR extent. Therefore, the physicochemical properties have greater changes with RF heating compared to WB heating. The RF MRPs film became more flexible and ductile with much higher thermal stability and much better light barrier property as well as lower oxygen transition rate. However, the WVP of the RF MRPs reduced with

the expended MR time probably due to the formation of new products (caramel products, AGEs) in the solution.

3. Consideration on the MRPs yield, the improvement of the gelatin film, also the energy efficiency and heat capacity, the optimal MR modification was treated by 1:1 MR system for 2 h with RF heating.

5.2 Future work

Maillard reaction (MR) was the first time applied to improve the physicochemical properties of the gelatin film. As the molecular weight of gelatin was unknown, the lactose amount was tough to confirm for the combination with gelatin. Therefore, the MR extent was hard to control. Moreover, the radio frequency (RF) heating was also unprecedented to treat the MR process. The mechanism of RF technique on MR was still not totally clear. Further research is necessary for the better understanding on the influence of RF to chemical reaction. In view of the above problems, the future works of this research are as follows.

1. RF heating had much better influence on MR modification to the gelatin film. However, during the MR process, it is more likely to generate caramel products and AGEs due to the huge energy. Reducing the MR time and optimizing the MR ratio furtherly to improve the MRPs yield, simultaneously to prevent the formation of caramel products, especially the AGEs as low as possible, would be the primary research in the future work.

2. After the modification by MR, the water vapor permeability (WVP) of gelatin films was increased because the molecular structure had been altered and the intermolecular force decreased as well as the intermolecular gaps increased. The addition of some edible additives to increase the molecular interaction would be feasible in the future research.
3. Except the antioxidative properties, the MRPs also possess many other unique characteristics, such as antibiotic, antimicrobial, and antiallergenic properties. Studying the effectiveness of these characteristics on the gelatin film and employing these natures of MRPs into the food industries would have a promising future.

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