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Effect of Amidated Low Methoxyl Pectin on the Mechanical Properties and Colour Attributes of Fish Mince

Rocio M. Uresti[1], Nancy López-Arias[1], José A. Ramírez[1] and Manuel Vázquez[1,2]*

[1]Unidad Académica Multidisciplinaria Reynosa Aztlán
Universidad Autónoma de Tamaulipas
Apartado Postal 1015, Reynosa, 88700 Tamaulipas, México

[2]Área de Tecnología de los Alimentos, Escuela Politécnica Superior
Departamento de Química Analítica, Universidad de Santiago de Compostela
Campus de Lugo, 27002 Lugo, Spain

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Summary

Pectins have been unsuccessfully applied to improve functionality of meat and fish products. Effect of amidated low methoxyl pectin (ALM pectin) levels on functionality of Mexican flounder (*Cyclopsetta chittendenii*) mince was studied. Changes in the firmness and work of extrusion of pastes, texture profile analysis (TPA) of gels, and colour parameters were determined. ALM pectin at 1 % decreased firmness and work of extrusion of fish pastes but increased hardness, chewiness and cohesiveness of the gels ($P<0.05$). The addition of ALM pectin increased slightly the whiteness and yellowness of mince gels. Chrome parameter indicated that gels remained in the grayish achromatic region. Therefore ALM pectin at 1 % could be employed to modify the textural properties of fish mince.

Key words: amidated low methoxyl pectin, gel, fish, restructured products

Introduction

Properties of myofibrillar protein can be modified using different additives. Vegetal or animal hydrocolloids, such as flour, starch, gums or proteins can influence the formation of the continuous matrix of the muscle during the formation of the paste or during the heat-induced gelation. Some additives interact with proteins to form a more structured system, while others acts as fillers just binding water and modifying the viscosity of the system (*1*). Vegetal hydrocolloids are typically added to meat, poultry and fish derived products for improving their functional properties. Starch is perhaps the most commonly used ingredient as filler in surimi products, fish based products as well as meat and poultry derived products. It increases firmness and gel strength (*1–5*). Other hydrocolloids like carrageenans (*6*), and locust bean/xanthan gum mixtures (*7*) have been reported to improve the mechanical properties of fish products. However, all the hydrocolloids are not compatible with meat, poultry and fish processed foods. While hydrocolloids such as starch, carrageenan and konjak are commonly used in surimi (*8*), other hydrocolloids such as alginates, xanthan and high methoxyl pectins have a disruptive effect on surimi gels (*1,7,9*).

Pectins are a complex family of polysaccharides, which are characterised by their degree of esterification (DE). High methoxyl (HM) pectin has a typical DE of 55–80 % and is generally gelled in the presence of sugar at low pH (*10*). Low methoxyl (LM) pectin may form

* Corresponding author: ramirez@qui-rey.nat.mx

gels at concentrations higher than 1 % in the presence of calcium over a wide range of pH with or without sugar (*11*). LM pectin is prepared by acid treatment in ethanol or isopropanol. Amidated pectins are mainly prepared by the reaction of ammonia with the alcoholic suspensions of pectin (*10*). Another method to obtain amidated pectins is an amino-dealkoxylation (aminolysis) using different amines. However, these pectins can show different physical and chemical properties that depend on the amide groups formed (*12*).

The formation of protein-polysaccharide complexes can be employed to improve functional properties of proteins. Covalent protein-pectin interactions have been induced between whey protein concentrate and LM pectin, improving solubility, gelling, emulsifying and foaming properties (*13*). Pectins were also used as a cryoprotectant in surimi (*14,15*). Recently, amidated low methoxyl pectin at 1 % has been used to improve the mechanical properties of surimi gels (*9*).

Mexican flounder (*Cyclopsetta chittendenii*) is an abundant fish species in Gulf of Mexico and high volumes of this species are incidentally caught as part of the shrimp by-catch and discarded due to their small size. A great interest has been paid in processing this abundant resource and several studies have been reported about its gelling properties (*16–19*). Jellyfish products could be an interesting alternative for this kind of fish resource. The objective of this work was to determine the effect of ALM pectin on the mechanical and colour properties of mince from Mexican flounder.

Materials and Methods

Chemicals and materials

Amidated low methoxyl pectin LM 35 Powder (27–33 % DE, Tic Pretested Pectin; Lot 0003655) was provided by Tic Gums (Belcamp, MD).

Preparation of mince

Mexican flounder or sole (*Cyclopsetta chittendenii*) was obtained from a fish market in Tampico, Tamaulipas, Mexico. Forty kg of whole fresh fish (ca 90 fishes) were washed and kept in ice until processing. The fish were processed about 6 h after being caught. Mexican flounder was beheaded, gutted and washed. The skin and bones were removed with a Bibun deboning machine (Model NF2DX, Fujiyama, Japan, purchased from Bibun Engineering Co., Ltd, Singapore) containing a drum with 5 mm diameter perforations. The whole fish paste was mixed with the cryoprotectant sucrose (8 %) in a Hobart mixer (model VCM, Troy, Ohio) and then packed into polyethylene bags (2 kg), frozen within 5 h at –30 °C in a Crepaco plate freezer (Model B-5854-AM12, Crepaco, Inc. Chicago, IL) and stored at –20 °C until used. Only one batch of whole paste containing 78 % moisture content was used.

Preparation of paste and gels

Mexican flounder mince (250 g) was selected from a 2-kg bag, partially thawed at room temperature reaching –5 to –2 °C during 4 h, cut into small pieces and chopped in a 5.5 L capacity Hobart cutter (Model 84145, Troy, Ohio) for 3 min with 2.5 % salt to solubilise the myofibrillar proteins. Final chopping temperature was maintained below 15 °C by the use of partially thawed samples. Pectin 1–5 % was added directly into the whole fish paste, in a dry form after myofibrillar proteins were solubilised. The paste was stuffed into stainless tubes (diameter = 1.87 cm; length = 17.75 cm) previously sprayed with commercial vegetable oil to prevent sticking. The tubes were capped before the thermal treatments: 40 °C for 30 min, followed by 90 °C for 15 min. After cooking, tubes were immediately removed, placed in cold water bath (1 °C) and cooled at 4–5 °C for 30 min. All gels were removed from the tubes and stored overnight at 4 °C in polystyrene bags, prior to testing.

Forward extrusion analysis

The effect of adding ALM pectin on firmness and work of extrusion (consistency) of fish pastes was evaluated using a forward extrusion cell. The compression force required to extrude 30 g of solubilised fish paste was measured using a TA-XT2i Stable Micro Systems Texturometer (Viena Court, England) with the forward extrusion cell (model HDP/FE).

Fish paste was extruded at 80 % of its initial height (mean height 14.4 mm, S.D. 0.98 mm) at 1 mm/s through a 10-mm diameter outlet in the bottom of a 50-mm diameter container. Before the analysis, all fish pastes were stored at room temperature (ca 26 °C) for 2 h. The samples were introduced into the cell avoiding the formation of air bubbles. Fig. 1 illustrates the forward extrusion analysis. After the surface was triggered, the piston was pushed onto the sample, compressing the sample to pack more tightly. In this stage, the force increased steeply and the extrusion commenced. Once the force increased to a maximum point a plateau was observed. Such plateau was considered as the force required to continue the extrusion. The fluctuation in

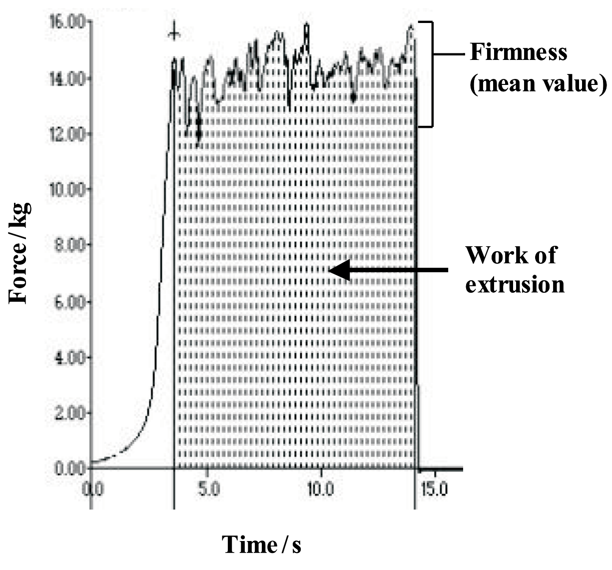

Fig. 1. Dynamics of force during the forward extrusion analysis of solubilised fish pastes

force produced on extrusion might be associated with the release of trapped air pockets or the presence of particles bigger than mean size particles obtained during chopping. To standardise such deviations, a mean value was considered. Such mean value can be obtained with the Texture Expert version 1.22 software included with the texturometer. The mean value was considered as the firmness of the paste and the area was reported as the work of extrusion. Six samples were analysed for each treatment. Each treatment was obtained with two replicates.

Texture profile analysis (TPA)

Gel samples with 3 cm length and 1.87 cm diameter were equilibrated at room temperature for 30 min into a plastic bag to avoid dehydration before textural analysis, which was determined using a TA-XT2i Stable Micro Systems Texturometer (Viena Court, England). Textural profile analysis (TPA) was performed using a cylinder probe (P50) with 50 mm in diameter. Samples were compressed to 50 % of initial height using a compression speed of 1 mm/s (*9*). Hardness, springiness, cohesiveness and chewiness were reported. Six samples were analysed for each treatment. Each treatment was obtained with two replicates.

Colour attributes

Spectral reflectance of restructured fish gels was determined using a HunterLab MiniScan XE Plus spectrocolorimeter (model 45/0-L; Hunter Assoc., Reston, Va., U.S.A.) calibrated against black and white tiles. CIE L* (Lightness), a* (redness), and b* (yellowness) values, chrome ($[a^{*2} + b^{*2}]^{1/2}$), and hue angle (arc tan b*/a*) were calculated based on illuminant C and the 2° standard observer. Six samples were analysed for each treatment. Each treatment was obtained with two replicates.

Statistical analysis

Statistical analysis was performed using a Statgraphics 5.0 (Software Publishing Corporation, Bitstream Inc.). LSD's multiple range tests were used to determine significant differences (P<0.05) among treatments.

Results and Discussion

Forward extrusion analysis

The effect of ALM pectin on the firmness and the work of extrusion of fish paste is shown in Fig. 2. The firmness value of fish pastes varied ranging from 16.4 to 21.3 kg. Fish paste control (without ALM pectin) had a firmness value of 20.4 kg. The firmness of the fish paste decreased (P<0.05) to 16.4 kg when 1 % ALM pectin was added, and it increased with the increase of the concentration of ALM pectin and reached the highest value (21.3 kg) with 5 % ALM pectin addition.

Work extrusion values of the fish pastes were in the range of 149 to 201 kg/mm. The pattern of this parameter was similar with that of firmness. The fish paste control had 162 kg/mm of work of extrusion. This value was lower in samples containing 1 % ALM pectin (149 kg/mm) (P<0.05). Higher concentration of ALM pectin

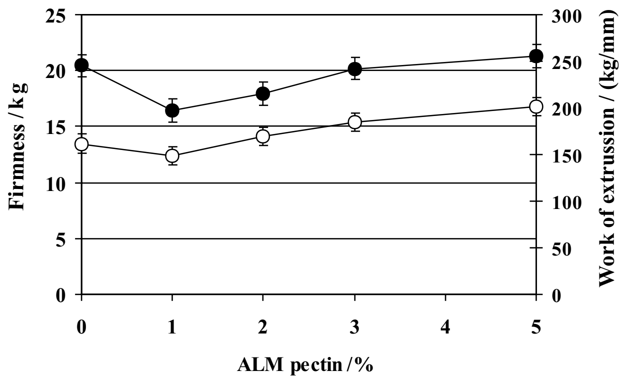

Fig. 2. Effect of the ALM pectin concentration on the firmness (●) and work of extrusion (o) of solubilised fish pastes (forward extrusion analysis). Bars show standard deviation from 6 determinations

resulted in the increased value of the work of extrusion. The work of extrusion of pastes containing 3 or 5 % ALM pectin was higher than the value of the control sample (P<0.05). Although forward extrusion analyses are not commonly used for fish paste, they were used to measure the textural quality of viscous liquids, gels, marmalades, meat pastes and other foods (*20*).

Firmness and consistency of fish pastes depend on protein-protein and protein-water interactions. Hydrocolloids are incorporated into protein products because of their ability to bound water with their hydroxyl groups. Beside the –OH groups, ALM pectins have three other groups: carboxylic acid (–COOH), amide (NH_2), and methoxyl ($–OCH_3$). At physiological muscle pH (near 7) the former groups can interact with proteins preferentially by hydrogen bonds, but methoxyl groups could interact just with proteins by hydrophobic interactions. However, hydrophobic groups in solubilised myofibrillar protein are buried until protein becomes denatured by heat, pH or another denaturating agent. Therefore, protein-pectin hydrophobic interactions are improbable. The initial decrease on firmness and consistency of the fish paste by adding 1–2 % ALM pectin could be associated with the increase in polar protein- pectin interactions over protein-water or pectin-water interactions. Increasing the ALM pectin concentration in the fish paste could induce a higher pectin-water interactions increasing the water holding capacity and consequently improving the fish paste firmness and consistency. These results indicate that ALM pectin modifies the physicochemical properties of fish pastes, changing the amount and availability of protein surface groups by forming protein-pectin interactions. Such changes might be responsible for the changes of mechanical properties of fish gels.

Texture profile analysis

Gelling of myofibrillar proteins makes the fish pastes change from a viscous semi-liquid state to a rigid semi-solid state. Protein interactions induce the formation of a three-dimensional matrix which trap the water. This system shows different levels of hardness, cohesiveness, springiness and chewiness. Such properties are different for each type of products. Surimi gels from Alaska pollock usually show high values of hardness, cohesiveness, springiness and chewiness. Modori phenomenon is a deteriorative phenomenon associated with proteolysis that induces a decrease in the value of all TPA parameters. Additives which increase TPA parameters are generally considered appropriate for use in fish products: MTGase, calcium ion, carrageenan and protease inhibitors. Additives which decrease TPA parameters (xanthan gum and high methoxyl pectins) are considered as inappropriate for use in fish meat products.

ALM pectin affected all TPA parameters of mince gels (Fig. 3). Hardness was increased (P<0.05) from 4.38 kg in the control to 5.43 kg by adding 1 % ALM pectin. When ALM pectin was added at higher levels (2–5 %), hardness decreased as a function of pectin level. The lowest value of hardness (3.43 kg) was obtained with 5 % ALM pectin. Cohesiveness and chewiness showed similar behaviour. With the addition of 1 % ALM pectin, cohesiveness and chewiness were increased from 0.78 and 698 (control) to 0.85 and 750, respectively. However, such increases were not significant (P>0.05). A marked decrease in the cohesiveness and chewiness was associated with the increase of the ALM pectin from 2 to 5 %. Both parameters were lower in samples containing 3 or 5 % ALM pectin. However, the control sample showed a low value of springiness (0.202). This property was not increased by adding 1 % ALM pectin (P>0.05) but decreased by adding 2, 3 or 5 % ALM pectin (P<0.05).

According to these results, addition of 1 % ALM pectin increased hardness, chewiness and cohesiveness of gels. However, higher levels of ALM pectin (2–5 %) had a detrimental effect on the TPA parameters. Initial decrease of firmness and consistency of fish pastes (Fig. 2) was consistent with the increase in textural properties of fish gels (Fig. 3). These results suggest that carbohydrate-water interactions are favoured when ALM pectin is added at low concentration (1–2 %). This behaviour induces a decrease of protein-water interactions and the increase of protein-protein interactions and it might be responsible for the improvement of the mechanical properties of fish gels (*9*).

Pectin-pectin interactions are discarded as responsible for the improvement of textural properties, because ALM does not form gels in absence of calcium ions and fish muscle contains low levels of calcium (*9*). However, pectin-protein interactions cannot be discarded as responsible for the improvement of textural properties. As previously mentioned, low methoxyl amidated pectin contains carboxylic, amino and methoxyl reactive groups, which can interact with corresponding groups in proteins to form electrostatic and hydrogen bonds, as well as hydrophobic interactions.

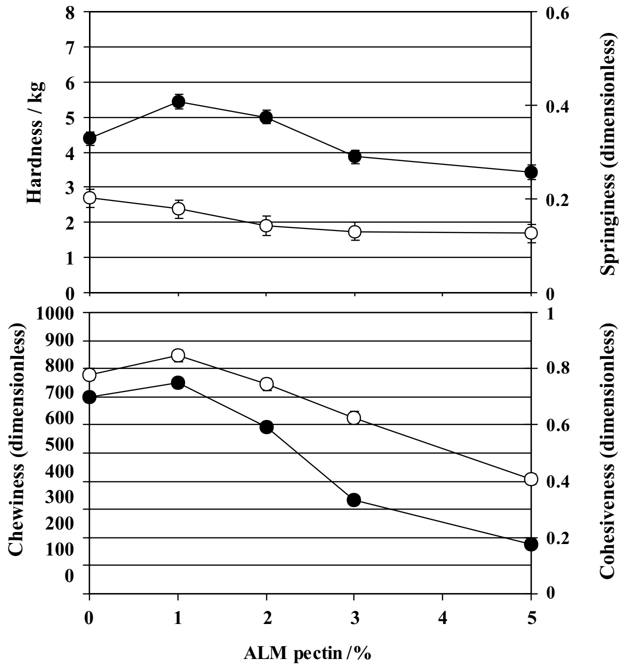

Fig. 3. Effect of the ALM pectin concentration on the Texture Profile Analysis parameters of fish gels. Hardness (●), Springiness (o), Chewiness (●), Cohesiveness (o). Bars show standard deviation from 6 determinations

The decrease in the textural properties of gels by adding 2–5 % of ALM pectin was coincidental with the increase in firmness and consistency of fish pastes. This behaviour might be associated with an increase in pectin-water interactions, inducing the swelling of the pectin and causing a disruptive effect on gel structure. This disruptive effect might be associated with a decrease in free water molecules (salting out effect) or just with the impediment to form the appropriate structure for steric hindrance.

However, the presence of pectin-protein interactions may be responsible for the negative effect, because protein and anionic carbohydrate can interact above the isoelectric point with electrostatic interactions and modify the native structure of proteins (*21,22*).

Table 1. Changes in the colour characteristics of gels from Mexican flounder mince as affected by different levels of ALM pectin

Pectin content / %	L*	a*	b*	Chrome	Hue
0	44.07±6.51 [ab]	–1.15±0.19 [a]	7.67±1.24 [a]	7.76±1.24 [a]	91.42±0.01 [a]
1	53.70±6.62 [b]	–0.89±0.15 [a]	10.23±1.29 [b]	10.27±1.28 [b]	91.48±0.02 [b]
2	51.38±9.19 [b]	–0.24±0.14 [b]	11.55±1.70 [b]	11.55±1.70 [b]	91.55±0.01 [d]
3	49.19±8.33 [ab]	–0.125±0.05 [b]	11.30±1.62 [b]	11.30±1.62 [b]	91.56±0.01 [d]
5	40.14±12.20 [a]	–0.445±0.20 [a]	10.50±2.37 [b]	10.51±2.37 [b]	91.53±0.01 [c]

[a, b, c] Values in the same column with different letters are significantly different (P<0.05)

Although both high and low methoxyl (HM and LM) pectins have been reported as not suitable for improving mechanical and functional properties of surimi (*9*), ALM pectin increased mechanical properties of surimi gels, possibly because amidation can give a more polar characteristic to LM pectin. Thus, hydrogen bonds between ALM pectin and protein might form a more compatible protein-carbohydrate system. In this work, hydrogen bond between amidated pectin and myofibrillar proteins could be associated with the increase of the mechanical properties of fish gels when ALM pectin was added at 1 %. Increasing the amount of ALM pectin (2–5 %) could modify the native structure of muscle proteins forming both hydrogen bonds and electrostatic interactions. The modification of native structure of muscle protein might be associated with the inducing of weak gels, and thus decreasing mechanical and textural properties. However, more studies are needed to elucidate the nature of these interactions.

Colour attributes

The effect of ALM pectin on colour attributes of the gels is shown in Table 1. The L* value of the control (44.07) was significantly increased by the addition of 1 % ALM pectin (53.70). Using higher levels of ALM pectin, a decrease in L* value was observed. The lowest L* value (40.14) was obtained with the addition of 5 % ALM pectin.

The a* value of the control was –1.15. This attribute was modified by adding ALM pectin, with the values ranging from –0.89 to –0.445, indicating that ALM pectin increased the redness of the gels only slightly (see Table 1). Gels containing 1–5 % ALM pectin had higher b* values ($P<0.05$), compared with the control (Table 1). The changes in the a* and b* values affected the Chrome and Hue parameters (Table 1). From the results, addition of the ALM pectin modified the hue value of the gels by increasing the greenness of the gels. Although the chrome value was significantly increased by the ALM pectin addition, the product remained in the grayish zone.

Conclusions

ALM pectin at level of 1 % increased the textural properties of the gels from Mexican flounder mince. Higher levels of the hydrocolloids showed a disruptive effect on the system. The effect on the colour attributes by addition of 1 % ALM pectin was very weak.

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Utjecaj amidiranog pektina s malim udjelom metoksila na mehanička svojstva i boju usitnjene ribe

Sažetak

Uporabom pektina nije poboljšana kakvoća mesa i ribljih proizvoda. Ispitan je utjecaj amidiranog pektina s malim udjelom metoksila (ALM pektin) na kakvoću usitnjenog meksičkog lista *Cyclopsetta chittendenii*. Utvrđene su promjene čvrstoće i sile pri ekstruziji riblje paste, zatim pri analizi teksture gela (TPA) i u nijansi boje. Količina AML pektina od 1 % smanjuje čvrstoću i silu ekstruzije riblje paste, a povećava tvrdoću, koheziju gela i jačinu žvakanja ($P<0,05$). Ustanovljeno je da su gelovi bili uglavnom sivkaste nijanse, a dodatkom ALM pektina neke su sive komponente postale bjelije ili žuće. Stoga se količina ALM pektina od 1 % može upotrijebiti radi promjene teksture usitnjene ribe.