Effects of native and modified starches on the physicochemical and textural properties of rainbow trout (Oncorhynchus mykiss) fish burgers

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ABSTRACT
Native and modified starch content, pH, water-holding capacity (WHC), malonaldehyde content, sensorial properties and texture profile of the rainbow trout fish burger (RTB) were investigated during 21 days of storage at 0°C. The addition of acid-modified starches (AMS) to RTB resulted in a high content of resistant starch (RS). The RTB with added native and modified starches showed a better stability of pH, WHC and texture profile values during the 21 days of storage. The lipid oxidation values of the RTB control showed higher values than the RTB with added native and modified starches. In the sensory evaluation test, the RTB supplemented with AMS have the highest score regarding acceptability. The result confirmed the potential of modified starches as a prebiotic food ingredient, since they improve the physicochemical and sensorial properties of RTB.

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Introduction
In the Food Guide Pyramid, meat is categorized as the primary source of protein along with poultry and fish. However, despite having a high protein content, meat lacks specific nutrients such as vitamin B12, folic acid and prebiotics. Currently, consumer demands have considerably changed the field of food production. Most consumers seek not only to satisfy their hunger but also to consume foods that provide them with the necessary nutrients to prevent nutrition-related diseases and improve their physical and mental well-being (Thachil, Chouksey, & Gudipati, 2014). Recently, it became possible to develop meat products with new potential health benefits by using strategies such as the incorporation of functional ingredients (Jiménez-Colmenero, Carballo, & Cofrades, 2001), dietary fiber, fructo-oligosaccharides and inulin (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006). The objective of these strategies is to provide a prebiotic effect to the consumers of meat burgers.

The use of fish meat has increased recently due to its content of proteins with high biological value and its fatty acid composition, which is especially rich in polyunsaturated fatty acids, particularly omega-3 (Del Nobile et al., 2009). Furthermore, there are reports that addition of bioactive compounds to fish meat burgers improved their chemical and sensorial properties (Spinelli, Conte, & Del Nobile, 2016). Meleiro, Nascimento, Barbosa Junior, Saldanha, and Barbosa (2016) studied the addition of carotenoids to fish burgers and reported a decrease in malonaldehyde (MDA) content during the time of storage as compared with the control sample. Another natural polymer that can be used as an additive for meat is starch (Resconi et al., 2016), which can be used as a substitute for fat. The addition of modified starches to fish meat products has been minimally studied. Modified starches such as resistant starch (RS) have been used primarily in bakery products. Some reports mention that RS can be considered a prebiotic because it contributes...
to reduction of the prevalence of colon cancer (Cummings & Stephen, 2007; Fuentes-Zaragoza et al., 2011).

However, there are few studies on fish-based products in general and no reports on the use of rainbow trout meat (Oncorhynchus mykiss). The Hidalgo state produced 277 tons of rainbow trout fish in 2017, consuming only 89% (CONAPESCA, 2017). Therefore, rainbow trout meat has the potential to be used as a meat product which could be supplemented with modified starches to increase the nutritional value. Most studies on fish products have focused on the effects of grilling and baking (Bainy, Bertan, Corazza, & Lenzi, 2015), on changes due to frozen storage (Tokur, Polat, Beklevik, & Özkütük, 2004) and on the effect of chilled and frozen storage on the oxidative and microbial stability of fish burgers (Corbo et al., 2009, 2008; Vanitha, Dhanapal, & Reddy, 2015). Further studies are needed regarding the effect of the addition of modified starches on fish product acceptance. The main objective in this study was evaluation of the addition of native and modified starches on resistant starch content, physiochemical parameters, malonaldehyde content, texture profile and sensorial properties of rainbow trout fish burgers during 21 days of storage at 0°C.

Materials and methods

Materials

Chayotextile (Shechium edule Sw.) and potato (Solanum tuberosum) tubers were collected in Tulancingo, Hidalgo. The Resistant Starch Assay Kit (K-RSTAR 09/14) was acquired from Megazyme International (Wicklow, Ireland). Butylhydroxytoluene, trichloroacetic acid, thiobarbituric acid, hexane and 1,1,3,3-tetraethoxypropane were acquired from Sigma Aldrich, Mexico.

Isolation and modification of starch

The starches of chayotextile and potato were isolated according to the methodology reported by Flores-Gorosquera et al. (2004). The acid modifications of the starches were made according to the procedure of Loksuwan (2007). Native starches were added to 3N H₂SO₄ in a ratio of 1:5 w/v. The mixture was stirred magnetically for 3 h at 60°C. After hydrolysis, the starch slurry was cooled and neutralized with saturated Na₂CO₃. The acid starch was filtered, washed with three volumes of distilled water followed by 100 mL ethanol (95%), and then dried at 50°C overnight. The dried starch was ground into a powder in a blender and then sieved through a standard 100 mesh. For thermal modification (Aparicio-Saguilán et al., 2005), 60 g of native starches were mixed with 210 mL of water, and the mixture was then pressure-cooked at 121°C for 1 h in an autoclave, cooled down to room temperature and stored at 4°C for 24 h. After three autoclave/cool storage cycles, the sample was freeze-dried and ground into fine particles (100 mesh U.S.). Both samples of modified and native starches were stored in a sealed container in ambient conditions until use.

Fish meat

Rainbow trout fish (Oncorhynchus mykiss) was purchased in Huasca de Ocampo, Hidalgo, Mexico, from the aquaculture production unit “La trucha del Zembo”. The trout fish was filleted in aseptic conditions in the meat workshop of the Institute of Agricultural Sciences of the Autonomous University of the State of Hidalgo. Subsequently, the trout meat was frozen at −18°C and ground for the production of hamburgers; the yield obtained was 47%.

Preparation of trout burgers

Trout burgers were supplemented with six types of starch: native chayotextile starch [NCHS], acid-modified chayotextile starch [AMCHS], thermally modified chayotextile starch [TMCHS], native potato starch [NPS], acid-modified potato starch [AMPS] and thermally modified potato starch [TMPS]. Hamburgers without the addition of starch served as control samples [Ctrl]. The following ingredients and proportions (w/w) were used: starch (5%), oil (2%), salt (1%), spice mix (0.4%) and ground trout meat (86.6% in hamburgers with starch treatments and 96.6% in Ctrl), following the method of (Meleiro et al., 2016). The trout fillets were ground (−18°C) using an electric mill (TorRey M12FS) until obtaining a homogeneous mass. After weighing the ground meat, the other ingredients were added to the mass, which was then homogenized manually for 5 min. Using a hamburger mold, the mass of fish meat was shaped into hamburgers of uniform weight (55 g), which were then individually packaged in low density polyethylene and stored under freezing conditions at 0 ± 1°C until analysis. Prior to each data analysis, the fish trout burger was conditioned at 5°C ± 1°C for 4 h.

Determination of resistant starch [RS]

This methodology was used for native and modified starches and rainbow trout burgers [RTB] supplemented with native and modified starches. The samples of rainbow trout burgers were lyophilized (12525, Virtis Company, Gardner, New York), ground and sieved through a 1-mm sieve before testing. A MEGAZYME Kit was used for the resistant starch, based on the approved method from AACC 32-40.01, in which each sample was measured at a wavelength of 510 nm in a UV–vis spectrophotometer (Thermo Scientific, Mod. Genesys 10 S VIS, USA).

Determination of pH

One piece of trout burger (15 g) was liquefied with an immersion blender (Ultra Turrax IKA T18). A 10 g sample was taken, combined with 90 mL of distilled water and measured with a potentiometer (Model HANNA 211). The pH was determined every 7 days during the 21 days storage period.

Water-holding capacity [WHC]

Approximately 5 g homogenized raw batter sample was placed in a 50 mL centrifuge tube with addition of 10 mL distilled water. Following 10 min centrifugation at 4500 rpm, 15°C, supernatant was decanted and the final sample mass was determined. The test was on storage days 1, 7, 14, and 21.

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\text{WHC} = \frac{\text{final sample weight} - \text{original sample weight}}{\text{Original sample weight(g)}}
\]
The higher WHC value (expressed by g H₂O absorbed/g meat) indicates that more water was bound, and, therefore, that the batter possessed a higher water-holding ability.

Color measurement
The color of the raw fish burgers was analyzed using a MINOLTA CR-300 colorimeter calibrated with a white ceramic plate (Bainy et al., 2015). The colorimeter was used with a D65 illuminant and an observation angle of 10°. The readings were performed in the CIELAB system (L*). Five measurements per hamburger disk were made, using 3 disks per treatment, for a total of 15 color measurements.

Measurement of thiobarbituric acid reactive substances levels
Thiobarbituric acid reactive substances [TBARS] levels (mg malonaldehyde/kg sample) were analyzed by the method proposed by Botsoglou et al. (1994). Two grams of sample was homogenized with an acid solution [8 mL of aqueous dissolution acid TCA (5 mL/100 mL) (trichloroacetic acid) and 5 mL of BHT (0.8 mL/100 mL) (butylhydroxytoluene) in hexane] by using an Ultra-Turrax T25 for 1 min at 9,500 rpm. After that, the samples were centrifuged for 10 min at 1,028 g. Later, the supernatant was removed and the volume recapitulated to 10 mL with the TCA solution. After centrifugation (1,028 g for 10 min), aliquots of 2.5 mL were taken from each sample in triplicate and 1.5 mL of thiobarbituric solution was added. All samples were placed into a water bath at 70°C for 30 min. After incubation, the samples were rapidly cooled in a water bath until room temperature (22 ± 2°C). Absorbance values were measured using a UV-vis spectrophotometer (Thermo Scientific, Mod. Genesys 10S VIS, USA) at 532 nm. TBARS were calculated using a 1,1,3,3-tetraethoxypropane standard curve.

Textural profile analysis
Texture profile analysis [TPA] was performed following the methodology of (Fernández-Martín et al., 2000) with slight modifications. The sizes of samples were 10 g and 24.4 mm diameter, 8–10 mm height; measurements were obtained by axial compression to 40% of their original height in a two cycle test. Force-distance curves were derived with a 30 kg load cell, at a crosshead speed of 50 mm min⁻¹, with a spherical P/1S addition connected to a TA.XTplus texturemeter (Stable Micro Systems, Surrey, U.K.)

Cooking procedure
The fish trout burgers were cooked using an electric grill (FARBERWARE G767) at 163°C (the distance between the heat source and the samples was 4 cm) for 16 min (8 min for each side of the meat burgers). Meat burger samples reached an internal temperature of 80°C.

Preference test
The preference test was developed according to (Utrilla-Coello et al., 2011), with some modifications, for preference assessment of trout burgers served on coded plates. Consumers were asked to assess their degree of sensorial satisfaction by paper ballot using the ranking preference test and the 9-point hedonic rating scale, where 9 indicates an extremely favorable impression and 1 indicates extreme distaste. One hundred consumers were briefed on the evaluation protocol and then proceeded to randomly evaluate the coded trout burgers. The treatment with the highest rating was assigned the attribute of preference regarding smell, color, taste or texture.

Statistical analysis
Experiments were arranged in a completely randomized design. An analysis of variance (ANOVA) was carried out with the statistical program Sigma-Stat version 12.5. Media comparison was conducted by Tukey’s multiple tests (P < 0.05).

Results and discussion
Determination resistant starch
The results of the fraction of resistant starch (RS) in modified starches and the content of resistant starch in rainbow trout burgers were determined. Native chayotextle and potato starch showed low values of RS of 3.40% and 0.92%, respectively. When the starch samples were subjected to heating-cooling-heating cycles, the RS content increased by ~6%. Similar results were reported by Aparicio-Saguilán et al. (2005) with modified banana starch, with percentages of RS of 1.51 to 16.02% in native and modified starch, respectively. Ashwar et al. (2016) mention that the increase in RS caused by the autoclaving-retrogradation treatment involves a two-step process. In the autoclaving step, the hydrated starch gelatinizes and amylose chains are leached out from the starch granules. In the cooling step, starch retrogrades and linear amylose chains recrystallize to form tightly packed double helices stabilized by hydrogen bonds, which are resistant to enzymatic hydrolysis. The fraction of RS in acid-modified starches was 33% in chayotextle and 30% for the potato. The modification of starch by acid hydrolysis increased the fraction of RS by up to 10 times as compared with the thermal modification of starch. Shin, Byun, Park, and Moon (2004) reported similar values of RS in different types of tuber starch; they reported that the content of RS depended on the type of treatment and the starch source. There are also reports that acid-modified starch had a larger RS fraction (Brumovsky & Thompson, 2001). The same behavior was observed when different types of modified starch were added to rainbow trout burgers (5% w/w). As expected, the burger samples with higher values of RS were those that contained starch modified by acid hydrolysis (~10%), and the lowest values in RS were observed in burgers with native starch added (~2%). The burgers with added thermally modified starches showed values of ~4% RS.

Determination of physicochemical parameters in rainbow trout burgers
pH values showed a decrease as the days of storage increased (see Table 1). However, the control sample showed the lowest pH values during the storage time (6.53 to 6.33). Dunajski (1980) mentions that fish meat has a neutral pH immediately after the fish death and that a decrease in the pH values is related to a degradation of glycogen by anaerobic metabolism, which causes the synthesis of lactic acid.
Rainbow trout burgers that were supplemented with native and modified starches showed higher and more stable pH values during the 21 days of storage (see Table 1). In these samples slight changes were observed in the pH values with respect to storage time. Statistical analysis showed that the addition of native or modified starches influenced the pH parameters when these samples were compared with the control ($P \leq 0.05$). This phenomenon indicates a slow...
degradation of glycogen, increasing the sensory quality in this fish burger. A similar behavior was observed by Schelegueta, Delcarlo, Glimme, and Campos (2016) who reported a stable pH during 22 days of storage of fish burgers supplemented with chitosan, nisin and sodium lactate.

Table 1 displays the water-holding capacity [WHC] values. These values decreased as the time of storage increased, and they were not affected by the addition of native and modified starches. Lonergan, Zhang, and Lonergan (2010) mentioned that the WHC is associated with the denaturation of myosin caused by the thawing of meat. The denaturation of myosin causes a detachment of the sarcocomplexes and gaps in the extracellular matrix, enlarging the intermyofibrillar space, which, in turn, reduces the water-holding capacity of cells (Olsson, Olsen, Carlehög, & Ofstad, 2003). The statistical analysis of this parameter showed that the addition of native or modified starches, as well as storage time have no influence on the WHC (P > 0.05).

Luminosity (L*) values showed a slight increase with the addition of chayote and potato starch as well as with acid-modified starches (see Table 1). These values were higher than those observed in the control rainbow trout burgers and in burgers supplemented with thermally modified starch, during the storage time. Milicevic et al. (2015) studied the use of starch as a supplemental material in chicken meat and sausage, respectively. They reported an increase in L* values of 56.75 in chicken meat and of 68 in sausage. This increase in L* values in meat is attributed to the high values of L* (91.9–94.5) of tuber starch (Soisonet al., 2014), which imparts whiteness to the meat mixture. Additionally, Rutenberg and Solarek (1984) mention that acid modification of the starch provokes the elimination of some pigments and proteins, producing whiter starches.

Figure 1 shows the lipid oxidation values in different burger samples during the 21 days of storage. Thioarbituric acid reactive substances [TBARS] were measured as an indication of lipid oxidation based on the mg of malonaldehyde per kilogram of burger sample. The initial amount of malonaldehyde in the control burger was 2.48 mg/kg, while the content in the samples with native and modified starches ranged from 1.06 to 2.43 mg/kg. Lipid oxidation values increased in all fish burgers with storage time. However, the addition of native and modified starches to fish burgers delayed the increase of lipid oxidation as compared to the control burger. The statistical analysis showed that storage time and the addition of native and modified starches had an effect on the content of malonaldehyde/kg of fish burgers (P ≤ 0.05). This decrease of TBARS in burger samples supplemented with starch may be due to the formation of amyllose-lipid complexes (Blazek, 2008), which complicate the extraction of lipids (Thachil et al., 2014) and the digestion process (Al et al., 2014). This bonding between amyllose and lipid molecules influences the reduction of lipid oxidation values. Thachil et al. (2014) reported the effect of using corn with different amyllose content (25 and 45%) in the preparation of corn-based puffed snacks by extrusion. They found that the snacks that were prepared with corn starch with high amyllose content (45%) had lower values of lipid oxidation.

### Texture measurements

Table 2 shows the texture profile analysis [TPA] of rainbow trout burgers. The texture properties in RTB were enhanced as the storage time increased. It was also observed that the addition of

| Time  | Control | NCHS | AMChS | TMChS | NPS | AMPS | TMPS |
|-------|---------|------|-------|-------|-----|------|------|
| T1    | 0.68 ± 0.13 | 0.74 ± 0.05 | 0.73 ± 0.00 | 0.74 ± 0.03 | 0.97 ± 0.29 | 0.75 ± 0.13 | 0.83 ± 0.06 |
| T7    | 1.04 ± 0.05 | 1.09 ± 0.19 | 1.07 ± 0.20 | 1.28 ± 0.14 | 1.04 ± 0.14 | 1.21 ± 0.22 | 1.25 ± 0.36 |
| T14   | 1.23 ± 0.01 | 1.36 ± 0.19 | 1.27 ± 0.15 | 1.48 ± 0.15 | 1.29 ± 0.10 | 0.99 ± 0.10 | 1.28 ± 0.08 |
| T21   | 1.35 ± 0.05 | 1.55 ± 0.06 | 1.49 ± 0.08 | 1.86 ± 0.06 | 1.38 ± 0.25 | 1.63 ± 0.40 | 1.44 ± 0.09 |

Means of five replicates ± standard error. Lowercase letters represent the effect of storage time in the same treatment. Uppercase letters represent the effect of the addition of starch, at the same storage time.

Means in the columns that do not share the same lowercase letters are significantly different (P ≤ 0.05).

Means in the rows that do not share the same uppercase letters are significantly different (P ≤ 0.05).

Control = Sample of burger without starch; NCHS = native chayote starch; AMChS = acid-modified chayote starch; TMChS = thermally-modified chayote starch; NPS = native potato starch; AMPS = acid-modified potato starch; TMPS = thermally-modified potato starch

Medias de cinco repeticiones ± error estándar. Las letrillas minúsculas representan el efecto del tiempo de almacenamiento en el mismo tratamiento. Las letras mayúsculas representan el efecto de la adición del almidón en el mismo tiempo de almacenamiento.

Medias en las columnas que no comparten las mismas letras minúsculas son significativamente diferentes (P ≤ 0.05).

Medias en la fila que no comparten las mismas letras mayúsculas son significativamente diferentes (P ≤ 0.05).

Control = Muestra de hamburguesa sin almidón; NCHS = almidón chayote nativo; AMCHS = almidón de chayote modificado en medio ácido; TMCHS = almidón de chayote modificado térmicamente; NPS = almidón de papa nativo; AMPS = almidón de papa modificado en medio ácido; TMPS = almidón de papa modificado térmicamente
native and modified starches caused an increase in the values of hardness, elasticity and gumminess when compared against the control trout burger and respective storage day ($P \leq 0.05$). Gianhão, Morcuende, and Estévez (2010) studied the effect of storage time (12 days) and the addition of different fruit pulps on pork burgers. They reported that the addition of pulp concentrates had a noticeable effect on the textural properties. Ağar, Gençcelep, Saricaoğlu, and Turhan (2016) reported the addition of sugar beet fiber to a meat emulsion, finding an increase in the hardness, gumminess and cohesiveness. Similar behavior was found in this study. Aktas and Gençcelep (2006) mention that the addition of starch into meat helps to form stronger structures in the protein gel matrix, which increase the structural strength and affects some texture properties. By contrast, elasticity and adhesiveness decreased with the addition of starch and with time of storage. Hardness was associated with elasticity in the rainbow trout burgers: an increase in hardness indicates a loss of elasticity.

The two-way statistical analysis showed that the storage time has more effect ($P \leq 0.05$) on these properties than does the addition of natives or modified starches.

**Preference test**

In the preference test, the modified starches with higher RS content (AMCHS and AMPS) and native control starches (NCHS and NPS) were used in the preparation of rainbow trout burgers. Figure 2 shows the acceptability of rainbow trout burgers using a hedonic scale. The results show that the burger samples supplemented with modified potato starch obtained the highest score (7.01), followed by burgers supplemented with NPS and AMCHS, which obtained respective scores of 6.86 and 6.58. However, these differences were not statistically significant ($P > 0.05$).

The scores obtained for the hamburger samples on the hedonic scale correspond to the point "like moderately". The control burger (without starch) had a score of 5.93, while rainbow trout burgers supplemented with NCHS had the lowest scores (3.43) in the hedonic scale. The attributes selected by the respondents regarding the burger samples with higher values of acceptability were the following: 85% flavor, 12% texture, 2% color and 1% odor. These results suggest that it is possible to incorporate potato and chayote native starches, and their modified starch forms, into trout meat to create a popular commercial product such as hamburgers.

**Conclusion**

The additions of nonconventional or modified starches to rainbow trout burgers seem to offer technically viable alternatives. Furthermore, the addition of starches exerted a strong effect against lipid oxidation, improving the color, textural quality, and oxidative and physicochemical stability during the storage time. According to the results obtained,
the acid-modified starch can be recommended in the production of trout burgers, since it increased the acceptability by consumers and showed higher resistant starch values, which would provide functional properties related to health.

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No potential conflict of interest was reported by the authors.

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