ABSTRACT

Natural starch (NS) was isolated from triticale flour (TF) and later modified by extrusion (MS) using acetic anhydride as a reactive agent. From each product (NS, TF, MS) a proximal analysis was performed as well as the characterization of its physicochemical properties (particle size determination (PSD), water absorption index (WAI) and solubility index (WSI), color L*, a*, b*, and viscosity), degree of substitution (DS), and thermal properties (differential scanning calorimetry (DSC)). Particle below sizes of 250 μm were obtained in the NS and MS treatments (42.91 and 36.03 %, respectively). The DS value (0.1) was found within the range allowed by the FDA for human consumption (0.01-0.2). MS presented a greater value in WAI (4.52), and the color parameter of b* (22.75) as well as a decrement in L* (70.61) and retrogradation viscosity (5.2 cP), being an indicator of greater functionality compared to NS. Through DSC, we found that MS experimented a decrement of 30.2 °C in the glass transition temperature compared with NS. Through isolation and the different modification treatments applied to triticale starch, we obtained functional ingredients with high potential, for use in food for microwave expanded and fried food as third generation snacks.

Keywords: Triticale starch, extrusion, modified starch.

RESUMEN

A partir de harina de triticale (HT) se aisló almidón (AN) para ser modificado por extrusión (AM), utilizando como agente reactivo anhídrido ácido. De cada uno de los productos (HT, AN, AM) se realizó su análisis proximal y se caracterizaron sus propiedades fisicoquímicas (determinación de tamaño de partícula (DTP), índice de absorción de agua (IAA) e índice de solubilidad en agua (ISA), color L*, a*, b* y viscosidad), grado de sustitución (GS), y propiedades térmicas (calorimetría diferencial de barrido (CDB)). Se obtuvieron tamaños de partícula en los tratamientos AN y AM por debajo de los 250 μm (42.91 % y 36.03 %, respectivamente). El valor del GS (0.1) se encontró dentro del rango permitido por la FDA para consumo humano (0.01-0.2). AM presentó mayores valores del IAA (4.52), así como del parámetro de color b* (22.75) y un decremento en los valores de L* (70.61) y de viscosidad de retrogradación (5.2 cP), siendo un indicador de una mayor funcionalidad en comparación con AN. Mediante CDB se encontró que el AM experimentó un decremento de 30.2 °C en la temperatura de transición vítreo en comparación con la AN. A través de la aislación y la modificación del almidón de triticale se obtuvieron ingredientes funcionales con un alto potencial para ser utilizadas en alimentos destinados a expansión en microondas o por freído como las botanas de tercera generación.

Palabras Clave: Almidón de triticale, almidón modificado, extrusión.

INTRODUCTION

Starch is the most important energy reserve of vegetal tissues like grains, fruits, roots and tubers. It is composed of a mixture of two polysaccharides: amylose and amylopectin, at approximately 20 % and 80 % of the starch content, respectively (Lupano, 2013). Starch is a very valued component in the food industry because it is used in many products such as baked and fried ones as well as desserts, sauces and other condiments. The most used starch in Mexico is corn starch (Ellasson, 2004). However, native starches have poor or unstable functional properties and can present undesirable reactions, such as retrogradation and syneresis (López et al., 2001). Therefore, the purpose of the modification is to confer functional properties to native starch, such as improved viscosity, cohesiveness, appearance, water absorption capacity, as well as storage stability (resistance to retrogradation) (Badui, 2006). One of the most used methods to modify the starches is the chemical one, but it requires more time and uses a high amount of reagents. Therefore, it is required to use alternatives to have a process of effective modification, shorter and environmentally friendly.

Triticale is a hybrid cereal obtained from wheat and rye, which offers two main advantages. First, triticale is tolerant to dry and nutrient-poor soils, as well as to low environmental conditions.
temperatures. Second, the triticale grain conserves the nutritional value of their progenitor’s cereals (Cornejo-Ramírez et al., 2015). The triticale starch corresponds to 60-70 % weight of the grain and contains approximately 23 % of amylose and 77 % of amylopectin (Burešová et al., 2010). The content of amylose and amylopectin can affect the functional properties of the starch, but also the presence of lipids or proteins can affect those properties. Usually, lipid or protein content of starches is null, but this may vary according to the isolation method. Proteins are located in and out of the starch granules and are known as granule-bound proteins, available for extraction at the same time of starch isolation (Makowska et al., 2014).

Extrusion is a thermal process where a material is transported at certain controlled conditions (temperature, pressure and speed). At the end of the transport, the material passes through a die of a certain geometry to obtain the final shape. Under this process, homogenization, mixing, partial or total cooking and material formation take place (Martínez-Meza et al., 2016). Modification by extrusion involves a low level of moisture (10 to 30 %) and heating at high temperature (90 °C to 120 °C) for short times. The starch treated by extrusion has greater thermal stability, less swelling, decreases maximum viscosity, resistance to retrogradation and decreases gelatinization temperature compared to native starch and other starch sources (Shah et al., 2016). The starch modification by extrusion process, besides fast and economical is versatile and usually environment friendly, as it does not uses any chemical reagent (Camacho et al., 2014).

The aim of the study was to evaluate the effect of extraction, chemical modification and extrusion modification of triticale starch on its functional properties.

MATERIALS AND METHODS

Materials

Triticale (Triticosecale) grains were obtained from CIRENa Department located at Salaiaca, Chihuahua, Mexico. Triticale kernels were ground on a hammer mill (Retsch, Gilson Company Inc., SK 100, Mexico) and sifted in a 60 mesh (250 μm) obtaining a thin triticale flour (TF). All reagents employed were analytical grade.

Starch extraction

Methodology used for obtaining starch was according to Aguirre et al. (2011). Fifty g of triticale flour were added to 0.25 L of a NaCl solution (5 g/L) (JT Baker, Mexico), agitated at 20 °C for 1 h, and centrifuged at 4000 rpm (Centra CL3R, Thermo IEC, USA) for 15 min (20 °C). The supernatant was collected, dried at 40 °C for 12 h in a forced air circulation oven (EcoShel 9053A, USA), ground on a commercial mill (Krups, Mexico), and sifted in a 60 mesh (250 μm), to obtain the native triticale starch (NS).

Starch modification

To obtain the acetylated starch we used the methodology reported by Mali et al. (2005) with some modifications, using acetic anhydride as an esterifying agent. A mixture of a sample of native starch (500 g), conditioned at 22 % moisture content, and 5.5 g of acetic anhydride was stored for 12 h at 5 °C and later the pH adjusted to 8.5-9 using a solution of NaOH (10 %). Next, sample was processed in a single-screw extruder (compression rate 1:1), with a temperature profile programmed for four heating zones was 60, 70, 80 and 100 °C (zones 1-4, respectively). The die diameter was 6.5 mm. The extruded obtained was dried in a forced air circulation oven (EcoShel 9053A, USA) at 45 °C until reaching 7 % of moisture content. Finally, the extruded sample was ground in a hammer mill (Retsch, Gilson Company Inc., SK 100, Mexico) and sift in a 60 mesh (250 μm), obtaining triticale modified starch (MS). The starch obtained was refrigerated at 5 °C until its use.

Physicochemical properties of starches and triticale flour

Particle size determination (PSD)

For PSD analysis, we carried out the methodology reported by Galicia-Garcia et al. (2010), in triplicate, placing seventy g of starch or flour on a stack of sieves with apertures of: 850, 600, 250, 180 and 150 μm (20, 30, 40, 60, 80, and 100 mesh, respectively) for 10 min on a Rotap equipment (WS Tyler, model RX-29, EUA). The retained portion on each sieve was weighted and related with the total weight, obtaining the result in percentage (%).

Water absorption index (WAI) and water solubility index (WSI)

These analyses were determined based on the methodology reported by Anderson (1982). Samples of starch or flour (2.5 g), weighed in 50 mL Falcon® tubes, and 30 mL of distilled water were agitated for 30 min at 30 °C in an incubator (Labnet International, Inc. USA). Then, centrifuged at 4000 rpm for 10 min (Centra CL3R, ThermoIEC, EU). The solid residue was weighted and the supernatant evaporated. The WAI was calculated by the relation with the weight gain and was expressed in grams of absorbed water for grams of dry sample. For the WSI analysis, the decanted and evaporated supernatant was related to the original sample and the result expressed in percentage (%). Both analyses were carried out by triplicate.

Color

This analysis was determined using a colorimeter (Konica minolta® CR-410, Japan). The evaluated parameters on the obtained flows were L* (luminosity), a* [green (-) to red (+) tendency] and b* [blue (-) to yellow (+) tendency]. This analysis, realized by triplicate, resulted in average values and standard deviations (ASTM D1544-04, 2001).

Proximate analysis

The included analyzes on this group, known as Weende proximate analysis, are applied to verify or determine certain components in different foods. These analyses were performed according to methodology reported by AOAC (2005). Determinations carried out were: moisture
content (method 7.003), raw protein content (total nitrogen) (2.057), raw fiber content (7.07), lipid content (922.06) and ash content (14.006). Carbohydrates were calculated by weight difference, subtracting to 100 % the sum of the other components of the proximal analysis (Almanza, 2013).

**Degree of substitution (DS)**

The degree of substitution is an indicator of OH groups for acetyl groups substitution. This determination was based on the methodology reported by Bello-Pérez et al. (2002). One g of starch modified by extrusion and acetylation was placed in a 250 mL flask, to which 50 mL of an ethanol-water solution were added (75 % v/v). This mixture was kept under stirring for 30 min at 50 °C. Next, it was cooled and with 40 mL of KOH (0.5N) and kept resting for 72 h with occasional agitations. Later, it was titrated with a 0.5N HCl solution, using phenolphthalein as an indicator. This was carried out by triplicate and with a control sample of native starch. The percentage of acetylation and the degree of substitution were obtained by the following equations:

\[
\% \text{Acetylation} = \frac{\text{mL(Blank)} - \text{mL(sample)}}{\text{weight of sample, g (dry base)}} \times 100
\]

\[
\text{DS} = \frac{162 \times \% \text{acetylation}}{4300 - (42 \times \% \text{acetylation})}
\]

**Thermal properties of starches and triticale flour**

**Viscosity analysis**

The viscosity profile of samples was obtained by the method 76-21.01 (AACC, 2000), where 3.5 g of sample were deposited in a container with 25 g of distilled water. Viscosity analysis was performed in a viscometer (Rheoplus/32 V3.40, USA) with Thermocline software. The profile test was performed in the next manner. First, a temperature ramp was conducted from 25 to 90 °C at a heating rate of 0.033 °C/s. Next, the sample was maintained at 90 °C for 5 min. After that, sample was cooled up to reaching 25 °C. The applied shear rate was 210 s⁻¹. Data plotted on Origin® program (Origin software, V 5.0).

**Differential scanning calorimetry (DSC)**

The thermal characterization was carried out with a DSC equipment (NETZSCH 200 PC, Germany) following the methodology reported by Umaña et al. (2013). Ten mg of flour samples were placed in 40 μL aluminum capsules and 20 μL of deionized water were added. Later the capsule was hermetically sealed and incorporated in a calorimeter previously calibrated with indium. The applied heating rate was 10 °C/min from 30 °C to 120 °C under a nitrogen atmosphere.

**Statistical analysis**

The data were analyzed through of Minitab 17® statistical software, using a one-way analysis of variance (p≤0.05), and for significant statistical differences, means were compared with the Tukey test (p<0.05).

**RESULTS AND DISCUSSION**

**Physicochemical properties of starches and triticale flour**

**Determination of particle size (PSD)**

Higher particle retention was obtained in mesh number 60 (250 μm), with a triticale flour percentage of 53.76 ± 4.05%, native starch of 42.91 ± 2.12 % and modified starch of 36.03 ± 0.07 % (Table 1). Particle size is a parameter of quality of flours and starches, in the CODEX Standard, for standard size of wheat flour. For the elaboration of food products, the general recommendation is the use of a 70 mesh, equivalent to 212 μm. In this case, with the use of triticale and their starches, it is more effective to use 60 mesh (250 μm) to avoid loss of raw material and have standardized the particles, what will give us as a result more cohesives doughs for the fabrication of different food products (Codex Standard, 1985). Fernández-Muñoz et al. (2008) reported that in PSD analyses the distribution in sieves of different sizes indicates the functionality that the flour presents as well as its potential use. For example, for the production of tortillas a fine particle size is necessary, while they recommend larger particle sizes for the elaboration of other products like tortilla chips.

A grinding performance of 95.80 % was obtained. This result is similar to the standard reported by Saxena et al. (1992) in wheat and recent varieties of triticale, where it exceeds 70 % in yield, and is one of the main causes to obtain grains with a greater density that favors their later extraction. Pena and Amaya (1992) reported that to increase the efficiency in obtaining wheat flour, it is possible to use 25 % triticale, where the blend retains its functional properties. Regarding starch extraction, the efficiency was 68.9 %, proving that the starch corresponds to more than half of the grain of triticale. This value relates to those reported by Bushuk (2004) and

| Sample | Mesh | μm | % Retention | WAI (g H2O/g) | WSI | Color Parameter |
|--------|------|----|-------------|---------------|-----|----------------|
| TF     | 60   | 250| 53.76±4.05 | 1.79±0.04     | 10.81±0.16 | 88.54±0.89 | 2.63±0.47 | 11.02±1.53 |
| NS     | 60   | 250| 42.91±2.12 | 1.78±0.07     | 2.40±0.07  | 80.39±0.99 | 4.45±0.16 | 13.53±0.27 |
| MS     | 60   | 250| 36.03±0.70 | 4.52±0.12     | 11.94±0.1  | 70.61±0.48 | 4.47±0.10 | 22.75±2.72 |

TF: triticale flour, NS: native starch, MS: modified starch. Average of three replicates of each one ± standard error. Means in the same column with different letters are significantly different (Tukey, α = 0.05). g of water/g of dry sample base.
Water absorption index (WAI) and water solubility index (WSI)

Table 1 shows that WAI values present a significant difference (p< 0.05) in the native starch and flour in relation with the modified starch, where a high index value was obtained (4.52±0.12). For WSI, high values were observed in the TF, as in the MS, the last one being significantly higher, which suppose a better solubility and facility to create a more uniform mix (α = 0.05) (Table 1). The highest value of modified starch’s WAI can be due to the addition of acetyl groups to the glucose molecule, associated to the modification with acetic anhydride and the extrusion process (Salcedo-Mendoza et al., 2016). On the other hand, the WSI highest value in MS can be the result of the addition of the same hydrophilic groups in the modification, as well as different components contained in the flour, without any extraction and modification, such as water-soluble proteins, and sugars can facilitate solubilization. Bello-Pérez et al. (2002) indicate that the WAI is a parameter that indicates mainly the availability of hydrophilic groups to bind water molecules (H₂O), which can delay retrogradation in starch-based foods products, as well as serve as a plasticizer in thermo-mechanical processes such as extrusion. Rodríguez-Sandoval et al. (2012) obtained WSI values of 20.09 % in wheat flour and indicated that the components in the flour affect the percentage obtained in WSI since the hydrophobic groups can also reduce this index. These indexes can also affect the behavior of the flour blend in the extrusion machine, making more homogeny dough, favoring the mixing of the ingredients.

Color

The Luminosity parameter (L*) for the MS (70.61) is the lowest one, indicating that MS is more opaque than the others. Also, the b* parameter for MS (22.75) is significantly higher, indicating that the color tends more to yellow (Table 1). The previous behavior could be attributed to the heat treatment applied in the extruder, where the interactions between various components of the material favor the hydrolysis of starch, which is characterized by a yellowish color. Previous behavior leaves simple sugars and amino acids available in the medium (flour material remainder), leaving the possibility of another reaction that leads to a browning color of the conditioning material. The above is known as the Maillard reaction (Gómez-López, 2013).

Proximate analysis

From moisture content analyses, there were significant differences in all the samples, with a highest value for the NS (8.80 %). The highest ash content was for the TF (1.43 %), as well as protein content (10.27 %). Lipids and fiber content decreased in MS (94.7 % and 69.29 %, respectively) and NS (83.62 % and 64.03 %, respectively) in relation with TF sample. The total carbohydrates reacted inversely, concentrating and obtaining the highest value in MS. In the proximal analysis of NS and TF, most of the components decreased after starch extraction, due to the same process and washed to obtain the starch samples. Martinez-Meza (2016) reported that through thermal treatment, the chemical components could diminish since moisture and proteins react to heat, evaporation of water, and protein denaturation.

The proximate analysis of obtained TF values were low in comparison to that reported by USDA-ARS (2012) in triticale: moisture content 10.51 %, ash content 2.23 %, protein content 13.05 %, lipid content 2.09 %, fiber content 2.23 % and carbohydrates content 72.12 %. Makowska et al. (2014) reported that lipid content present in triticale starch is around 0.204-0.299 %, and protein content values ranged from 0.055 to 0.068 %; only NS sample presented similar lipid content, however, the protein content obtained were higher in MS and NS (5.12 and 6.26 %, respectively) due to conditioning in the extraction. Baldwin (2001) reported that an important part of proteins present in starch is found in the superficial part of granule conforming an association with other components such as lipids and have a direct influence on their characteristics such as the hydration rate of the granule (Debet and Gidley, 2006).

Degree of substitution (DS)

The degree of substitution obtained was 0.10 ± 0.007 and the percentage of acetylation was 2.65 ± 0.18 %. These values are within range of DS approved by the FDA, as suitable for human consumption (0.01 a 0.2), being an indication that the intake of the modified starch obtained does not represent a risk to health. Murua-Pagola et al. (2009) reported values

Table 2. Proximate analysis of starch and triticale flour.

| Sample | H | A | P | L | F | CH |
|--------|---|---|---|---|---|----|
|        | % | % | % | % | % | %  |
| TF     | 7.72±0.04b | 1.43±0.03a | 10.27±0.19a | 1.71±0.38a | 1.14±0.13a | 77.7±0.21c |
| NS     | 8.80±0.07b | 0.33±0.02b | 6.26±0.25b | 0.28±0.03b | 0.41±0.01b | 83.89±0.33b |
| MS     | 7.0±0.39a | 0.03±0.00a | 5.12±0.65a | 0.09±0.01a | 0.35±0.01a | 87.38±0.80a |

TF: triticale flour, NS: native starch, MS: modified starch. H: moisture, A: ashes, P: protein L: lipids, F: fiber CH: carbohydrates. Average of two measurements and three replicates of each one ± standard error. Means in the same column with different letters are significantly different (Tukey, α = 0.05).

USD-ARS (2012) with a triticale starch value of 60 %, and Makowska et al. (2014) with a range of 63 to 65.8 % in five varieties of triticale.
of 0.033 in the degree of substitution with the same process of chemical modification. We found that extrusion is a good alternative to obtain starch-based foods products. The above is due to the extrusion modification process, which employs less reagent than the used in the chemical modification, and it does not require washing step, making extrusion a fast, effective and environmentally friendly method.

**Rapid viscosity analysis (RVA)**

Viscosity was obtained in each of the phases of the profile test (Table 3). In the heating stage, an increase in viscosity was observed for all the treatments, obtaining a Peak Viscosity of 6230 cP for NS, followed by the MS (4100 cP) and the TF (1507.5 cP), respectively. In NS, this increment indicates a higher interaction of the isolated starch with water molecules. The same effect was presented in MS, where the difference is due to the thermomechanical effect to which it was previously subjected (Figure 1). In the cooling stage, MS treatment provoked a decrease of 57 % of retrogradation viscosity (Setback viscosity) compared to NS, mainly due to the inclusion of acetyl groups that enhance the retention of water, providing a greater stability in the cycles of cooling, and avoiding in a smaller proportion, water outlet of the structure (syneresis). This same trend was present when evaluating the gelatinization temperature in which the treatment of MS presented a difference of 18.32 °C lower than the treatment of NS. This is probably because chemical and thermomechanical modification favored the interaction and retention of water molecules, which allowed a greater absorption of starch granules and their subsequent gelatinization. Nuñez-Santiago et al. (2010), reported a decrease in the retrogradation viscosity values of barley and maize starch treatments subjected to acetylation (32.5 % and 47.6 %, respectively).

Merino (2013), discussed that the WAI relates to viscosity, since in the modified starch is a greater capacity of glucose molecules to join water due to the addition of acetyl groups, so viscosity decreases. It is also known that a higher viscosity is an indicator of a high proportion of starch without gelatinizing, while a lower viscosity indicates a proportion of starch already gelatinized, which is reflected in the NS and MS by acetylation and extrusion (Gómez-López, 2013). Márquez-Gómez (2016) shows a viscosity reduction of modified starches, justified by breakdown or disorganization of the crystalline structure of the starch granule. When modified (in this case, by acetylation and extrusion of starch), the amount of soluble starch increases and with new molecules of low molecular weight, the hydration capacity decreases and cannot swell like a native starch. This may be a reason for the lower viscosity values for MS versus NS. Figure 1 presents the behavior of the MS viscosity curve, exhibiting a normal behavior for starches, which shows that the modified starch remains functional; it can be also observed that TF presents smaller viscosity values, compared to starches. This probably attributed to swelling starch tendency during the heating, causing a gelatinization and consequently, an increase in viscosity takes place. About peak viscosity, it is lower in MS than in NS, as well as the gelatinization temperature.

**Thermal properties of starches and triticale flour**

**Differential Scanning Calorimetry (DSC)**

The transition temperature values obtained in TF, NS and MS were 67.50 ± 2.40 °C, 66.80 ± 3.68 °C and 36.60 ± 0.28 °C, respectively (Table 3), having a lowest significant

![Figure 1. RVA Viscoamylogram of triticale flour and starches. a) TF: flour, b) NS: native starch, c) MS: modified starch.](image)

**Figure 1.** RVA Viscoamylogram of triticale flour and starches. a) TF: flour, b) NS: native starch, c) MS: modified starch.

![Viscoamylogram of triticale flour and starches. a) TF: flour, b) NS: native starch, c) MS: modified starch.](image)

**Table 3.** Viscosity and thermal properties of starch and triticale flour.

| Sample | Peak viscosity  | Minimum viscosity | Final viscosity | Setback Viscosity | Gelatinization temperature | Glass transition temperature (Tg) by DSC |
|--------|----------------|-------------------|----------------|-------------------|----------------------------|----------------------------------|
|        | cP             | cP                | cP             | cP                | °C                         | °C                               |
| TF     | 1507.5±3.5°C  | 336.5±13.44°C     | 830±42.4°C     | 3493.5±28.99°C    | 64.35±0.49°C               | 67.50±2.40°C                     |
| NS     | 6230±665°C    | 2510±325°C        | 14650±360°C    | 12140±3280.97°C   | 65.67±0.46°C               | 66.80±3.68°C                     |
| MS     | 4100±42.4°C   | 2305±35.4°C       | 7515±63.6°C    | 5210±28.28°C      | 47.35±1.06°C               | 36.60±0.28°C                     |

TF: triticale flour; NS: native starch, MS: modified starch Tg: glass transition temperature. Average of three replicates of each one ± standard error. Means in the same column with different letters are significantly different (Tukey, α = 0.05).
The glass transition values obtained in MS present similar behavior to gelatinization temperature (evaluated in viscosity analysis) with a decrement of 30.2 °C compared to NS sample. Rivas-González et al. (2009), propose that the chemical modification of starches, results in the destabilization of the granule ordered structure, due to de addition of new molecules. This can increase water absorption capacity of the starch and in consequence, the energy needed to start the gelatinization, or the glass transition of the granules will decrease. Therefore, it can be seen WSI and WAI are significantly different in the MS compared to NS and TF (Table 1), indicating the disorganization of the granule structure. When comparing the glass transition obtained by the DSC analysis with the gelatinization temperature through the RVA analysis, values present some differences but the same tendency.

CONCLUSIONS

In this study, the application of extrusion technology besides a chemical agent for the modification of triticale starch, was possible. Significant differences were found in the characterizations performed, highlighting the thermal ones and the substitution degree, which indicates that the modification was successfully carried out and safe for use on starch-based foods products and human consumption. In addition, we can take advantage of the starch extraction method, since NS and MS presented high protein values that can be useful to the formulation of different starch-based food products with high protein content. Finally, with the help of the thermal characterization, WAI and WSI, it is concluded that the triticale modified starch has good viscosity characteristics, presenting a decrease in its gelatinization and glass transition temperatures; in this way it is indicated that the functional properties have been changed during the modification process, but they are still useful even when heat was applied. The snacks development in this research presented a high potential for use as third generation snacks.

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