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Physicochemical characterization of starch from hexaploid triticale (*X Triticosecale* Wittmack) genotypes

Caracterización fisicoquímica de almidón de genotipos de triticale hexaploide (*X Triticosecale* Wittmack)

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The starch granules of complete and substituted triticale genotypes were analyzed for morphology and size distribution using scanning electron microscopy (SEM) and laser diffraction, respectively. A quantitative comparison of total carbohydrate, starch, and amylose contents was also performed. The results showed that the complete triticales contained 8.8% more total carbohydrate content and 13.8% more starch content than the substituted ones. No significant differences in the average amylose content (22.8%) were observed between the two sets of triticales. However, the A- and B-type starch granules of the substituted triticales showed significant differences in amylose content. Starch granule distribution profiles showed that the sizes of the A- and B-type starch granules of complete triticales were larger (in the range of 18–41 μm and 2–13 μm , respectively) than those of substituted triticales (in the range of 8–38 μm and 0.5–6 μm , respectively). This study demonstrated that the starch in triticale genotypes differs in physicochemical properties.

Keywords: triticale; SEM; laser diffraction; starch; amylose; carbohydrate

Se analizaron los gránulos de almidón de genotipos de triticales completos y sustituidos en morfología y distribución de tamaño utilizando microscopía electrónica de barrido (SEM, por sus siglas en inglés) y difracción láser, respectivamente. Se realizó una comparación cuantitativa del contenido de carbohidratos totales, almidón y amilosa. Los resultados mostraron que los triticales completos contienen 8,8% más en contenido de carbohidratos totales y 13,8% más de contenido de almidón que los triticales sustituidos. No se observaron diferencias significativas en el contenido de amilosa (promedio 22,8%), sin embargo, los gránulos de almidón tipo-A y tipo-B de los triticales sustituidos mostraron diferencias significativas en el contenido de amilosa, pero no los respectivos gránulos de los triticales completos. El análisis del perfil de distribución de los gránulos de almidón mostró que los gránulos de almidón tipo-A y tipo-B de los triticales completos son de mayor tamaño (rango de 18–41 μm y 2–13 μm , respectivamente) que el de los triticales sustituidos (rango de 8–38 μm y 0,5–6 μm , respectivamente). Se concluye que el almidón de los genotipos de triticales difieren en las propiedades fisicoquímicas estudiadas.

Palabras clave: triticale; SEM; difracción láser; almidón; amilosa; carbohidrato

Introduction

Triticale (*X Triticosecale* Wittmack) is an artificial cereal crop created from a cross between wheat (*Triticum* sp.) and rye (*Secale cereale* L.) (Kulp & Ponte, 2000), which is tolerant to low temperatures, drought, and acid and alkaline soils (Royo, Rodríguez, & Romagosa, 1993; Varughese, Barker, & Saari, 1987). However, triticale is sensitive to water stress (He, Goyal, Laroche, Zhao, & Lu, 2012) and, as other cereals, it may also be sensitive to high development temperatures. Triticales with genomic constitutions AABBRR (hexaploid) or AABBDDRR (octaploid) are known as “complete” (Lukaszewski, 2006; Varughese et al., 1987). Breeders have used genetic manipulation to modify the genomic characteristics of the grains of triticales by replacing the R chromosome of rye by a D chromosome of bread wheat in both hexaploid and octaploid triticales. The resulting improved triticales with genomic constitutions AABBDR and AABBDDRD are known as “substituted” triticales (Varughese et al., 1987).

Starch is by far the most abundant component of cereal grains, and its synthesis and composition are influenced by the genotype and environment (Hurkman et al., 2003; Liu et al., 2011). In general, triticales contain around 62.4–70.9% of starch with approximately 20.8–26.4% of amylose content (Burešová, Sedláčková, Faměra, & Lipavský, 2010). According to previous reports, substituted triticales contain lower amounts of amylose than complete triticales (Navarro-Contreras et al., 2014), which have been related to the size of starch granules (Ao & Jane, 2007). Starch granules of triticales show a bimodal size distribution (Ao & Jane, 2007; Stoddard, 1999; Wilson, Bechtel, Todd, & Seib, 2006) and are identified as A- and B-type starch granules. A-type granules are large and disk-like or lenticular in shape (Ao & Jane, 2007; Li et al., 2011) with a diameter around 10–35 μm , whereas the B-type granules are small and spherical shaped with a diameter of about 2 μm (Ao & Jane, 2007). The A-type starch granules have a higher amount of amylose and higher average branch chain length

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than the B-type starch granules, which show broader ranges of gelatinization temperatures and display lower retrogradation rates than the A-type granules (Ao & Jane, 2007).

Starches extracted from various sources contain different amounts of amylose and amylopectin, which influence physicochemical properties such as gelatinization, retrogradation, water absorption, and paste viscosity (Charles, Chang, Ko, Sriroth, & Huang, 2005; Jane & Chen, 1992; Mangalika, Miura, Yamauchi, & Noda, 2003; Sandhu & Singh, 2007). The different sizes and shapes of the A- and B-granules of wheat, triticale, and barley starches are important characteristics that determine their end uses (Ao & Jane, 2007). Characteristics such as amylose content and starch granule size distribution influence the starch degradation degree (Stevnebø, Sahlström, & Svihus, 2006), pasting behavior (Shinde, Nelson, & Huber, 2003), and viscosity and gelatinization temperatures (Ao & Jane, 2007). These properties are important in the commercial starch industry (Shinde et al., 2003) as well as in the bread-making industry (Park, Wilson, & Seabourn, 2009).

The triticale is an important crop for places with undesirable environmental factors (Martinek, Vinterová, Burešová, & Vyhnanek, 2008), which makes it a feasible alternative to traditional crops as food and feed as well as for the non-food industry (Oettler, 2005). Scientists and breeders have studied the triticale grains for their genetic (Lukaszewski, 2006), agronomic (Martinek et al., 2008), and baking (Naeem, Darvey, Gras, & MacRitchie, 2002; Tohver, Kann, Täht, Mihhalevski, & Hakman, 2005) characteristics. However, few studies have been conducted to compare and contrast differences in certain physicochemical properties of starches among different triticale genotypes. Starches extracted from complete and substituted triticales might have different physicochemical characteristics due to the differences in their genetic background, and their elucidation would allow selection of the most appropriate one(s) to tailor products with desirable characteristics. In order to provide new insights into properties of starch extracted from triticales and predict their possible end uses, the present work was carried out to evaluate and compare the starch content and the A- and B-granules of three complete and three substituted triticales in some physicochemical properties.

Materials and methods

Raw materials

For this study six triticale varieties were employed. Three of them were complete triticales (Beagle, Eronga, and Fahad), and three substituted triticales (Armadillo, Panda, and Yoreme). Seeds of triticales were kindly donated by the Wellhausen-Anderson Plant Genetic Resource Center located at the International Maize and Wheat Improvement Center (CIMMYT; El Batán, México). The triticales were planted in the 2011–2012 planting season at the same time and under the same environmental conditions at the University of Sonora Agricultural Experiment Station in Mexico. A completely randomized design with three replications was used. Once harvested, samples of triticales were collected and stored at -20°C until analysis.

Starch preparation

For SEM and starch granules' size distribution analyses, starch was prepared from single kernels according to the method of Bettge, Morris, and Greenblatt (1995). The embryo and bran of each kernel were removed with a razor. The remaining kernel

was lightly crushed between two sheets of waxed paper with the help of a hammer, transferred to a standard 1.5 mL polypropylene microcentrifuge tube, and steeped twice at room temperature for 30 min in 500 μL petroleum ether with occasional gentle agitation. After centrifugation at 10,000 g for 10 min and solvent removal, the precipitate was steeped twice in 500 μL of 0.1 M NaCl at room temperature for 30 min with occasional gentle agitation. Gluten was formed by lightly kneading the endosperm for 2 min inside of the microcentrifuge tube and manually removed. The remaining starch was washed twice with 500 μL of deionized water to remove excess of salt and centrifuged at 10,000 g for 10 min. The pelleted starch was suspended twice in 1 mL acetone, centrifuged, and finally dried at ambient temperature.

To determine the amylose content in A-type and B-type starch granules, samples of mature triticale grains were treated according to the method of Stevnebø et al. (2006) to isolate starch and fractionate it into A-type and B-type starch granules. Ten grams of starch were combined with 100 mL of deionized water, stirred for 10 min at ambient temperature, and rested for 90 min to allow the large A-type granules to sedimentate. The supernatant containing the small B-type starch granules was filtered through a Büchner funnel (Whatman filter, 40 μm). The pellets of both fractions were washed with acetone, centrifuged, and air-dried at room temperature.

Starch content

The total starch content in mature triticale kernels was determined following the amyloglucosidase/ α -amylase method 76-13 of the American Association of Cereal Chemists (AACC, 2000) using the Megazyme Total Starch Assay Kit (Megazyme International Ireland Ltd., Bray Business Park, Bray Co., Wicklow, Ireland).

Amylose content

The apparent amylose content in the A- and B-type starch granules was determined following the modified method of Knutson and Grove (1994). Starch samples (10 mg) were dispersed in 100 μL of 3 M CaCl_2 and thoroughly mixed in a vortex mixer. After standing for 10 min, 900 μL of dimethyl sulfoxide containing 6.7×10^{-3} M iodine was added. The starch suspensions were stirred and sonicated for 15 min in a Branson M3800 H water sonication bath (Branson Ultrasonics Corp., Danbury, CT, USA) set at 70°C . Aliquots of 100 μL of the resulting sonicated suspensions were taken and combined with 900 μL of dimethyl sulfoxide containing 6.7×10^{-3} M iodine. A volume of 8 mL of deionized water was added to each tube to form the blue amylose–iodine complex. The color intensity formed was measured at 600 nm. Apparent amylose values were first calculated from a standard curve and corrected for amylopectin according to the procedure of Knutson (1986).

Total carbohydrate content

The total carbohydrate content was determined using a modification of the phenol-sulfuric assay of Dubois, Gilles, Hamilton, Rebers, and Smith (1956) as reported by Masuko et al. (2005). An aliquot of 50 μL of the blue complex solution formed for the determination of amylose content was taken to determine the total carbohydrate content. The presence of iodine causes no

interference with the assay at this concentration as reported by Knutson and Grove (1994). Volumes of 150 μL of concentrated sulfuric acid and 30 μL of 5% phenol (w/v) were added in rapid succession to the blue complex solution and heated at 90°C for 5 min. After cooling to ambient temperature, the color intensity of the samples was measured at 490 nm using a NanoDrop 8000 UV/Vis spectrophotometer (Thermo Scientific, Waltham, MA). The total carbohydrate concentration was calculated from a standard curve prepared for commercial glucose.

Starch granule morphology

Scanning electron microscopy (SEM) was used to analyze the morphology of starch granules following the method of Jane, Kasemsuwan, Leas, Zobel, and Robyt (1994). Starch was spread out on silver tape, metal-shadowed with gold/palladium (60/40), and mounted on a brass disk. Samples were examined at 1500 \times magnification under low vacuum using a JEOL JSM-5400LV scanning electron microscope (Peabody, MA, USA) at an acceleration voltage of 15 kV.

Size distribution of starch granules

The size distribution of starch granules was measured using laser diffraction in a Coulter LS 100Q (Beckman Coulter, Miami, FL, USA). A small quantity (20–30 mg) of starch was suspended in 2 mL of deionized water and vortexed before analysis. The suspension was placed in the flow system and pumped through the optical chamber. A GB500 standard, which consisted of a glass bead sample with a nominal mean particle size of 500 μm , was used as reference.

Statistical analysis

All analyses were run in triplicate. The results were expressed as mean values \pm standard deviation. Data were subjected to an analysis of variance following general model procedures (SAS 2005). The resulting mean values were compared using Tukey's multiple range test with significance defined at $P \leq 0.05$.

Results and discussion

Total carbohydrate, starch and amylose content

The results of determination of the total carbohydrate, starch and amylose content in complete (Beagle, Eronga, and Fahad) and substituted (Armadillo, Panda, and Yoreme) triticales are shown in Table 1. It was observed that the complete triticales showed higher total carbohydrate contents than the substituted ones ($P \leq 0.05$) with average values of $80.4 \pm 1.0\%$ and $73.3 \pm 1.0\%$, respectively, which were concomitantly related to the starch content of the two sets of triticale genotypes. The complete triticales showed higher starch content than the substituted triticales ($60.6 \pm 0.9\%$ and $52.2 \pm 0.9\%$, respectively), which showed a difference of 8.4%, on the average, between the two sets of triticale genotypes. Carbohydrates available in cereal grains, mainly starch deposited in the endosperm, amount to 56–74% (Koehler & Wieser, 2013). The difference in starch content between the triticale sets might be attributed to the carbohydrate accumulation in the grain during development and dependent on the quantity of sucrose available during anthesis and the activity of starch synthase (Fernandez-Figares, Marinetto, Royo, Ramos, & Garcia Del Moral, 2000; Keeling, Bacon, & Holt, 1993). The complete triticales used in this study had on the average a starch content value that was in the range reported for the “cool-season” cereals (wheat, rye, barley, and oats) (Koehler & Wieser, 2013). However, the lower starch contents in substituted triticales suggest that the sowing temperature would probably not have been the most appropriate for their vernalization and maturation, affecting the enzymatic synthesis and accumulation of total carbohydrates and starch. High growing temperatures adversely affect starch accumulation in barley (MacLeod & Duffus, 1988), corn (Jones, Roessler, & Ouattar, 1985; Tester, South, Morrison & Ellis, 1991) and wheat (Chowdhury & Wardlaw, 1978; Liu et al., 2011; Tester et al., 1995), leading to reduction in starch accumulation and grain size. Hurkman et al. (2003) observed that high temperatures during grain filling of wheat caused a decrease in starch content primarily due to the reduction in the duration of starch accumulation. Temperatures higher than 25°C adversely affect the activity of soluble starch synthase in the wheat endosperm, reducing the

Table 1. Total carbohydrate, starch and amylose contents determined in complete and substituted triticales.

Tabla 1. Contenido de carbohidratos totales, almidón y amilosa determinados en triticales completos y sustituidos.

Triticale	Total carbohydrates	Total starch	Amylose in starch granules ¹		
			A-Type	B-Type	Average
Complete					
Beagle	79.9 \pm 1.8 ^a	61.4 \pm 0.9 ^a	23.8 \pm 1.4 ^{a,A}	22.2 \pm 0.7 ^{a,A}	23.0 \pm 1.4 ^a
Eronga	80.1 \pm 0.1 ^a	59.8 \pm 1.1 ^a	23.3 \pm 0.4 ^{a,A}	22.3 \pm 0.8 ^{a,A}	22.8 \pm 0.8 ^a
Fahad	81.2 \pm 0.3 ^a	60.1 \pm 0.5 ^a	24.6 \pm 1.0 ^{a,A}	23.0 \pm 0.8 ^{a,A}	23.8 \pm 1.2 ^a
Mean	80.4 \pm 1.0 ^a	60.6 \pm 0.9 ^a	23.9 \pm 0.7 ^{a,A}	22.5 \pm 0.4 ^{a,A}	23.2 \pm 0.6 ^a
Substituted					
Armadillo	73.8 \pm 1.3 ^b	52.6 \pm 1.2 ^b	23.4 \pm 0.9 ^{a,A}	21.2 \pm 0.8 ^{a,B}	22.3 \pm 1.4 ^a
Panda	73.0 \pm 1.2 ^b	51.6 \pm 0.5 ^b	23.3 \pm 0.9 ^{a,A}	21.1 \pm 0.8 ^{a,B}	22.2 \pm 1.4 ^a
Yoreme	73.0 \pm 1.1 ^b	52.2 \pm 0.8 ^b	23.4 \pm 0.3 ^{a,A}	22.5 \pm 1.3 ^{a,B}	23.0 \pm 1.0 ^a
Mean	73.3 \pm 1.0 ^b	52.2 \pm 0.9 ^b	23.4 \pm 0.1 ^{a,A}	21.6 \pm 0.8 ^{a,B}	22.5 \pm 1.6 ^a

Notes: Mean values are expressed in percentage \pm standard deviation. Values followed by the same lowercase letter in the same column are not significantly different ($P \leq 0.05$, $n = 3$).

¹Mean values of amylose content in A-type and B-type starch granules are expressed in percentage \pm standard deviation. Values followed by the same capital letter in the same row are not significantly different ($P \leq 0.05$, $n = 3$).

Notas: Los valores promedio están expresados en porcentaje \pm desviación estándar. Los valores seguidos por la misma letra en minúscula en una misma columna no son significativamente diferentes ($P \leq 0.05$, $n = 3$).

¹Los valores promedio del contenido de amilosa en los gránulos de almidón tipo-A y tipo-B están expresados en porcentaje \pm desviación estándar. Los valores seguidos por la misma letra en mayúscula en la misma hilera no son significativamente diferentes ($P \leq 0.05$, $n = 3$).

duration of starch accumulation (Hawker & Jenner, 1993; Keeling et al., 1993) as well as the expression of gene encoding enzymes for starch biosynthesis (Hurkman et al., 2003). Differences in starch content between the triticale genotypes might also be due to the existence of null alleles on the short arms of chromosomes 7A (*Wx-A1*) and 7D (*Wx-D1*) and the long arm of chromosome 4A (*Wx-B1*), all derived from wheat, which code for the synthesis and expression of starch synthase (Fujita et al., 1996; Yamamori, Nakamura, Endo, & Nagamine, 1994). This adverse effect would have probably taken place in substituted triticales used in our study because they contain genomes A and B from durum wheat, and genome D from hexaploid wheat.

The values of amylose content in both complete and substituted triticales used in the present study showed similar percentage values (Table 1) and were in the range 12.8–35.1% as previously reported (Dennett, Schofield, Roake, Howes, & Chin, 2009). The average amylose content in both sets of triticale genotypes showed similar percentage values, and although that of the substituted triticales was slightly lower, the difference was not of statistical significance (Table 1). A similar result was observed between the amylose contents of the A-type and B-type starch granules of the complete triticales. However, the starch granules of the substituted triticales differed in amylose content ($P \leq 0.05$), although the differences observed were not too large. Matsuki, Yasui, Kohyama, and Sasaki (2003) reported that the amylose contents in several wheat varieties were not affected even when their starch contents decreased as maturation temperatures increased. Silencing the genes that code for the synthesis of the starch branching enzymes *SBEIIa* and *SBEIIb* leads to an increase in amylose content (Konovalov, Shaturova, Mitrofanova, & Kudryavtsev, 2012), which, according to our results, might not have occurred during the development of the complete and substituted triticales of our study.

The lower amylose content observed in the B-granules of substituted triticales led us to think that the granule bound starch synthase I (*GBSS I*), the enzyme responsible for amylose synthesis and called *waxy* protein, was expressed in lower amounts in

substituted triticales (Yamamori & Quynh, 2000). Debiton, Bancel, Chambon, Rhazi, and Branlard (2010) demonstrated that variations in amylose content in near isogenic wheat lines were directly related to the amounts of *GBSS I* found in the wheat seeds. Zhang et al. (2010) observed that *GBSS I* was expressed during the mid to late filling stage of wheat grains under development. However, at that stage of kernel maturation the environmental temperatures start to increase, affecting the enzyme activity as well as gene expression (Hurkman et al., 2003). Therefore, it is supposed that *GBSS I* might have been less active or in lower amounts possibly due to the sensitivity of substituted triticales to high temperatures at which they were grown (Liu et al., 2011; Zhao, Dai, Jiang, & Cao, 2008) in comparison to complete triticales. However, we were unable to demonstrate that because the kernels of the triticale genotypes were analyzed in a mature state and no information about the environmental growing conditions under which they were grown was available.

The low starch accumulation in substituted triticales suggested that high growing temperatures might have caused an earlier cessation of starch synthesis such as that reported for wheat (Hurkman et al., 2003; Liu et al., 2011). Low starch contents, such as those observed in substituted triticales although with similar amylose contents to the complete triticales, open the possibility that the bread wheat *SBEIIb* genes might have been silenced with no effect on amylose synthesis as has been observed in wheat (Regina et al., 2005). For years triticale has been successfully used as feed due to its high protein and carbohydrate content (Peña, 1996). According to our results, complete triticales might be the choice for feeding purposes over substituted triticales as they contain higher carbohydrate contents.

Starch granule morphology

The scanning electron micrographs showed that the unfractionated starch granules of complete triticales (Figure 1A, B, and C for Beagle, Eronga, and Fahad, respectively) and substituted

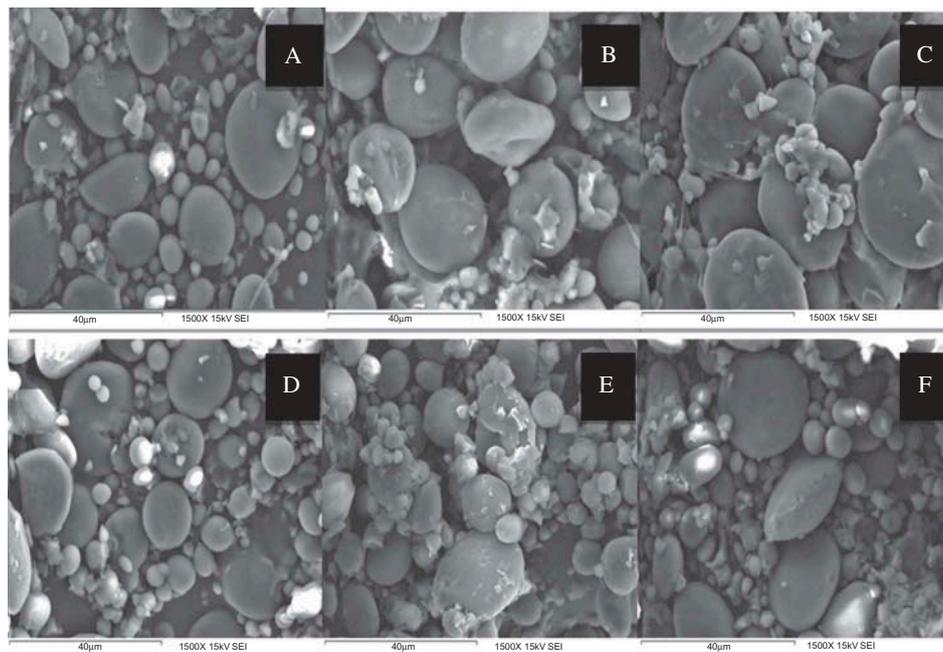


Figure 1. SEM images of starch granules of complete triticales (A, B, and C for Beagle, Eronga and Fahad, respectively) and substituted triticales (D, E, and F for Armadillo, Panda and Yoreme, respectively). Scale bar = 40 µm.

Figura 1. Imágenes de microscopía electrónica de barrido de gránulos de almidón de triticales completos (A, B y C para Beagle, Eronga y Fahad, respectivamente) y de triticales sustituidos (D, E y F para Armadillo, Panda y Yoreme, respectivamente). Barra de escala = 40 µm.

triticales (Figure 1D, E, and F for Armadillo, Panda, and Yoreme, respectively) displayed bimodal size distributions. The A-type starch granules of complete and substituted triticales displayed disk shapes whereas the B-type starch granules displayed a more spherical-type shape such as those reported by Wilson et al. (2006) and Ao and Jane (2007). SEM also showed that the starch of substituted triticales contained spherical or elongated B-type starch granules in higher number than those observed in the complete triticales. Bechtel, Zayas, Kaleikau, and Pomeranz (1990) and Wilson et al. (2006) also observed the presence of smaller starch granules in hard red winter wheat starch. Therefore, the presence of a high amount of small starch granules in substituted triticales might be due to their genomic composition, which includes the D genome from hexaploid wheats.

Starch granule size distribution

The granule size distribution of complete (Beagle, Eronga, and Fahad) and substituted (Armadillo, Panda, and Yoreme) triticales is shown in Figure 2. All triticales showed a bimodal starch granule distribution as reported previously (Ao & Jane, 2007; Stoddard, 1999; Wilson et al., 2006). However, starches from complete and substituted triticales showed significant difference in size distribution as well as in the volume per cent of the granules (Table 2). The complete triticales showed bigger diameter size distribution of A- and B-granules (range of 18–41 μm and 2–13 μm , respectively) than those of the substituted ones (range of 8–38 μm and 0.5–6 μm , respectively). Table 2 also shows that the volume per cent of the A- and B-granules was also different for the complete and substituted triticales. The average volume per cent of the A-granules of complete triticales was higher than that of the substituted triticales (80.1 ± 0.03 and 75.0 ± 0.03 , respectively). In contrast, the B-granules of the complete triticales showed lower volume per cent values (19.8 ± 0.2) than those of the substituted triticales (25.0 ± 0.3). Differences in granule size and volume per cent of each starch granule type have been related to their synthesis rates, which have been attributed to several factors. The A-granules start to form in the wheat amyloplast at about 4–5 days after anthesis (Bechtel et al., 1990; Parker, 1985) and continue to increase in size until reaching a maximum diameter at physiological maturity of the kernel. In contrast, the B-granules are initiated at 12–14 days after anthesis and continue to enlarge until 21 days post-anthesis (Bechtel et al., 1990). However, according to Li et al.

Table 2. Starch granule size distribution and volume (%) of A- and B-type starch granules of complete and substituted triticales.

Tabla 2. Distribución del tamaño y volumen (%) de los gránulos de almidón tipo-A y tipo-B de triticales completos y sustituidos.

Variety	A-Type		B-Type	
	Size (μm)	Volume (%)	Size (μm)	Volume (%)
Complete triticales				
Beagle	18–40	79.8 ± 0.02^c	2–11	20.2 ± 0.02^d
Eronga	18–40	80.4 ± 0.03^a	2–11	19.6 ± 0.03^f
Fahad	18–41	80.2 ± 0.05^b	2–13	19.8 ± 0.05^e
Average		80.1 ± 0.30^a		19.8 ± 0.20^b
Substituted triticales				
Armadillo	8–37	75.1 ± 0.04^e	0.8–3	24.9 ± 0.04^b
Panda	8–36	75.2 ± 0.04^d	0.8–4	24.7 ± 0.04^c
Yoreme	12–38	74.6 ± 0.05^f	0.5–6	25.4 ± 0.05^a
Average		75.0 ± 0.32^b		25.0 ± 0.30^a

Note: Mean values are expressed in percentage \pm standard deviation. Values followed by the same letter in the same column are not significantly different ($P \leq 0.05$, $n = 3$).

Nota: Los valores promedio están expresados en porcentaje \pm desviación estándar. Valores seguidos por la misma letra en la misma columna no son significativamente diferentes ($P \leq 0.05$, $n = 3$).

(2011) starch granules rapidly accumulate in the triticales endosperm after 6 days after anthesis, of which some grow rapidly (A-granules) whereas others do not (B-granules), making it difficult to observe a distinction in synthesis initiation of the last ones. Hurkman et al. (2003) observed that starch synthesis in wheat starts earlier at high growing temperatures ($>24^\circ\text{C}$) causing low starch accumulation in the mature grains and altering the size distribution of the A- and B-granules. However, it appears that the synthesis of the A-type starch granules in the complete triticales started earlier after anthesis, leading to an increase in the size of the A-granules more than those in the substituted triticales. Therefore, considering that the B-granules enlarge towards the end of the grain-filling period when high temperatures prevail, their synthesis might have been more affected in the substituted triticales than in the complete ones. High environmental temperatures from anthesis to maturity reduce the duration of starch accumulation, inducing significant changes in starch granule size distribution of grains in wheat (Chi, Seib, & Bernardin, 1994; Hurkman et al., 2003; Zhao et al., 2008), leading to a significant reduction in amylopectin and starch contents with no amylose content alteration (Lu et al.,

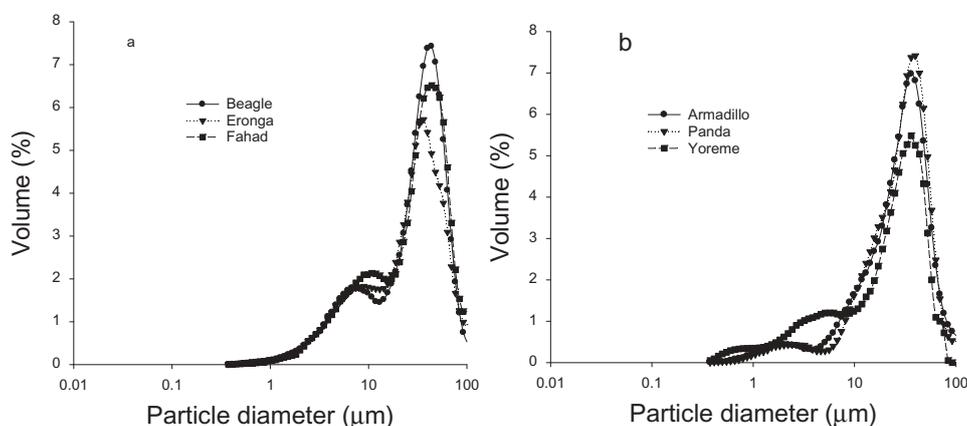


Figure 2. Granule size distribution of starches from (a) complete and (b) substituted triticales.

Figura 2. Distribución del tamaño de gránulos de almidón de triticales (a) completos y (b) sustituidos.

2014). Additionally, water stress also affects the starch granule size distribution and amylose content of wheat cultivars (Dai, Yin, & Wang, 2009; He et al., 2012) causing a decrease in the contents of starch and amylose in wheat grains. Generally there is an increase in the proportion of A- to B-granules in response to high temperatures (Savin & Nicolas, 1999). When wheat was held at 40°C for 3 days before anthesis, the early initiated A-granules were overly affected, being lower in number relative to B-granules and showing morphological deformation and fissuring (Savin & Nicolas, 1999). In our study, the A- to B-granules ratio in mature seeds of complete and substituted triticales was approximately four and three, respectively, indicating that the volume percentage of the A-granules was lower in substituted triticales. Taking into consideration previous reports and the results of the present study, it appears that the substituted triticales are more affected by growing temperatures, decreasing the size and volume per cent of the A-granules. Consequently, the complete triticales are more tolerant to higher temperatures for their development than the substituted triticales, which may be corroborated in appropriate future studies.

Another evidence that high temperatures affect the development of grains is the weight of mature kernels. Liu et al. (2011) observed that the weight of wheat grains exposed to temperatures above 25°C was lower than that of grains grown at normal temperature after anthesis (DAA). In our study, the 1000 kernel weight test showed that the complete triticales had significantly higher average grain weight (58.0 g) than that of the substituted triticales (38.0 g), which suggests that the last ones might be more affected in the yield level and quality by growing conditions, especially high temperatures.

Conclusions

The complete and substituted triticales showed differences in carbohydrate and starch contents. A concomitant relationship was observed between total carbohydrate content and starch content, suggesting that the activity of the enzymes involved in the starch synthesis in both sets of triticale genotypes is different. Based on the results of size and volume per cent of the starch granules, the complete triticales preferentially synthesize larger A- and B-type starch granules than the substituted triticales. These differences in starch properties between the two sets of triticale genotypes can be used to select those that fulfill the industrial and food needs.

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