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CHARACTERIZATION AND EVALUATION OF MODERN TRITICALE (X *Triticosecale* Wittmack) LINES FOR MALT PRODUCTION AND CRAFT BEER BREWING

CARACTERIZACIÓN Y EVALUACIÓN DE NUEVAS LINEAS DE TRITICALE (X *Triticosecale* Wittmack) PARA LA PRODUCCIÓN DE MALTA Y ELABORACIÓN DE CERVEZAS ARTESANALES

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Abstract

Triticale has proved to be immensely popular as a renewable source of energy both for animal feed as well as in the food industry. The aim of this study was to characterize and evaluate ten lines of triticale so as to identify and select the best one suited for malt production and brewing. The ten lines of triticale exhibited a protein (11.97-14.52%) and carbohydrate (77.24-82.87%) content good for malt production. The starch viscosity for these lines varied from 33.33 to 104.33 RVU. Four lines (PM-1, PM-3, PM-6 and PM-8) were selected further for malt production. These lines had a high malt extract content of 89.62 to 97.64% and diastatic power similar to some previously known barley malts (86.19-190.19 °L). The soluble protein content (4.56%-5.66%) was observed to be more than that of barley malts and the viscosity was high. Two triticale malts, PM-1 and PM-3, were selected for further fermentation with barley malt at varying concentrations (100, 80, 70, 50, 30, and 0%, respectively). Triticale malts (100%) have the potential to generate an optimum fermentation profile (~72%). From our study, it can be concluded that triticale malts have the potential to produce high-quality craft beer.

Keywords: triticale, barley, malt, wort, fermentation.

Resumen

Triticale ha demostrado ser inmensamente popular como fuente de energía renovable tanto para la alimentación animal como en la industria alimentaria. El objetivo de este estudio fue caracterizar y evaluar diez líneas de triticale para identificar y seleccionar la más adecuada para la producción de malta y cerveza. Las diez líneas de triticale mostraron un contenido de proteína (11.97-14.52%) y carbohidratos (77.24-82.87%) bueno para la producción de malta. La viscosidad del almidón para estas líneas varió de 33.33 a 104.33 RVU. Cuatro líneas (PM-1, PM-3, PM-6 y PM-8) se seleccionaron para la producción de malta. Estas líneas tuvieron un alto contenido de extracto malta de 89.62 a 97.64% y poder diastático similar a algunas maltas de cebada conocidas previamente (86.19-190.19 °L). Se observó que el contenido de proteína soluble (4.56% -5.66%) era mayor que el de las maltas de cebada y que la viscosidad fue alta. Dos maltas de triticale, PM-1 y PM-3, se seleccionaron para una fermentación adicional con malta de cebada a concentraciones variables (100, 80, 70, 50, 30 y 0%, respectivamente). Las maltas de Triticale (100%) tienen el potencial de generar un perfil de fermentación óptimo (~72%). Se puede concluir que las maltas de triticale tienen el potencial de producir cerveza artesanal de alta calidad.

Palabras clave: triticale, cebada, malta, mosto, fermentación.

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1 Introduction

Triticale is a hybrid cereal resulting from the combination of wheat and rye; This grain is mainly used as food for animals, however, in recent years it has gained some interest in the food industry for its nutritional properties, being able to be used in the preparation of baking products, yogurt, biocompatible films, pasta, brewing adjunt and malts (McGoverin *et al*., 2011; Arendt *et al*., 2013). Triticale has the potential to become a major food crop if it were to be used as a human food grain in addition to that for animal feed, particularly if this were so on a commercial scale (Gustafson *et al*., 1991; Peña, 1996; FAOSTAT, 2017).

Some studies have shown that unmalted triticale may also be suitable as a brewing adjunct (Glatthar *et al*., 2005). Most non-malt adjuncts contribute neither enzyme activity nor soluble nitrogen to the wort; however, this is not the case with triticale. The ability of triticale to produce high alpha-amylase activity could be advantageous in the production of triticale malt which in turn can be used as an additive in the food industry or in the brewing process. In the aforementioned case, triticale malt has been found to be acceptable with respect to amylolytic activity and wort yields (Lersrutaiyotin *et al*., 1991). One possible hurdle to the use of triticale could be its marginally higher proteolytic activity that results in higher levels of solubilized protein. This has the potential to interfere with the fermentation and storage (protein precipitation) process and could also impact the color (dark) of the beer. Despite this drawback, several studies have recently been carried out where different time and temperature conditions are evaluated during the triticale germination and malting process in order to improve the organoleptic and sensory characteristics of the beer made from this cereal (Biernacka and Wardencki, 2012). Although triticale exhibits variability in malting quality, breeding specifically for this trait may be difficult because there is no methodology that allows for rapid and simultaneous screening of both protein solubilization as well as carbohydrate modification (Homes, 1989; Lersrutaiyotin *et al*., 1991; Pomeranz *et al*., 1970).

On the other hand, commercial large-scale breweries prefer to use of barley malt and other starchy adjuncts as these raw materials are of the industry standards for high scale beer production. Craft beer, is highlighted by its novelty and innovation, every own brewer has a unique recipe and fermentation time, as craft brewers are known to interpret historic styles with unique twists that enable the development of new styles that have no precedent with new flavors and raw materials.

Table 1. The pedigree of Triticale lines used in this study.

Line	PEDIGREE
PM1	BAT*2/BCN//CAAL/3/ERIZO_7/BAGAL_2//FARAS_1
PM2	BW32−1/CENT.SARDEV/7/LIRON_2/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/BULL_10/MANATI_1/8/MERINO/ JLO//REH/3/HARE_267/4/ARDI_4/5/PTR/CSTO//BGLT/3/RHINO_4−1/4/ HARE_7265/YOGUI_3/6/BULL_10/MANATI_1
PM4	FD−693/2*FAHAD_4//POLLMER_4/3/POLLMER_2.1/4/FARAS/ CMH84.4414/6/RHINO_3/BULL_1−1/5/CMH77.1135/CMH77A.1165// 2*YOGUI_1/3/IBEX/4/JLO 97/CIVET
PM5	POPP1_2/TAHARA/4/DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9
PM6	POLLMER_2.2.1*2//FARAS/CMH84.4414/4/DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9
PM7	POLLMER_2.2.1*2//FARAS/CMH84.4414/5/BANT_4//HARE_7265/YOGUI_1/ 3/SUSI_2/4/MASSA/NIMIR_3/3/YOGUI_1/TARASCA 87_3//HARE_212
PM8	POLLMER_3.5.1//ERIZO_15/FAHAD_3/3/POPP1_1/4/POLLMER_2.2.1*2// FARAS/CMH84.4414
PM9	GAUR_2/HARE_3//JLO 97/CIVET/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/ KER_3/6/150.83//2*TESMO_1/MUSX 603/7/POPP1_1/8/BULL_10/ MANATI_1*2//FARAS/CMH84.4414
PM10	LIRON_2/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/BULL_10/ MANATI_1*2/7/TUKURU

Craft beer is generally made with traditional ingredients like malted barley, hop, water and yeast, but they use sometimes in non-traditional ingredients like fruits, chocolate, spices and other herbs, can also be added for distinctiveness (Brewers Association, 2015). Craft beer production is on the upswing around the world and consumers are known to enjoy the variety of taste and texture that this type of beer offers. Therefore, this is a potential market where triticale malt could be used. Zhu (2018) mentioned that the triticale genotype may also influence the malt properties, which remains to be studied. Furthermore, the resulting malts from the studies remain to be used for beverage production. Therefore, the present work was carried out to study the physicochemical properties of ten modern triticale lines and select those that are best suited for malt production and can potentially be used in craft brewing.

2 Materials and methods

2.1 Raw material

The ten triticale modern lines used (Table 1) in this study were grown in Polotitlan, Mexico. These lines were kindly provided by the International Maize and Wheat Improvement Center-CIMMyT. "Esmeralda", a type of barley used in this study, was grown in Apan, Mexico. For the Apparent Attenuation Limit (AAL) test a commercial barley base malt (Briess®) was used; this was produced in the U.S.A. from AMBA/BMBRI recommended 2-Row malting varieties.

2.2 Analytical methods for triticale grain

All samples were analyzed in triplicate. All tests relevant to the study were done as follows: Grain moisture and total protein contents were determined using the Barley 5-A and Barley 7-A methods of the American Society of Brewing Chemists (ASBC, 2004), while ash (method 923.09), grain fat (method 920.39), total fiber content (method 962.09) were determined with the Association of Official Analytical Chemists (AOAC, 2000) methods, respectively. Total carbohydrate content was determined by the weight difference of all other components (Blanco *et al*., 2000).

2.3 Environmental scanning electron microscopy (ESEM)

Five kernels of each sample of triticale and barley were placed into a rubber mold. The kernels were embedded in an acrylic disk made of a two-component acrylic resin: methylmethacrylate and 2-hydroxyethylmethacrylate (AcryFix, Struers, Denmark). The sample was allowed to rest for 12 h before removing it from the rubber mold. A longitudinal sectioning of the kernel structures was performed by mounting the samples on a Struers Rotopol-25 polishing tool and subsequently using various polishing pads to polish until the surface appeared shiny. To study the bran morphology, an ESEM model XL30 (Phillips, Research Laboratories, Eindhoven, The Netherlands) equipped with a 30 kV beam and a GSE detector was used. In accordance with the methodology proposed by Figueroa *et al*. (2011) micrograph images of the bran layers were taken at 20 kV to 350. The bran layers examined were those located in the curved dorsal region of the grains at their maximum height. The thicknesses of bran layers in the micrographs were measured at 5 different points using the software ImageJ version 1.33o.

2.4 Pasting properties

The pasting properties of the triticale starch were analyzed on triticale flour using a Rapid Visco-Analyzer (RVA) 3C (Newport Scientific PTY LTD, Sydney, Australia). Each starch suspension (8%, w/w, dsb; 28 g of total weight) was equilibrated at 50 °C for 1 min, heated at a rate of 6 °C/min to 95 °C, maintained at that temperature for 5 min, and then cooled to 50 °C at a rate of 6 °C/min. A constant rotating speed of the paddle (160 rpm) was used in this test (Jane *et al*., 1999). The analysis was conducted in duplicate for each sample.

2.5 Malting

Steeping: Samples (300 g, dry basis) of triticale grain that had previously been washed with neutral liquid soap were immersed for 2 h in a solution of $Ca(ClO)_2$ g/L (1:1). Subsequent to this the grain samples were placed for steeping in the germinating chamber for 18 h in a glass beaker containing 250 mL of normal water at 18 °C (Electrolux, Mod. ERWW084MSKBM, China). At the end of this period, the excess water was drained and it was observed that the samples had attained 45% grain moisture. Prior to the next step

in the procedure, the grains were allowed for a 4-h respiration period at room temperature. Germination: the samples were transferred to trays and placed in the germinating chamber at 18 °C and 100% RH in darkness for 3 days until the acrospires reached a size three-fourths the size of the grain (Figueroa, 1985). During this step, three manual agitations were performed to separate roots. Kilning: was carried out using an oven (Felisa, modelo FE-291). The samples were treated at 35 °C, 45 °C, 65 °C and 30 °C for 19h, 24h, 25h, and 14 h respectively. At the end of this stage, the malt reached moisture content of 3%-6% (López-Perea *et al*., 2008). The malting process was conducted in triplicate for each sample.

2.6 *Malt Processing Efficiency and Grinding*

The roots generated during germination were eliminated from the malt by shaving, and the clean samples were weighed. The samples were ground using method of 935.30 of AOAC (2000) as well as the method prescribed by Figueroa (1985).

2.7 *Malt Analysis*

ASBC (2004) methods were used for malt analysis as follows: Malt moisture (Malt-3), specific gravity and °Plato (Wort-2), extract content (Malt-4), wort viscosity (Wort-13), diastatic power (Malt-6) and soluble protein (Beer 11-A).

2.8 *Apparent Attenuation Limit (AAL)*

Two triticale malts were selected for the Apparent Attenuation Limit. The selected malts were mixed with barley malt in the following proportions (%): 100/0, 80/20, 70/30, 50/50, 30/70 and 0/100, respectively. The mixes were used for fermentation following the EBC method 8.6.2 (European Brewery Convention. Analytica-EBC, 2003). This analysis was performed in triplicate.

2.9 *Statistical analysis*

The statistical software SAS Institute (SAS User's Guide, version 8. SAS Institute Inc., Cary, N, 2006), was used to analyze the data. The analysis was done using one-way ANOVA and statistical significance ($\rho < 0.05$) was determined by comparing mean values using Tukey's test.

3 Results and discussion

3.1 *Characterization of the triticale grain samples*

It was observed that all the triticale lines used in this study had similar moisture content (Table 2). The line PM8 showed a marginally higher content (12.02%) while PM1 exhibited a slightly lower value (11.22%). Previous studies have reported that the moisture content for triticale is approximately 13% (Serna, 2001).

Table 2. The chemical composition of the modern triticale lines; the contents (%) of ash, fat, fiber, protein and carbohydrates are on dry matter basis.

Sample	Moisture	Ash	Fat	Fiber	Protein	Carbohydrates
PM1	11.47 ± 0.20^{bc}	1.86 ± 0.07^{b}	1.34 ± 0.25^{ab}	2.83 ± 0.07^{a}	13.25 ± 0.99^{a}	80.77 ± 0.67^{ab}
PM2	11.58 ± 0.09^{bc}	1.68 ± 0.04^{b}	2.47 ± 0.74^{a}	2.17 ± 0.25^{a}	12.53 ± 0.67^{a}	80.15 ± 0.47^{abc}
PM3	11.71 ± 0.07^{ab}	1.59 ± 0.05^{b}	2.10 ± 0.26^{ab}	1.49 ± 0.13^{a}	11.97 ± 1.30^{a}	82.87 ± 1.24^{a}
PM4	11.46 ± 0.16^{bc}	2.13 ± 0.54^{b}	2.60 ± 0.16^{a}	2.69 ± 0.86^{a}	14.52 ± 0.31^{a}	77.92 ± 1.04^{bc}
PM5	11.71 ± 0.12^{ab}	3.65 ± 0.22^{a}	2.28 ± 0.06^{ab}	3.06 ± 0.55^{a}	13.79 ± 0.32^{a}	77.24 ± 0.39^{c}
PM6	11.54 ± 0.17^{bc}	1.72 ± 0.12^{b}	2.39 ± 0.11^{ab}	1.93 ± 0.49^{a}	12.87 ± 0.30^{a}	81.03 ± 0.01^{a}
PM7	11.50 ± 0.19^{bc}	1.83 ± 0.16^{b}	2.20 ± 0.16^{ab}	2.65 ± 0.46^{a}	13.13 ± 0.97^{a}	80.16 ± 1.45^{abc}
PM8	12.02 ± 0.22^{a}	1.77 ± 0.16^{b}	1.08 ± 0.06^{b}	2.03 ± 0.07^{a}	12.81 ± 0.01^{a}	82.26 ± 0.31^{a}
PM9	11.22 ± 0.03^{c}	1.72 ± 0.01^{b}	1.74 ± 0.31^{ab}	2.63 ± 0.36^{a}	13.31 ± 1.00^{a}	80.61 ± 0.33^{ab}
PM10	11.37 ± 0.02^{bc}	1.75 ± 0.03^{b}	2.42 ± 0.25^{ab}	2.32 ± 0.14^{a}	12.50 ± 0.33^{a}	81.01 ± 0.25^{a}

*The results are the average of three determinations ± the standard deviation.

**Values within columns followed by different letters are significantly different ($p < 0.05$).

According to NOM-FF-043-SCFI-2003, the moisture content standard for malting grains is between 11.5 and 13.5%; the grain lines PM1, PM9 and PM10 had moisture contents lower than 11.5%. Low moisture levels are needed in order to inactivate the enzymes involved in seed germination and discourage the growth of disease-causing microorganisms (Fox *et al*., 2003). Thus, moisture content has the potential to affect grain quality as well as germinative capacity. The triticale lines were determined to have moisture content suitable for storage and/ or subsequent use in the malting processes. Only PM5 showed significant difference in ash content ($\rho < 0.05$). The rest of the samples showed non-significant differences for ash (1.68-2.133%) or fiber (1.49-3.06%) content ($\rho > 0.05$) the values being similar to those reported previously by Heger and Eggum (1991). A low-fat content in malting grains is desirable and for the triticale samples, this parameter ranged from 1.08 to 2.60% (Table 2). The fat values observed in this study were similar to those reported in previous studies on triticale (1.06-2.38%) (Rakha *et al*., 2011; Peña, 2004) and much lower as compared to the barley fat content of 1.5 to 6.0% (López *et al*., 2007; Dendy *et al*., 2004; Hoseney, 1991).

Protein content is one of the important parameters for selecting malting barley. Protein content is known to be affected by factors such as genotype, cultural practices, and growing environment (Fox *et al*., 2003). In the triticale lines used in this study, the protein content was in a range from 11.97-14.52% and non-significantly different were observed across lines ($\rho > 0.05$) (Table 2).

High protein content is known to result in lower extracts as it slows down water uptake during steeping which can potentially affect the final malt quality (Briggs *et al*., 2004). On the other hand, a very low protein level less than 8%, results in a deficiency of enzymes necessary to modify the barley kernel and to break down the starch during mashing. Low protein also impairs the brewing performance due to poor yeast amino acid nutrition. The desirable range of protein is between 9.0-11.0% for 2-rowed barley and 9.0-11.5% for six-rowed (Fox *et al*., 2003; Hoseney, 1991). Triticale has had changes in the protein content for several years from 18% to 12% and is still high compared with malt barley standars, maximum limit 11.5%. The triticale lines with high protein content were not selected for produce malt, because those could produce turbidity in the wort and beer. It has also been reported that during germination the protein content can increase (Servín de la Mora-López *et al*., 2018).Triticale lines showed high carbohydrates content (range 77.24-82.87%) regarding to the reported by the United States Department of Agriculture Agricultural Research Service (USDA, 2017), which ranges in value from 72.13 to 77.31%.

Table 3. The pasting properties of modern triticale lines.

Sample	Pasting temperature (°C)	Viscosity (RVU)[a]	
		Peak	Breakdown
PM1	65.25±0.02^{a}	104.33±5.51^{a}	7.00±3.00^{a}
PM2	63.40±0.08^{a}	79.00±10.00^{c}	5.67±1.53^{a}
PM3	64.70±0.28^{a}	101.50±2.12^{ab}	8.00±8.49^{a}
PM4	62.65±0.04^{a}	72.67±6.51^{c}	4.67±2.52^{a}
PM5	63.77±0.03^{a}	52.67±4.93^{d}	5.00±1.00^{a}
PM6	62.65±0.01^{a}	47.00±0.20^{de}	1.50±0.71^{a}
PM7	64.03±0.25^{a}	82.50±0.71^{bc}	3.00±1.41^{a}
PM8	62.83±0.25^{a}	49.00±0.09^{de}	2.00±1.41^{a}
PM9	61.67±0.20^{a}	40.33±9.02^{de}	7.67±5.86^{a}
PM10	62.12±0.38^{a}	33.33±1.53^{e}	5.67±3.06^{a}

[a] = Measured in Rapid Visco-Analyzer units

*The results are the average of three determinations ± the standard deviation.

**Values within columns followed by different letters are significantly different ($p < 0.05$).

3.2 Pasting properties of triticale grain

High carbohydrate content in triticale lines used in brewing was favorable because starch is converted to simple sugars by amylolytic enzymes (Heger *et al*., 1991; Mathlouthi *et al*., 2002) having effect in physichochemical and sensory characteristics. The pasting properties of various triticale starches have been summarized in Table 3. The triticale lines were observed to vary significantly with respect to pasting properties. All triticale starches exhibited a gradual increase in viscosity with an increase in temperature ramp. This temperature dependent increase in viscosity can be attributed to the removal of water from the exuded amylose by the granules as they swell (Ghiasi *et al*., 1982). Peak viscosity for triticale starches ranged between 33.33-104.33 RVU to PM10 and PM1 respectively. Zihua and Jane (2007), reported peak viscosity in the range of 105 and 123 RVU for selected triticale cultivars.

Breakdown viscosity (a measure of when the cooked starch disintegrates) was found to be the lowest for PM6 and the highest for PM3 starch. Pasting temperature (temperature at the onset of a rise in viscosity) for various triticale starches ranged between 61.67 and 65.25 °C (Table 3); the lowest pasting temperature was seen for PM9 and the highest for PM1 starch. The high pasting temperature of PM1 indicated its higher resistance toward swelling. Zihua and Jane (2007), reported that triticale starch has pasting temperatures in the range of 76-85 °C; for barley starch, the same has been reported to be between 86 and 94 °C (Zihua and Jane, 2007; Pycia *et al*., 2015). Therefore, it can be concluded that, as compared to barley starch, triticale starch is likely to have an improved response to swelling which in turn would facilitate the collapse of the starch granule at the mashing temperatures which usually range between 43 and 75 °C (Wolfgang, 1999), and the pasting properties could depend of the cultivar conditions.

Pasting properties of starch are affected by factors such as the size of starch granule, amylose and lipid content, and amylopectin structures. According to Cornejo *et al*. (2015), triticale presented more A-type starch around 80% than B-type 20%. The A-type starch granules triticales displayed disk shapes and bigger size (18-40 μm), whereas the B-type starch granules displayed a more spherical-type shape and smaller size (2-11 μm) (Wilson *et al*., 2006; Ao and Jane, 2007; Navarro-Contreras *et al*., 2014). Amylopectin is primarily responsible for granule swelling whereas amylose and lipid restrict the same (Tester and Morrison, 1990). B-granule starch has more lipids than the A-granule starch and, as lipids form helical complexes with amylose, this restricts granule swelling. Therefore, the predominance of A-starch in triticale facilitates water absorption and grain swelling, which is advantageous for the mashing process.

3.3 Malt quality

Based upon the higher protein and carbohydrate content as well as pasting properties of the grain, four lines of triticale (PM1, PM3, PM6, and PM8) were selected for malting. The formulation of a quality beer is dependent upon the generation of superior wort which in turn is a direct consequence of the quality of the malt (Glatthar *et al*., 2002). The analytical parameters for the malted triticale samples are shown in Tables 4 and 5, respectively. Specific gravity (sg) is a reflection of the soluble sugar content in the wort which is crucial for the growth of yeast. In triticale malt, this parameter was observed to be approximately 1.04 g/mL (Table 4), which is higher than that seen for barley malts (1.02 g/mL).

Table 4. Functional properties of triticale malts analyzed in relevance to brewing practice.

Triticale Sample	Specific Gravity (g/mL)	ºPlato	Extract content (%) db	Viscosity (cP)
PM-1 (n=3)	1.04 ± 0.00^{a}	10.33 ± 0.14^{a}	97.64 ± 1.24^{a}	1.89 ± 0.02^{a}
PM-3 (n=3)	1.03 ± 0.00^{bc}	9.84 ± 0.14^{bc}	91.52 ± 1.91^{b}	1.75 ± 0.07^{ab}
PM-6 (n=3)	1.04 ± 0.00^{b}	9.98 ± 0.02^{b}	92.99 ± 0.50^{b}	1.83 ± 0.03^{a}
PM-8 (n=3)	1.03 ± 0.00^{c}	9.59 ± 0.14^{c}	89.62 ± 1.29^{b}	1.60 ± 0.09^{b}

*The results are the average of three determinations ± the standard deviation.

** Values within columns followed by different letters are significantly different ($p < 0.05$).

db = Dry basis.

Specific gravity (sg) is a reflection of the soluble sugar content in the wort which is crucial for the growth of yeast. In triticale malt, this parameter was observed to be approximately 1.04 g/mL (Table 4), which is higher than that seen for barley malts (1.02 g/mL). For wheat malt, this value ranges from 1.03-1.05 g/mL (Holmes, 1989). From this, it can be concluded that the behavior of the triticale malts is similar to that of their ancestor. The various lines of triticale malts exhibited significant differences in specific gravity and °Plato, with PM-1 exhibiting the highest value and PM-8 the lowest value (Table 4). The extract content is a factor of major importance in the brewing industry. This is because the fermentable sugars present in the extract are soluble in the wort and are the most complex malting quality trait in terms of biochemistry and genetics (Fox *et al*., 2003). This attribute was measured in the triticale extract at five different time points and was invariably observed to exhibit high levels (Table 4). Triticale malts had 89.62% to 97.64% of extract content with PM-1 malt exhibiting a significantly different higher value ($\rho <$ 0.05). The values obtained are greater than those obtained by Kunze (2006), who reported an extract content of barley malts between 75% and 82%, these values could give the guideline to obtain a higher concentration of fermentable sugars. While this may result in higher alcohol content after the fermentation process, the actual value will be dependent upon the type of fermentable sugars present in the wort as well as the concentration of the same (Fox *et al*., 2003; Kunze, 2006).

The results obtained of malt extract of triticale in this work are in agreement with the reported by Lersrutaiyotin *et al*. (1991). Trombos and Briggs (1984) hypothesized that the reduced aleurone layer in combination with the increased starchy endosperm may be one of the reasons why triticale has higher malt extract as compared to barley. Figure 1 shows the different layers of the triticale and barley kernel; the aleurone layer averaged 19.72 μm for triticale and 24.10 μm for barley which supports the hypothesis that this layer is thicker in barley as compared to triticale. In triticale the average thickness of the pericarp layer was 9.88 μm while for the testa it was observed to be 10.41 μm; for barley, the same was seen to be 6.05 μm and 13.48 μm, respectively. Lautering of the wort or mash filtration, is a solid - liquid separation and serves to separate the compounds of malt dissolved during mashing from the insoluble parts. This is a critical step in the production of beer because the lautering speed depends of the extract viscosity.

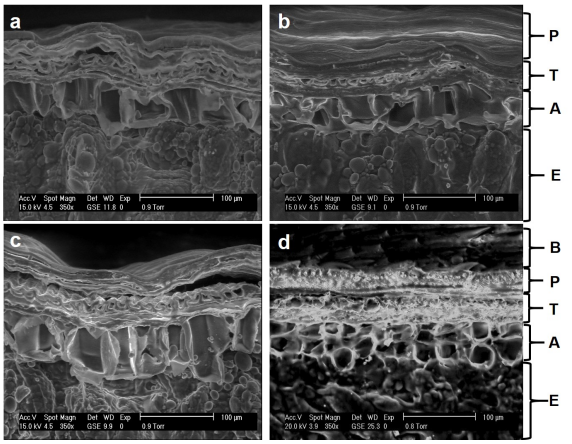

Fig. 1. ESEM images of the different kernel layers: Bran (B), Pericarp (P), Testa (T), Aleurona (A) and Endosperm (E). Triticale kernel PM2 (a), PM8 (b), PM6 (c) and malting barley (d).

The viscosity of the extract is affected by the hydrolysis of β-glucans and arabinoxylans during the mashing of the malt. In case of the triticale malts, a high viscosity in the wort (1.89-1.60 cP) (Table 4) was observed and the values varied significantly depending upon the line analyzed ($\rho <$ 0.05). It is likely that the rising viscosity of the triticale wort was affected by the high molecular weight of the arabinoxylans (Glatthar *et al*., 2002; Cleemput *et al*., 1993) which is indicative of poor activity by the cytolytic group of the enzymes (Schuster *et al*., 1999). Previous studies on unmalted triticale have reported high viscosity levels of 2.14-2.38 g/mL (Glatthar *et al*., 2003), while using enzymes on unmalted triticale, the values were 1.50-1.75 g/mL (Glatthar *et al*., 2002) and in malted triticale, the values were 1.89-2.12 g/mL (Blanchflower and Briggs, 1991; Grujic *et al*., 2007). In barley malts, it is known that for a good lautering that is also economical with respect to time, the viscosity should be less than 1.50 g/mL (Kumar *et al*., 2013). A study by Grujic *et al*. (2007) reported a lautering time of 8 min in barley wort and 14-24 min for triticale wort.

Table 5 shows that the malt moisture contents (4.12%-5.05%) of the triticale lines were non-significantly different ($\rho >$ 0.05). In contrast, significant differences were found between the lines in the soluble protein contents (4.56%-5.66%) (Table 5). Triticale malts with the highest soluble proteins were traced to lines PM-1 and PM-3. In barley malts, the soluble protein fraction was found to be between 3.90% and 4.70% (Hans, 2009).

Table 5. Moisture, soluble protein, and enzyme activity of the different triticale malts.

Sample	Malt moisture (%)	Soluble protein (%) db	Diastatic power ºL
PM-1 (n=3)	5.05 ± 0.73^{a}	5.66 ± 0.16^{a}	190.19 ± 13.69^{a}
PM-3 (n=3)	4.12 ± 0.51^{a}	5.60 ± 0.07^{a}	168.99 ± 16.56^{a}
PM-6 (n=3)	4.17 ± 0.23^{a}	4.56 ± 0.02^{b}	86.19 ± 16.49^{b}
PM-8 (n=3)	$4.71\pm0.26a^{a}$	4.91 ± 0.01^{b}	145.97 ± 14.12^{ab}

*The results are the average of three determinations ± the standard deviation.

** Values within columns followed by different letters are significantly different ($p < 0.05$).

db = dry basis, °L = Grados Lithner

Triticale malts showed a slightly higher content of soluble protein in their wort compared with reports of barley wort. During the germination process, some of the proteins are converted into their soluble forms. In the mash, high molecular weight proteins that escaped hydrolyzation during the malting process, are modified by proteolytic enzymes to form simpler compounds such as polypeptides and amino acids (Hoseney, 1991). In this study, the soluble proteins in wort derived from triticale malt were determined to be comparable to that derived from barley malts, which leads to the conclusion that the fermentation process should yield similar results. An important attribute influencing fermentation capacity and beer characteristics is the amino acid composition of the sweet wort; this is because free amino acids represent the major source of nitrogen for the brewing yeasts (Pomeranz *et al*., 1970; O'Connor-Cox and Ingledew, 1989; Pierce, 1982).

The diastatic power of malt represents the collective activity of several starch degrading enzymes that accumulate or are activated during the process of malting. Amylolytic enzymes such as α-amylase, β-amylase, limit dextrinase and β-glucosidase are known to be active during the process of malting and mashing (Fox *et al*., 2003). Malts have a high diastatic power of 125-170 °L as a result of which if there is a deficiency of diastatic enzyme activity there will be no optimum conversion of starch to reducing sugars (Holmes, 1989). The triticale malts exhibit a good diastatic power. PM-1 exhibited the highest value (190.19 °L) but was non-significantly different ($\rho > 0.05$) from PM-3 and PM-8 (Table 5); PM-6, which had a value lower than the other lines (86.19 °L), was significantly different to PM-1 and PM-3. The analysis of triticale malts showed that they have an excellent profile with respect to amylolytic enzymes. Tombros and Briggs (1984) have reported that triticale malts have diastatic power in the range of 199-198 °L.

In accordance with the results shown above, triticale cultivars PM-1 and PM-3 were selected for further downstream fermentation. These lines were selected as they presented with high diastatic power (the triticale malts have enough amylolytic enzymes to produce fermentable sugar) in combination with a good extract content and optimum soluble protein which would ensure that the yeast would have all nutrients essential to the fermentation process.

3.4 Fermentation

Different concentrations of triticale malt were selected to replace barley malt (Table 6) in the fermentation process. The objective of this exercise was to analyze the feasibility of producing beer with an all-triticale malt at an efficiency and quality comparable to that of the conventional barley malts. The fermentation process was measured in terms of percentage of Limit Attenuation of Fermentable Carbohydrates (AAL), which is the ratio of effectively metabolized fermentable carbohydrates in relation to the total fermentable carbohydrate content of the sweet wort. The value of AAL ranged from 72.23% to 83.79% and there are significantly different for different mixes ($\rho < 0.05$). There was non-significantly different ($\rho > 0.05$) between 100% triticale malt (both lines) and all-barley malt as both were seen to produce good quality worts for yeast fermentation. From this, it can be concluded that a good concentration of fermentable sugars can be obtained by using the triticale malts alone without the addition of any barley malt.

The analysis showed that PM-3, at a 30% concentration, presented with the most optimum results and that further increase of triticale malt concentration (50% and 70%) had no significant impact on the result. In case of PM-1, no significant difference could be found between different mixes regardless of the percentages.

Table 6. Means values of Apparent Attenuation Limit in different levels of triticale and barley malt.

Sample	Triticale malt (%)	Barley malt (%)	AAL (%)
PM-1	100	0	72.23 ± 0.15^{b}
	80	20	76.61 ± 9.37^{ab}
	70	30	78.00 ± 2.12^{ab}
	50	50	79.90 ± 1.77^{ab}
	30	70	81.28 ± 2.17^{ab}
PM-3	100	0	72.63 ± 1.14^{b}
	80	20	73.84 ± 1.02^{b}
	70	30	78.95 ± 1.08^{ab}
	50	50	79.61 ± 0.49^{ab}
	30	70	83.79 ± 1.74^{a}
Barley malt	0	100	77.07 ± 0.15^{ab}

*The results are the average of three determinations ± the standard deviation.

** Values within columns followed by different letters are significantly different ($p < 0.05$).

AAL = Apparent Attenuation Limit

A positive interaction between triticale and barley malt could be observed as it was noticed that all-barley malt had less fermentation as compared to the mixes that had triticale malt in combination. This behavior can be attributed to the fact that although barley malts have more fermentable sugars for yeast metabolism during fermentation as compared to triticale malts, the amylolytic activity of the triticale malts helps hydrolyze the starch of the barley malt thus increasing the concentration of fermentable sugars in the wort.

Similar results were found by Glatthar *et al.* (2003) who reported that triticale acted like an adjunct, and the AAL was higher when 70% of triticale unmalted was used as compared to the all-barley malt. However, it should be noted that the authors of the abovementioned study used alternate mashing regimens and they also suggested that triticale substantially contributes to the assimilable nitrogen content of wort during fermentation which in turn aids yeast propagation and improves fermentation capacity. A study of a similar nature, wherein different combinations of triticale with barley malts were used, reported the behaviors contradictory to those described above (Grujic *et al*., 2007).

Conclusions

Modern triticale lines have moisture, ash, fat, fiber, protein and carbohydrate content comparable to triticale cultivars reported almost two decades ago. This leads to the conclusion that the chemical composition of this cereal grain is highly stable. Triticale lines have a protein and carbohydrate content that is suitable for malt production as well as the production of good quality wort. The results show that the starch pasting properties can exhibit behavior that is different between different triticale lines as some lines were seen to have a high viscosity while others had very low values for the same (33.33 RVU). Fortunately, these properties do not interfere with the mashing process and do not negatively impact the production of high quality and quantity of malt extract. Triticale malts derived from modern cultivars have been proved to be of enough high quality to be used in a brewery. From the standpoint of large industrial breweries, high viscosity values observed in the wort could represent a lautering problem, and the final beer could have slight turbidity. Thus, this study proposes the use of triticale malt for the production of craft beer where the turbidity and alternate taste associated with triticale beer could find good acceptance. Beer can be produced using either 100% triticale malt or by using a combination of triticale malt (30%) with barley malt. Both processes produce wort with a higher concentration of fermentable sugars. Our results show that the modern triticale cultivars have been modified insofar as the protein content of the kernel is concerned, but the high viscosity in the wort is still a problem.

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