

RESEARCH ARTICLE

Influence of temperature and germination time on diastatic power of blue and red maize (*Zea mays* L.) malts

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ABSTRACT

Maize endosperm consists of about 70 % starch, which makes it an excellent substrate for fermentation. However, due to its low diastatic power, it is used in brewing as an adjunct, mainly. In order to include both red and blue maize as an enzyme source in the brewing process, the effect of temperature and time germination on the diastatic power of malts was studied. The research consisted in a completely randomized three-factor experimental design where the involved factors were colour of maize (blue and red), germination temperature (15, 20, and 25 °C), and germination time (3, 4, 5, 6, 7, 8, and 9 days). The response variables were germination percentage, acrospire length, malting yield, and diastatic power. Data was analysed through Analysis of variance and Comparison Multiple Tukey's Test. Results showed that both temperature and germination time encouraged the acrospire length, which had a negative effect on malting yield. Regarding to diastatic power, it maintained an increase from third to seventh germination day, at the three tested temperatures. Additionally, as the germination temperature increased, the diastatic power also increased. The highest diastatic power for blue and red maize malts were 39 and 42 °L, respectively, and it was reached when these malts were germinated for 7 days at 25 °C. It was concluded that, by germinating both blue and red maize under the resulting optimum conditions, the obtained malts would be capable of converting their own starch.

Keywords: Blue and red maize; Diastatic power; Germination temperature; Germination time; Malting yield

INTRODUCTION

Malting is a controlled germination of grains in moist air, and the process can be divided into three stages: steeping, with which the acquiescent grain imbibes water and hydrates the embryo and endosperm; germination, by which enzymes are synthesized, activated, and mobilized, and the embryo begins to develop; finally, kilning, wherewith grain growth is halted using a heat treatment, which dries the grain to constant and low moisture for storage. The aims of germination, among others, are to convert or modify the physical structure of the grain and allow synthesis or activation of a series of enzymes (MacLeod & Evans, 2016). Diastatic power is the combined activities of α -amylase, β -amylase, limit dextrinase, and α -glucosidase, which solubilize starch to fermentable sugar during malting (Ribeiro Jr et al., 2016). In other words,

diastatic power is a measure of how effective the malt is at converting starch to sugar (Farber & Barth, 2019), and is an important quality trait for malt used in brewing and distilling (Looseley et al., 2017).

There are different methods to measure diastatic power. Among them, the rapid Henry's method (Henry, 1984), which is characterized by both using a little quantity of sample and being a quick method compared to either American Society of Brewing Chemists (ASBC) or the Institute of Brewing (IoB) method.

Barley is the most preferred grain for brewing all over the world, among other things, due to its high diastatic power. However, if it is considered that the endosperm of maize consists of approximately 70 % starch embedded in a protein matrix, this cereal is an excellent substrate for

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fermentation (Chaudhary et al., 2014). For this reason, maize has been used since time immemorial as part of the carbohydrate material in beer brewing, but mainly as an adjunct prepared in different forms such as flakes, grits, and flour (Iwouno & Ojukwu, 2012), due to its little diastatic power (Meußdoerffer & Zarnkow, 2009).

In México there are a broad diversity of maize, and at least 59 races have been described (Vielle-Calzada & Padilla, 2009), among them the 'Chalqueño' race, which has variations in grains colour such as white, yellow, blue, and red (Herrera-Cabrera et al., 2004), and predominates in the High Valleys of the Mexican Central Plateau for irrigation crops (Arellano-Vázquez et al., 2014).

For many years, malting of maize for using as a major source of hydrolytic enzymes required for brewing purposes has received little attention, but during the last years many researchers have focused their efforts on optimizing the maize malting process (Chaudhary et al., 2014). Their results have shown the influence of malting conditions such as steeping time, germination time or kilning temperature on the diastatic power. However, these works have been focused on white and yellow varieties, mostly (Eneje et al., 2004; Iwouno & Ojukwu, 2012; Awoyinka & Adebawo, 2008). As for both blue and red maize malts, several research projects about brewing with these malts have been carried out in the Universidad Autónoma Metropolitana, in México (Romero-Medina et al., 2020; Flores-Calderón et al., 2017). Also, a patent about the coloured-maize brewing process has been developed (Verde-Calvo et al., 2019); however, the diastatic power of the malts has not been reported. In addition to this, the coloured-maize beers reported in these projects had low alcohol content, about 2 % and 3 % alcohol by volume, which could be related to the poor diastatic power of the malts.

Considering all the above, and in order to include coloured maize as an enzyme source in the brewing process, a study of maize germination conditions was performed. The aims of this research were to study the influence of temperature and germination time on the diastatic power of maize malts, as well as to find the conditions under which the malts have the highest diastatic power.

MATERIALS AND METHODS

Sample purchase and preparation

The maize used in this experiment were both blue and red colour of the Mexican race 'Chalqueño', purchased from a local producer in Milpa Alta, Mexico City. The maize of each colour was separately selected to eliminate

those kernels that looked like having either mechanical or microbiological damage.

Proximate composition

The methods used to perform the proximate composition of red and blue maize were those stipulated by the Mexican norms named in Table 1.

Weight of 1000 kernels

One thousand kernels of each colour of maize were randomly selected, taken in by hand, and weighted on an analytic balance (Explorer Ohaus, Switzerland) as reported by Deivasigamani and Swaminathan (2018). This process was made in triplicate for each colour of maize.

Germination

For the germination experiment, a completely randomized three-factor experimental design was used. The involved factors were colour of maize (red and blue), germination temperature, and germination time. The experiment was performed as follows: all maize of the same colour was steeped together and at once in fresh water (at a rate of 1:2, maize: water). The steeping lasted for 42 hours (Iwouno & Ojukwu, 2012), at room temperature (18-25 °C). During the steeping time, the water was drained every 12 hours and the maize had dry periods of 3 hours to allow oxygenation. After this, the soaked maize was distributed among experimental units (EU), which each one consisted of 150 g of soaked maize placed inside a clear hinged-lid plastic container whose both bottom and roof were previously prepared with a layer of wet paper. Along the germination time, the maize was aspersed with fresh water every 12 hours to keep the moisture of the kernels; also, it was aerated every 8 hours to remove the heat formed by grains metabolism. The germination was carried out at three different temperatures: 15, 20, and 25 °C for 3, 4, 5, 6, 7, 8, and 9 days. Every treatment was made in triplicate, thus having a total of 126 EU. All of EU corresponding to the same temperature were put inside an environmental chamber (Scorpion Scientific, Mod. 50620-IL), at 80 % of humidity and in light absence, where remained until their germination time was fulfilled. Once it occurred, the percentage of sprouted kernels and the length of acrospires

Table 1: Methods used to perform the proximate composition of maize

PARAMETER	METHOD
Moisture (%)	NOM-116-SSA1-1994
Crude fibre (%)	NMX-F-613-NORMEX-2017
Crude protein (%)	NMX-F-608-NORMEX-2011
Crude fat (%)	NOM-086-SSA1-1994
Ash (%)	NMX-F-607-NORMEX-2013
Total carbohydrate (%)	Calculated by difference (Ramdath et al., 2020)
Reducing sugar (%)	NOM-086-SSA1-1994

were determined. After that, radicles and acrospires were removed from the kernels before being kilned, so as these did not interfere on the drying process, and then all kernels of the correspondent EU were put inside a drying oven at 55°C for 24 hours to stop their germination process (Iwouno & Ojukwu, 2012). Finally, the malt yield of each EU was calculated.

Determination of the sprouted kernels percentage

Once the germination time was fulfilled, the correspondents EU were removed from the germination chamber and the sprouted and non-sprouted grains in each EU was counted to calculate the germination percentage, as reported by Saba *et al.* (2014), by using the next formula:

$$GP = \frac{S}{T} \times 100$$

Where:

GP = percentage of sprouted grains

S = number of sprouted grains

T = total number of grains

Determination of acrospire length

The determination of acrospire length was performed as reported by Dahiya *et al.* (2018). In each EU, acrospire was measured by taking the average of sprout length of twelve grains which were randomly selected, from base to tip, using a cotton rope because of the irregular shape of the acrospire.

Malting yield

Once dried, the malted grains (all grains, the sprouted and non-sprouted) were weighted on an analytic balance. To calculate the malting yield, a five-point linear calibration curve was built in order to know how many grams of raw maize were equal to the 150 grams of soaked maize used to perform the germination experiment. This curve was made using 90, 100, 110, 120, and 130 g of raw maize, which were separately soaked, and in the same conditions as the maize used for the germination experiment. The malting yield calculation was performed by using the next formula as described by Odo *et al.* (2016):

$$\%My = \frac{Mw}{mw} \times 100$$

Where:

%My = percentage of malting yield

Mw = dried malt weight (in grams)

mw = raw maize weight (in grams)

Correlation between the acrospire length and malting loss

A linear calibration was built to calculate the rate of malting loss, as the dependent variable (y), due to the acrospire

growth, as the independent variable (x). The curve was built by using the two mentioned response variables of each EU, and the correlation was calculated by using the Pearson's correlation.

Diastatic power

Henry's method

The Henry's method (Henry, 1984) with some modifications was used for measuring diastatic power of the experimental maize malts.

Reagents

Buffered starch solution, sodium hydroxide solution, p-hydroxybenzoic acid hydrazide solution (PAHBAH), and ammonium hydroxide solution were prepared as described for Henry (Henry, 1984). All reagents used were analytical grade.

Enzyme extraction

10 g of maize malt was ground in a manual fluted-disc-grain-grinder in such way that it passed through a 0.84 sieve. 0.0250 g of ground malt was directly weighted into a plastic centrifuge tube. 5 ml of 6 mM ammonium hydroxide was added and manually mixed by gentle swirling. This mixture was incubated in a water bath at 25 °C for 10 min and then centrifugated at 2500 g (Solbat, Mexico) for 3 min.

Diastasis

0.2 ml of enzyme extract was added to a 30 ml Pirex glass test tube containing 20 ml of a 2% w/v soluble starch solution buffered to pH=4.6. It was let stand for 10 min at 20 °C for starch digestion. After this time, the digestion was stopped by adding 1.2 ml of 0.5 M sodium hydroxide.

Blank correction

To prepare the blank correction solution, 1.2 ml of 0.5 M sodium hydroxide was added to 0.2 ml of enzyme extract before the addition of the starch solution. The blank solution was treated in the same way as the starch solution undergoing diastasis.

Determination of reducing sugars

0.2 ml of the digested starch solution was added to a 20 ml Pirex glass test tube containing 5 ml of 0.5% w/v PAHBAH, it was mixed and immersed in a boiling water bath for exactly 4 min. After this time, the tube was removed from the boiling water bath and placed in a 20 °C water bath for 10 min. Then, 10 ml of distilled water was added to the tube and mixed by vortexing. The blank solution was treated in the same way. Finally, absorbance was read at 415 nm (Thermo Spectronic, Mod. BioMate3, USA), and reducing sugars were calculated by using a dextrose calibration curve. Diastatic power by Henry's method was reported as mg of dextrose/l.

Linear calibration for diastatic power determination

Due to diastatic power of commercial malts is mainly reported either in Lintner (°L) or Windisch-Kolbach (°WK) degrees as well as the minimum diastatic power for malt to convert its own starch is reported in °L, when the Henry's method is used to measure diastatic power it is advisable to convert the Henry's units into °L or °WK to know, according to the literature, if the malts are suitable for brewing. For this reason, analogously to the recommendation of the ASBC (ASBC, 2004), a linear calibration of the Henry's method was made to convert the Henry's method units into Lintner degrees, instead of ASBC degrees. To make this, the diastatic power of seven different malts, from maize, wheat, rye, and barley were determined, in duplicate, through the Henry's method (as described for the maize malts) as well as the IoB method (Anon, 1982) with some modifications.

Institute of brewing method

Reagents

Buffered starch solution, sodium hydroxide solution, Fehling's solutions, and ammonium hydroxide solution were prepared as described for the IoB (Anon, 1982). All reagents used were analytical grade.

Enzyme extraction

11 g of maize malt was ground in a manual fluted-disc-grain-grinder and 10 g of this ground malt was directly weighted into a 500 ml glass beaker. 200 ml of 6 mM ammonium hydroxide was added and manually mixed by gentle swirling. Then, the mixture was incubated for 2.5 h in a water bath at 20 °C with stirring every half hour. After this time, a portion of this extract was taken and allowed the grains to settle for 30 min.

Starch digestion

The required volume of the enzyme extraction liquid (from 1 to 20 ml, depending on the malt) was added to a 200-ml flask containing 100 ml of 2% buffered soluble starch solution, so that the quantity of digested starch solution needed to reduce 5 ml of Fehling's solution was between 15 and 30 ml. The flask was shaken, and it was let to stand 1 h at 20 °C for starch digestion. After this time, the digestion was stopped by adding 6 ml of 0.5 N sodium hydroxide and made up to 200 ml with distilled water at the same temperature.

Titration of Fehling's solution

5 ml of mixed Fehling's solution was pipetted into a 150-ml Erlenmeyer flask and added a volume almost sufficient of the digested starch solution to reduce the Fehling's solution. The content of the flask was mixed by swirling and heat until moderate ebullition over a ceramic hot plate, where was kept for 2 min. Then, 3 drops of methylene blue

were added and the titration was completed in one minute maintaining ebullition.

Blank correction

The undigested 2% starch solution was titrated against 2 ml of mixed Fehling's solution as described in the "Titration of Fehling's solution" section.

Diastatic power calculation

Diastatic power of the malts was calculated by using the next formula:

$$D.P. = \frac{2000}{xy} - \frac{200}{xs}$$

Where:

D. P.= Diastatic power, expressed in IoB degrees (°IoB)

x= number of ml of malt extract taken for the digestion

y= number of ml of the digested starch solution required for reduction of 5 ml of Fehling's solution

s= titre of starch blank

The °IoB obtained were converted into °L by using the formula reported by the European Brewery Convention (Home & Sharpe, 2007):

$$^{\circ}\text{L} = (^{\circ}\text{IoB}) \times 1.1$$

Results were expressed as °L to the nearest whole number.

Then, a linear calibration was performed by calculating the regression line equation for the data using the units obtained from the Henry's method (reducing sugars produced, reported as g of dextrose/l) as the independent variable (x), and diastatic power (°L) as the dependent variable (y). The equation was used to determine the diastatic power of the maize malts in °Lintner (ASBC, 2004).

Determination of diastatic power of the experimental maize malts

As previously mentioned, the diastatic powers of the experimental malts were determined through the Henry's method. Finally, the obtained concentration units from this method were converted into °L by using the calibration curve equation.

Data analysis

The data obtained from the thousand-kernel weight was analysed through one-way ANOVA, where colour of maize was the only factor. The data obtained from the germination experiment (germination percentage, acrospire length, malting yield, and diastatic power) was analysed through both three-way ANOVA and Tukey's test by using the statistical software NCSS 2020 Version 20.0.3 (NCSS 2020 Statistical Software, 2020).

RESULTS AND DISCUSSION

Proximate composition

As it is shown in Table 2, the proximate composition of both red and blue maize are similar and their values fall within those reported for other Mexican varieties of maize. For example, Peña-Betancourt et al. (2017) and Sánchez-Madrugal et al. (2014) reported values of 7.09 % - 10.16 % of protein, 4.44 % - 5.81 % of fat, and 1.28 % - 1.39 % of ash for blue varieties. In the same way, Sánchez-Madrugal et al. (2014) reported 9.60 % of moisture, 75.23 % of carbohydrate, and 2.26 % of crude fibre. With respect to red varieties, it was not found proximate composition reported for Mexican races, but Adeniyi and Ariwoola (2019) reported values of 1.29 % and 2.24 % of fat, 0.51 % and 1.0 % of ash, 12.82 % and 9.32 % of protein, 0.86 % and 1.55 % of crude fibre, as well as 71.48 % and 74.40 % carbohydrate for two red Nigerian maize varieties.

Weight of 1000 kernels

Results showed that the thousand-kernels weight of the blue and red maize were 548.6 g and 520.9 g, respectively. So, in general, the blue ones were about 5.4% heavier than the red ones (p -value = 0.001), which indicates a larger size of the first ones. With respect to this Sulewska et al. (2014) reported the weight of three different fractions (small, medium, and large) of the 'Boruta' maize variety, and the thousand kernel weight of the large fraction was 410 g. Agama-Acevedo et al. (2011) reported the thousand kernel weight of different samples of blue-grain 'Chalqueño' race maize whose values were between 367.6 and 509.8 g. These values indicate that this race of maize has larger grains in comparison to other varieties, as reported by Rocandio-Rodríguez et al. (2014), who stated that this race is characterized by having large and wide grains.

Data analysis of the germination experiment

Table 3 shows the ANOVA results of each response variables for the three factors involved in the germination experiment as well as the interactions. As it can be seen, only the interaction of maize colour (Maize) with germination days was no significant for both germination percentage and acrospire length.

Table 2: Proximate composition of red and blue 'Chalqueño' race maize

Parameter	Red Maize	Blue Maize
Moisture (%)	11.92	11.32
Crude fibre (%)	1.50	1.59
Crude protein (%)	8.52	8.69
Crude fat (%)	5.08	4.93
Ash (%)	1.25	1.29
Total carbohydrate (%)	71.73	72.18
Reducing sugar (%)	2.97	3.05

Determination of the sprouted kernels percentage

Although the germination percentage of the blue maize was only about two percentual point greater than that of the red maize (91.4% and 89.3%, respectively), the ANOVA (Table 3) showed significant difference between them (p -value < 0.001). Concerning the temperature, as it is shown in the Table 4, the greatest germination values were at 20 °C, meanwhile the lowest were observed when the maize was germinated at 15 °C (p -value < 0.001). With respect to this Silva-Neta et al. (2015) tested 4 germination temperatures (10, 15, 20, and 25 °C) and their results showed that for some of the tested varieties as the temperature rose, the germination percentage also increased; however, in some varieties it was observed a decrease of this value when germinating at 20 °C in comparison to germination at 15 °C. Thus, the decrease in the germination percentage of the maize used in this research, when germinating at 25 °C in comparison to the germination at 20 °C, could be associated to the 'Chalqueño' race itself.

In the mentioned research, Silva-Neta et al. (2015) obtained germination percentages from 86 to 100 % when germinating at 20 and 25 °C. In addition, Zakeyeldinn et al. (2018) reported final germination percentages between 90.5 and 98 in five different hybrid maize varieties. Thus, the low germination percentages obtained in this research for 'Chalqueño' race maize could be related to a long storage, since it is reported that as the seed storage increases, both germination rate and germination index decrease (Garoma et al., 2017).

Acrospire length

As expected, the acrospire length increased as the germination time did, thereby being the 9-day malts having the longest acrospires; on the other hand, the 3-day malts were those with the shortest acrospires (p -value < 0.001). With reference to this, Evans et al. (2009), in a study about four different cereals, reported a significant relation between germination time and the acrospire vigour, showing a significantly increase in the growth of acrospire over the time. Concerning the germination temperature, the acrospire length increased as the temperature rose. So, the malts germinated at 25 °C had the longest acrospires, followed by those germinated at 20 °C, and finally the ones germinated at 15 °C (p -value < 0.001). As for the maize colour, the red maize had longer acrospires than the blue maize (p -value < 0.001), being their means lengths of 6.71 and 6.21 cm, respectively. These results agree with those of Akinuoye and Modi (2015), who reported longer shoot lengths when the germination temperature of maize increased from 20 to 30 °C. Similarly, Ennen and Jeschke (2020) reported higher shoot growth rates when maize germinated at higher temperatures. For example, the shoot growth rate when germinating at 15 °C was about

Table 3: ANOVA results of the germination experiment

Factor or interactions of factors	Response variables							
	Germination percentage		Acrospire length		Diastatic power		Malting yield	
	F-Ratio	P-value	F-Ratio	P-value	F-Ratio	P-value	F-Ratio	P-value
Maize	63.59	<0.001	38.98	<0.001	23.89	<0.001	385.09	<0.001
Germination temperature (GT)	32.13	<0.001	4297.12	<0.001	377.38	<0.001	8599.32	<0.001
Germination days	5.31	<0.001	1089.16	<0.001	342.21	<0.001	3068.08	<0.001
Maize*GT	16.77	<0.001	5.90	<0.001	4.81	0.0106	47.41	<0.001
Maize*Germination days	0.78	0.59	1.23	0.30	9.23	<0.001	8.48	<0.001
GT*Germination days	4.15	<0.001	141.03	<0.001	6.19	<0.001	166.04	<0.001
Maize*GT*Germination days	2.73	0.004	2.46	0.008	4.23	<0.001	3.76	<0.001

a) Bold numbers refer to those factors and/or interactions that resulted significant for the response variables

Table 4: Germination percentages of the red and blue maize at the three different germination temperatures

Colour of maize	Temperature (°C)			p-value
	15	20	25	
Red	87.03 ^{Bb}	90.89 ^{Ba}	89.98 ^{Aa}	< 0.001
Blue	91.29 ^{Ab}	92.77 ^{Aa}	90.39 ^{Ab}	< 0.001
Average ^(b)	89.16 ^c	91.83 ^a	90.19 ^b	< 0.001

(a) Capital letters indicate subgroups for temperatures (columns), and lowercase letters indicate subgroups for maize colour (rows).

(b) Germination average of both maize colours, just considering the effect of the factor temperature

0.3 mm/hour whereas it was around of 1.4 mm/hour when germinating at 30 °C. The effect observed on the length acrospires could be due to both rate of plant growth and development depend upon temperature surrounding the plant (Hatfield & Prueger, 2015), since all biological processes respond to it (Pietruszka & Haduch-Sendecka, 2016). It has been reported that the speed of germination is driven by accumulated temperature, or degree-days (GRDC, 2016). In addition to this, some studies have shown that plants adjust their growth and development as a response to even small differences in temperature. The set of morphological and architectural changes induced by high ambient temperatures, below the heat-stress, is collectively called thermomorphogenesis (Quint et al., 2016).

Malting yield

As it can be seen in Figs. 2 and 3, the malting yield decreased in both colour of maize malts as the germination time increased, being the 3-day germinated malts those with the highest, and the 9-day germinated malts those with the lowest malting yield (p -value < 0.001). Regarding to this, Ndife et al. (2019) reported malting losses of 10.6 % and 19.52 % in maize malts germinated for three and five days, respectively. There are some other studies about malts of different cereals in which a malting yield decrease was reported over the time. For example, Lekjing and Venkatachalam (2020) reported a malting yield decrease in rice malts as the germination time increased. Olugbile et al. (2015) reported a malting yield of 77.86 % in rice malts germinated for three days and 45.46 % in malts germinated 12 days, which clearly showed a malting yield decrease over

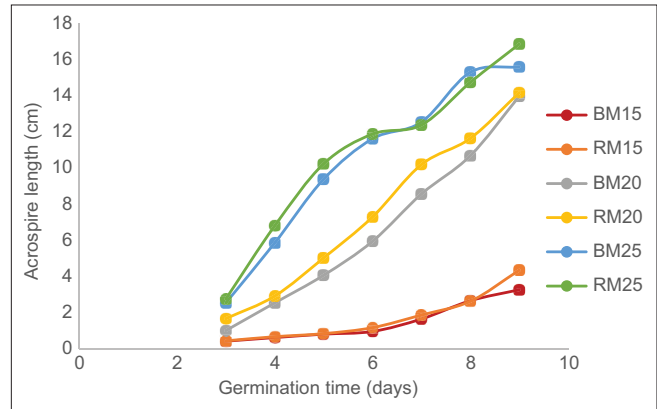


Fig 1. Effect of temperature and germination time on acrospire length of red and blue maize malts. The letters BM and RM refer to Blue Maize and Red Maize, respectively, and the numbers 15, 20, and 25 refer to the germination temperature.

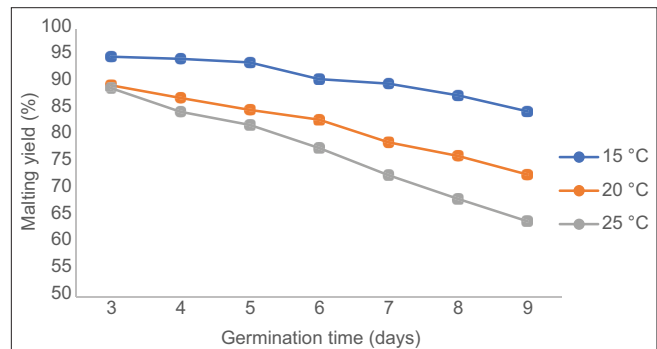


Fig 2. Effect of temperature and germination time on malting yield of red 'Chalqueño' race maize malts. The numbers 15, 20, and 25 refer to the germination temperature.

the germination time. Farzaneh et al. (2017) reported a malting yield of 86% in barley malts germinated for three days, which dropped until 78% in malts germinated for seven days. Eburuche et al. (2019) reported malting loss between 2 % and 23 % for both red and white sorghum malts, which increased as the germination time increased.

With respect to the germination temperature, the malting yield decreased as this factor increased. In this way, as it is shown in Figs. 2 and 3, the malting yield percentages of

the malts germinated at 15 °C were significantly higher than those germinated at 20 and 25 °C, being this last one which had the lowest percentages (p-value < 0.001). A similar result was reported by Claver et al. (2010), for sorghum malts, who proposed a mathematical model in which was shown that, in addition to the germination time, any increase in temperature would significantly increase malting loss.

These drops in malting yield are mainly caused by metabolic activity during the germination process as starch is the main source of stored energy in the quiescent grains, and degradation of starch is essential for heterotrophic growth in the germinating seed (Vinje et al., 2015). It is reported that, during seed germination, the stored reserves support growth of the seedling until it becomes photosynthetically active. So, the high-molecular-weight reserves, like starch, contained within the seed storage organs are converted into easily transportable low-molecular-weight metabolites that are mobilized to the growing regions in support of the energy-producing and synthetic events therein (Derek, 2001).

As for maize colour, the blue maize malts had a higher malting yield than the red maize malts (p-value < 0.001), whose values were 84.8 % and 83.2 %, respectively. This difference could be related to the longer acrospires of the red maize germinated under some of the treatments (combination of temperature and germination time), as it is shown in Fig. 1.

Correlation between the acrospire length and malting loss

As it is shown in Fig. 4, the Pearson's correlation coefficient (r) was 0.96, which indicates that malting loss was strongly related to the acrospire length, and as this latter increased, the former also did.

The obtained linear regression equation indicates that, under the conditions used in this experiment, the malting loss was at rate of 1.47 % per cm of acrospire growth. Besides, the y-intercept indicates the malting loss that could be attributable to moisture loss in malt after kilning ($\approx 5\%$), in comparison to the initial moisture of raw maize (11-12%).

Linear calibration to calculate diastatic power

Determination results of the diastatic power of different malts, through both methods, are shown in Fig. 5. As it can be seen, some variations were found in both methods, which could be attributed to errors in the determinations. However, it was observed a linearity between those, and the curve showed a correlation coefficient of 0.96. This value is higher than that reported by Henry (1984), and it is within

the range of correlations coefficients obtained by a group of 12 collaborators, who everyone performed a linear calibration between the Henry's method and the ASBC method, and whose values were between 0.91 and 0.99 (ASBC, 1990). It is important to mention that in the present work the range encompassed lower diastatic power values than in those cited studies. So that, the maize experimental malts fell within this. Therefore, the obtained equation, shown just below, was useful to convert the Henry's method units into Lintner degrees. In Table 5 is shown the diastatic power of some maize malts calculated by using the obtained equation.

$$^{\circ}L = \left[291.4 * \left(g \frac{\text{dextrose}}{l} \right) \right] - 0.0348$$

Diastatic power

Results showed that the diastatic power values of the maize malts maintained an increase from 3rd day to 7th day of germination at the three tested temperatures, and after the seventh day it was shown a decrease of these values (Fig. 6). Consequently, the greatest values for all experimental malts were observed on the seventh day of germination, regardless of the germination temperature (p-value < 0.001). These results agree with those reported by Singh and Bains (1984), who studied the effect of alkali pre-

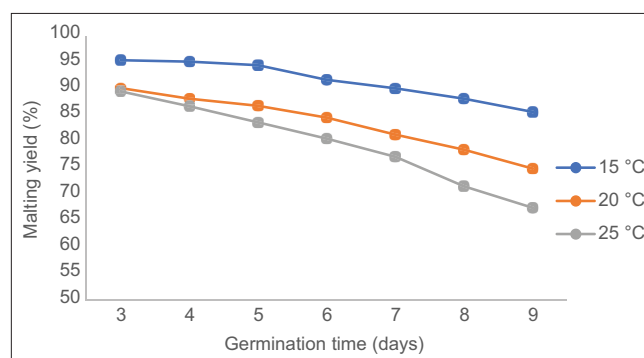


Fig 3. Effect of temperature and germination time on malting yield of blue 'Chalqueño' race maize malts. The numbers 15, 20, and 25 refer to the germination temperature.

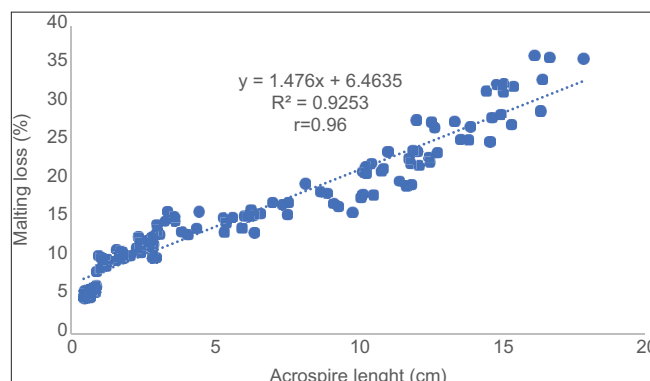


Fig 4. Linear regression of malting loss as a function of acrospire length .

Table 5: Diastatic power of some experimental malts expressed as g of dextrose/l, as well as in °L (rounded to the nearest whole number) calculated by means of the obtained formula

Malt	g of dextrose/l	DP (°L)
15°RM-6D	0.088	26
15°RM-7D	0.105	30
15°BM-6D	0.071	21
15°BM-7D	0.088	26
20°RM-6D	0.103	30
20°RM-7D	0.127	37
20°BM-6D	0.091	26
20°BM-7D	0.119	35
25°RM-6D	0.122	35
25°RM-7D	0.143	42
25°BM-6D	0.105	31
25°BM-7D	0.135	39

(a) Numbers 15°, 20°, and 25° refer to the germination temperature in degrees Celsius (°C). (b) Letters BM and RM refer to Blue Maize and Red Maize, respectively. (c) 6D and 7D refer to 6 and 7 days of germination, respectively

treatment of grains on some quality parameters of malts from two distinct maize varieties and found that the greatest diastatic power values of the malts were obtained when maize was germinated for seven days. In the same way, Farzaneh et al. (2017) reported both the highest enzymatic activity and diastatic power for barley malts germinated for seven days. On the other hand, the results of the present research differ from those reported by Iwouno and Ojukwu (2012), for malts of yellow Nigerian maize varieties, whose greatest values were reached on the fifth day of germination and showed values of about 30 °L. Also, the obtained results differ from those reported by Eneje et al. (2004), for malts of white and yellow Nigerian maize varieties, which their greatest diastatic power values were reached on the sixth germination day. Nevertheless, since the germination in the two last mentioned experiments lasted five and six days, respectively, and they were performed at different temperatures, the discrepancies with the results of this research could have been due to either differences in the metabolic properties among the maize varieties or to the differences in malting conditions. In other research about amylolytic enzymes, Helland et al. (2002) reported the maximum activity of α -amylases when maize was germinated for seven days; whereas in another one about Nigerian maize cultivars, the highest α -amylase activity was on the third day of germination, while the highest β -amylase activity was on the fifth day of germination (Awoyinka & Adebawo, 2008).

With reference to germination temperature, it was observed an increase in the diastatic power values as the germination temperature was increased (Fig. 6). In such way, the greatest values were obtained when germinating at 25 °C for both maize colours (p-value < 0.001). This temperature falls within the optimum temperature of maize, which is reported between 24 °C and 28 °C (Hatfield and Prueger,

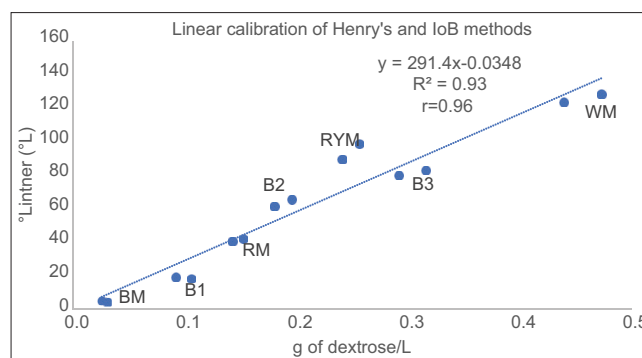


Fig 5. Correlation curve of the loB and Henry's method. BM=Blue-maize malt; B1=Caramel barley malt; RM=Red maize malt; B2=Vienna malt B3= Pale Ale malt; RYM=Rye malt; WM=Wheat malt

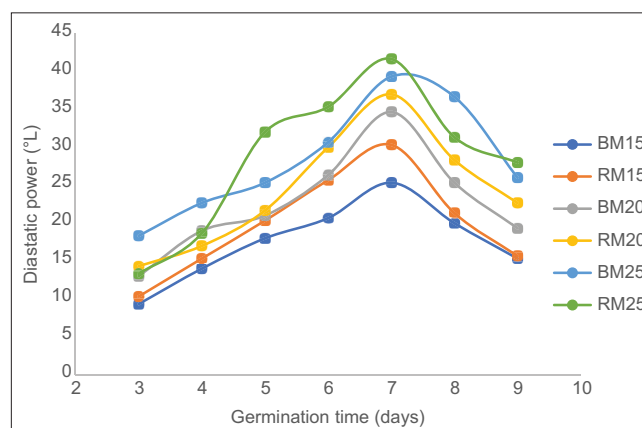


Fig 6. Effect of temperature and germination time on diastatic power of red and blue 'Chalqueño' race maize malts. The letters BM and RM refer to Blue Maize and Red Maize, respectively, and the numbers 15, 20, and 25 refer to the germination temperature.

2015). Aniche and Okafor (1989), reported a similar effect on the diastatic power of rice malts, whose values had a rapid increase when the germination temperature was rising from 22 to 28 °C. Conversely, the lowest diastatic power values were obtained when the maize was germinated at 15 °C. In relation to this, it is reported that low temperature tends to slow synthesis of enzymes, their diffusion through the endosperm and their action (Lewis & Young, 2001).

With respect to the colour, the red-maize malts reached greater values of diastatic power than the blue ones when germinating at 15 °C and 20 °C (p-value < 0.001), but there was no significant difference at 25 °C (Fig. 6). Regarding the blue maize varieties, Meußdoerffer and Zarnkow (2009) reported a diastatic power of 72 °WK (about 25 °L) for malts germinated for six days at 15 °C. In the case of red-maize malts, it was not found any report of neither diastatic power nor other malt properties for these.

Considering the three involved factors, and since there was no significant difference between the malts, both red and blue maize malts germinated during seven days at 25 °C had

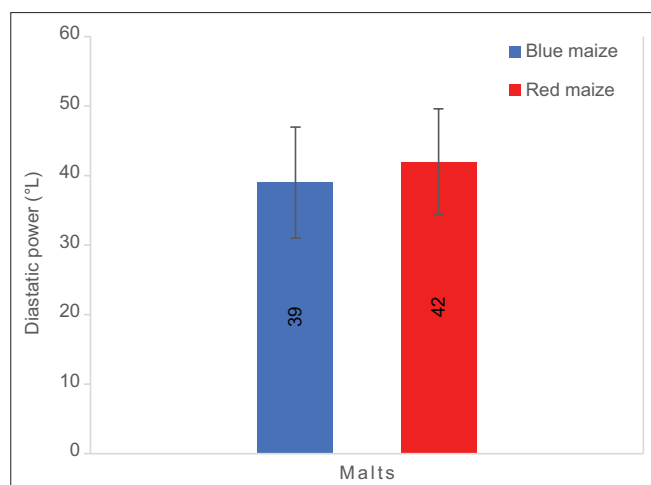


Fig 7. Diastatic power of blue and red maize malts germinated for 7 days at 25 °C

the greatest diastatic power, these values were 42 and 39 °L, respectively (Fig. 7). This means that both malts have about the minimum diastatic power to convert their own starch, which is 40 °L (Farber & Barth, 2019). In counterpart, the lowest values were obtained by the malts of both maize colour when malting three days at 15 °C, whose value was 9 and 10 °L for blue and red, respectively.

The greater diastatic power values of the ‘Chalqueño’ race maize malts, compared to those reported from other varieties, could be related, besides to the mentioned malting conditions, to the larger grain size of this race, since in a research about barley malts, conducted by Agu et al. (2007), it was found a positive correlation between the proportion of large-size grains contained in a malt and its diastatic power.

It is reported that maize have adequate activity of α -amylases (Dziedzoave et al., 2010), and they are responsible for most of amylolytic activity in maize seeds. Also, maize malts have relatively low activity of β -amylases (Dziedzoave et al., 2010; Evangelista-Oliveira et al, 2013), which are considered the most important enzymes for diastatic power in malts (Arends et al., 1995). However, Awoyinka and Adebawo (2008) reported that some maize cultivars were high in alpha amylolytic activity and some others were high in beta amylolytic activity. Thus, based on this research, both blue and red maize belonging to the ‘Chalqueño’ race could be high- β -amylase-activity maize cultivars, which would be responsible for their higher diastatic power in comparison to other maize cultivars. Nevertheless, it is needed a research to confirm this.

CONCLUSIONS

There was a positive effect on the diastatic power of the maize malts when the germination temperature

was increased. However, this factor did not modify the germination time in which the maximum values of diastatic power of the malts were reached. Likewise, under the mentioned optimum conditions of malting, according to the reported minimum diastatic power value for a malt, the malts obtained from blue and red ‘Chalqueño’ race maize are capable to convert their own starch. Nevertheless, it is necessary to carry out more studies in which other malting factors are considered, such as grain moisture, in order to control the acrospire growth and thus reduce malting loss.

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Conflict of interest

The authors declare there are no conflicts of interest.

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