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Optimization of Germination Conditions of Blue Corn (*Zea mays* L.) by Taguchi Orthogonal Array Methodology

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Abstract: A Taguchi L₉ orthogonal array methodology was used to optimize the conditions for maximal germination percentage in blue corn grains. The factors studied included steeping time, ratio of steeping water to corn grain, germination temperature and time. The optimal conditions included a steeping time of 24 h, an amount of 100 g of grain steeped in 200 mL of water and a germination time of 96 h at 25°C for a predicted germination of 90.6%. Under these conditions, 94% of the grains germinated reaching a moisture content of 43%. An increase in the range of 154 to 160% for total phenolics and 43% for anthocyanins was obtained after 96 h of germination. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) antioxidant activity increased slightly during the germination process. In conclusion, the process of germination was beneficial due to the increase in antioxidant compounds in the blue corn grain.

Key words: Germination, blue corn, optimization, Taguchi, anthocyanins, antioxidant activity

INTRODUCTION

Germination is the first step in the production of a native white or yellow corn beer called *tesgüino* which is consumed by several ethnic and mestizo groups in Mexico. *Tesgüino* is mainly consumed during ritualistic ceremonies by the Tarahumara and some other native tribes in Chihuahua but some light versions of the product are sold as refreshing beverages in the streets of several towns in Northern Mexico (Quintero-Ramírez *et al.*, 1999; Blandino *et al.*, 2003). For the preparation of *tesgüino*, dark-germinated corn is ground to obtain a dough which is boiled in water. Liquid portions are transferred to a clay pot and a catalyst is added. This mixture is allowed to ferment for two or three days to obtain the final product. The bacteria and yeasts isolated from *tesgüino* include mainly *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Saccharomyces* (Quintero-Ramírez *et al.*, 1999).

No studies are reported about the use of blue corn, which has a high content of anthocyanins, in the elaboration of *tesgüino*. Anthocyanins are hydrosoluble vegetable pigments responsible for the red, blue and purple color of many fruits, flowers and seeds. They

occur mainly as glycosides (Wu *et al.*, 2006). Cyanidin 3-glucoside has been identified as the major anthocyanin (75%) in Mexican blue corn from Toluca, which contains 321 mg kg⁻¹ of these compounds and 451 mg kg⁻¹ of total polyphenolic compounds (Del Pozo-Insfran *et al.*, 2006). These compounds may have an important role in scavenging the active oxygen species which are generated during seed germination (Bailly, 2004) and have been reported to be important in the prevention of obesity and in the amelioration of hyperglycemia in mice (Tsuda *et al.*, 2003). The use of this particular cereal could produce a *tesgüino* with a good content of antioxidant compounds.

Many cereals can be satisfactory malted, but corn is rarely used for this purpose because its fat becomes rancid during storage (Hough, 1991). However, it has been reported that some yellow corn varieties had good malting performance (Eneje *et al.*, 2004) and no problems have been detected when the corn malt is used immediately after its preparation for the elaboration of beer-like products like *chicha* or *tesgüino* (Quintero-Ramírez *et al.*, 1999). The main purpose of malting is the modification of the seed storage starch and protein reserves into assimilable materials for adequate yeast growth and fermentation (Ogbonna *et al.*, 2003).

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Germination is a process that has been optimized for some cereals (Capanzana and Buckle, 1997). Germination starts once a seed takes up water. Seed steeping is a known strategy to increase the uniformity of seed germination, but in the case of corn and rice, water-steeping also accelerates the germination process (Horii *et al.*, 2007). Steeping is normally completed in two days and then the grains are drained and spread in germination trays or incubators. There are studies indicating that the steeping and germination time and temperature affect the malting performance and fermentability of grains (Eneje *et al.*, 2004; Balogun *et al.*, 2006).

Most published optimization procedures involve response surface methodology, however, when the number of variables is high, the number of experimental runs is also very high. Reports are available on the use of Taguchi orthogonal matrix method in biotechnology, since this method examine the effects of several process variables and identify the factors which have major effects on the process using just a few experiments (Seyedeh *et al.*, 2007).

This study reports the manipulation of steeping time, amount of steeping water and germination time and temperature for the optimization of germination percentage of blue corn using the Taguchi method. Since germination can modify the phenolic composition of seeds (López-Amorós *et al.*, 2006), the changes in phenolic compounds and anthocyanins during germination of blue corn were also studied.

MATERIALS AND METHODS

The study was conducted in 2007 at the Escuela Nacional de Ciencias Biológicas of the Instituto Politécnico Nacional in Mexico City, DF, Mexico.

Analytical methods: The blue corn grain was purchased in the town of San Mateo Tepopula, State of Mexico (near Mexico City) and was analyzed for moisture (44-16), fat (30-10), protein (46-10), fiber (32-10), ash (08-01) and carbohydrates (80-60) by the approved methods of the AACC (AACC, 2000). The grains were physically and biologically characterized with the following parameters: hectoliter weight (Salinas-Moreno *et al.*, 2007), seed viability by the tetrazolium test (Rao *et al.*, 2006), germinative energy (Langrell and Edney, 2006), germinative capacity (Langrell and Edney, 2006) and amount of impurities (Piedade *et al.*, 2002). Phenolics were extracted with acidic methanol and measured with the Folin-Ciocalteu technique (Veliloglu *et al.*, 1998). Anthocyanins were extracted with acidic ethanol and its

Table 1: L₉ orthogonal array for the germination process

Run	Component				Germination (%)
	A (h)	B (mL)	C (°C)	D (h)	
1	72	200	20	96	52
2	24	100	20	24	10
3	48	100	25	96	74
4	24	400	30	96	68
5	72	400	25	24	36
6	48	400	20	48	44
7	24	200	25	48	76
8	48	200	30	24	34
9	72	100	30	48	30

A: Steeping time; B: Amount of steeping water per 100 g of corn grain; C: Germination temperature; D: Germination time

absorbance measured at 535 nm (Abdel-Aal and Hucl, 1999). The content of these compounds was also evaluated in white corn from the same region. The antioxidant activity was measured as DPPH (1,1-diphenyl-2-picrylhydrazyl)-radical scavenging activity. Samples (0.2 g fresh weight) were ground in a mortar at 4°C in 2.0 mL of absolute ethanol. One-half milliliter of the solution was mixed with 0.25 mL of 0.5 mM DPPH in ethanol solution and 0.5 mL of 100 mM acetate buffer (pH 5.5). The absorbance of the mixture was measured at 517 nm after 30 min (Kang and Saltveit, 2002) and compared with the absorbance of a solution without sample. The inhibition (%) of the color formation was reported. All the results are shown as the average of three determinations.

Optimization of the germination conditions using the Taguchi L₉ orthogonal array methodology: The design for the L₉ orthogonal array was developed and analyzed using the Design Expert 7.0.3 software (Stat-Ease Inc., Minneapolis, MN 55413). The factors studied included steeping time (24, 48 and 72 h), amount of steeping water (100, 200 and 400 mL water) used per 100 g of corn grain, germination temperature (20, 25 and 30°C) and time (24, 48 and 96 h). Table 1 shows the dark-germination conditions and the L₉ orthogonal array used in this study. The seeds were steeped in water and then placed into Petri dishes containing two discs of Whatman No. 1 filter paper which were moistened with distilled water to make certain that the moisture was adequate for germination (Koduru *et al.*, 2006). Controlled temperature chambers were used for seed incubation at the different temperatures. Three replicates of 100 seeds were used for each assay.

RESULTS AND DISCUSSION

Blue corn composition and properties: The results of the proximal analysis of the blue corn grain are shown in Table 2. These results are very similar to those previously reported for white and yellow corn (Haard, 1999; Eneje *et al.*, 2004).

Table 2: Blue corn grain composition

Component	Percent (w.b.)
Moisture	13.1
Fat	5.3
Protein	7.6
Fiber	2.6
Carbohydrates	70.0
Ash	1.4

Table 3: Some physical and germinative characteristics of the blue corn seeds

Characteristic	Blue corn
Impurities (%)	2.3
Hectoliter weight (kg HL ⁻¹)	70.4
Grain viability (%)	92.0
Germinative energy (%)	70.4
Germinative capacity (%)	93.0

Some other characteristics of the corn seeds are shown in Table 3. The hectoliter weight falls in the normal range for corn (61.0-73.6 kg HL⁻¹) reported by Greenway *et al.* (1977). The grain viability was relatively high. A viable seed is one, which is capable of germinating under the proper circumstances, so 92% viability could be correlated with a high probability of germination. The germinative energy and capacity for the blue corn grains (70.4 and 93%, respectively) were low compared with the values reported for white (95 and 97%) and yellow (97 and 99%) corns (Eneje *et al.*, 2004). More studies about these parameters using different varieties of blue corn from several places would be necessary to conclude adequately about the germinative properties of this pigmented seed.

The amount of anthocyanins was, as expected, considerably higher in blue than in white corn. The concentration of anthocyanins in the blue corn used in this study (540 mg kg⁻¹ corn) was higher than the content reported by Del Pozo-Insfran *et al.* (2006) for American (307 mg kg⁻¹) and Mexican (321 mg kg⁻¹) blue corns. The total polyphenolic content of the white corn (1741 mg kg⁻¹) was similar to that reported by Adom and Liu (2002) for yellow corn (1758 mg kg⁻¹), however, the concentration of polyphenols in the blue corn in this study (1528 mg kg⁻¹) was considerably lower than the content indicated by Del Pozo-Insfran *et al.* (2006) for a Mexican white corn from Monterrey (4899 mg kg⁻¹). The antioxidant activity for the blue corn allowed a 52% of inhibition of the DPPH color formation.

Optimization of the germination process: The optimal conditions predicted by the software included a steeping time of 24 h, an amount of 100 g of grain steeped in 200 mL of water and a germination time of 96 h at 25°C for a predicted maximal grain germination of 90.6% (Table 1). The four factors had an equal statistical importance level (25%). When these conditions were tested experimentally,

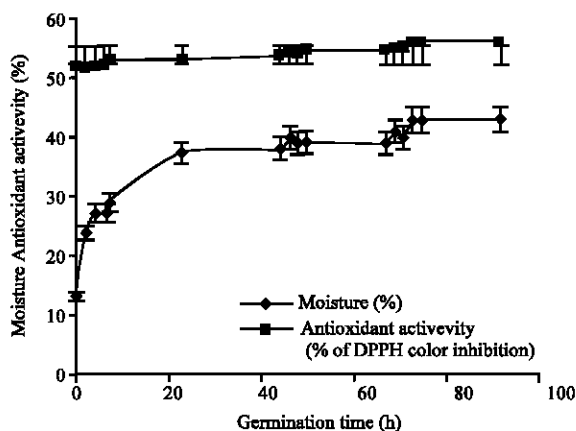


Fig. 1: Time courses of moisture content (%) and antioxidant activity (% of DPPH color inhibition) during the germination process of blue corn at 25°C

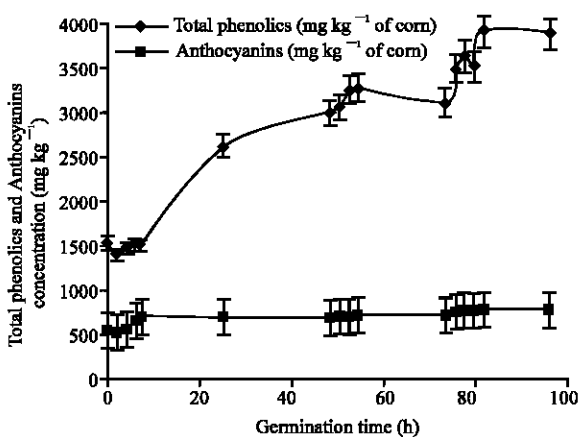


Fig. 2: Time courses of total phenolic content (mg kg⁻¹) and anthocyanins content (mg kg⁻¹) during the germination process of blue corn at 25°C

94% of the grains germinated reaching a final moisture content of 43%. The 2:1 water/grain ratio is the same proportion recommended for malting sorghum (Ogbonna *et al.*, 2003).

After the 24 h steeping period, moisture increased from 13.1 to 37.41%, phenolics increased from 1528 to 2600 mg kg⁻¹ of corn (expressed as ferulic acid) and anthocyanins increased from 540 to 678 mg kg⁻¹ of corn (expressed as cyanidine-3-glucoside). The grains were then germinated for 96 h. Moisture, phenolics and anthocyanins increased to important final contents of 43.01%, 3800 and 775 mg kg⁻¹, respectively (Fig. 1, 2). The antioxidant activity increased to 56% of inhibition of the DPPH color formation due to an increase in the content of phenolics. These results confirm the findings

of Randhir and Shetty (2005) about the developmental stimulation of total phenolics and their antioxidant activity in dark germinated sweet corn. They found that the phenylpropanoid pathway was stimulated during dark-germination of corn and consequently the total phenolic content increased to a value (4000 mg kg^{-1}) similar to the one found in this study for blue corn. They also noticed an increase in antioxidant activity to 81% DPPH inhibition after 8 days of germination. Similar increases in phenolics and antioxidant activity have also been observed during the germination of wheat, fava bean and *Lupinus angustifolius* (Yang *et al.*, 2001; Shetty *et al.*, 2003; Fernández-Orozco *et al.*, 2006). However, in the case of sorghum, germination did not affect the content of total phenolic compounds (Dicko *et al.*, 2005).

The increases in moisture, phenolic compounds and anthocyanins during the germination process could be fitted to the following equations:

$$\text{Moisture content (\%)} = \frac{1}{(0.03595 - 0.0023 \ln t)}; R^2 = 0.942$$

$$\text{Total phenolic content (\%)} = 73.974 + 2.693t - 0.127t^2; R^2 = 0.96$$

$$\begin{aligned} \text{Anthocyanins content (\%)} = & 56.465 + 0.6284t - 0.0092t^2 \\ & + 0.000054t^3; R^2 = 0.81 \end{aligned}$$

where, t is germination time in hours and R^2 is the determination coefficient of the equation fitting the data in Fig. 1 and 2.

It can be concluded that the content of anthocyanins is, as expected, considerably higher in the blue corn with respect to the white grain. However, the content of total phenolic compounds is slightly higher in the white corn. The germination process was shown to be beneficial due to the increase in anthocyanins, total phenolics and antioxidant activity in the blue corn malt. The optimization of the germination process can ensure a maximal proportion of sprouted grains and with it a higher yield of blue tesgüino. This research provides a new tool for the optimization of processes such as germination.

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