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β-Glucanase Addition in Brewing Malt Produced by Reduced Time of Germination

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HIGHLIGHTS

- The addition of β-glucanase reduced the germination time of the malt.
- The beer produced with malt germinated with β-glucanase had good sensory acceptance.
- Concentrations of 25 and 50 mg.kg-1 of β-glucans resulted in adequate β-Glucan content.
- The addition of β-glucanase to malt germination did not affect the quality of the beer.

Abstract: The β-Glucans content has straight influence on the quality of malt and beer, mainly during the filtration step. Barley presenting high β-Glucan content demands longer germination time at malting. The application of commercial β-Glucanase is an alternative to accelerate the process and preserve the quality of malt. This work aimed to evaluate the effect of commercial β-Glucanase addition in malt produced within reduced germination time (64 h). Micro-malting was conducted with BRS-Caue and Elis barley cultivars at germination time 64 h and 96 h. The β-Glucanase concentration applied were 0, 25, 50 and 100 $mg.kg^{-1}$. Barley, malt and wort samples were analyzed to check their physical-chemical features. Beers were produced with BRS-Caue malt and the physical-chemical and sensory attributes were analyzed. The commercial enzyme addition in BRS-Caue and Elis (64 h), at concentration 25 and 50 $mg.kg^{-1}$, resulted in wort presenting proper β-Glucan content (≤ 178 $mg.L^{-1}$). The beer produced with malt germinated for 64 h and added with 50 $mg.kg^{-1}$ of β-glucanase was the one showing the largest number of physical-chemical and sensory parameters similar to the beer made with malt germinated for 96 h (conventional process). Commercial β-glucanase application in malt allowed accelerating the malting process without affecting the quality of the malt for beer production.

Keywords: β-glucans; germination time; commercial enzyme.

INTRODUCTION

In the world, Brazil is the third largest beer producer. In 2016, the country produced approximately 14.1 billion liters of beer [1]; therefore, the demand for raw material is high. The country produces approximately 43% of the malt needed to supply brewer industries [2]. In order to fill the lack of raw material to brewing industries, many countries replace malt and barley by rice, rye, corn, oats and sorghum [3,4,5,6,7]. Although these adjuncts present lower production cost adjuncts materials can affect the physical-chemical and sensory properties of the beer [8].

Unmalted barley stands out among the adjunct materials due to its intrinsic features, which facilitates its incorporation to brew processing in beer industries. The gelatinization temperature of barley starch is similar of malted barley starch. Another advantage is the possibility to apply the barley straw to help the filtration process [9]. Using unmalted barley or the partial malt replacement by barley, or by malted barley presenting shorter germination time has been reported [10,11,12,13].

The malting process includes steeping, germination and kilning [14]. The β -Glucanases (1→3, 1→4 β-D- Glucan, 4-Glucan hydrolase, E.C.3.2.1.73) are the main enzymes produced in the germination stage. These enzymes hydrolyze the components in the cell walls of barley grains. Thefore, the total or partial replacement of malt by unmalted barley can affect the brewing process due the insufficient action of β-Glucanases and high β-Glucan content [9,15]. The high β-Glucan content increases wort viscosity, which causes reduced filtration and unwanted beer turbidity [16,17]. Thus, one of the requests from brewers is malt with β-Glucans content lower than 178 $mg.L^{-1}$ [18].

The brewing industry has interest to improve malting process to reduce cost and energy and water consume [11]. But changes in maceration, germination or drying steps may affect

the quality of the malt for beer production. The enzymes addition may improve the malt quality and it is already commercially available. Enzymatic preparations with microbial β-Glucanases are reported as promising to reduce the content of high molecular-weight β-Glucans in barley [9,19]. The present study aimed to assess the quality of brewer's malt produced within a shorter germination time and added with commercial β-Glucanase in order to reduce malt production costs and to accelerate its production for brewers.

MATERIAL AND METHODS

Raw Material

The barley BRS-Caue and Elis cultivars were grown and harvested in Guarapuava-PR, Brazil in 2013. A total mass of 50 kg of barley were sampled and stored at 25 ^{o}C for two months, until experimental use. The barley moisture (EBC 3.3) and protein (EBC 3.3.2) were analyzed according to the standards methods of the European Brewery Convention (EBC) [19]. Germination power was analyzed as described by Mitteleuropäische Brautechnische Analysen Kommision (MEBAK) [20]. The β-Glucan concentration was measured according to the methods described by the American Association of Cereal Chemists (AACC) [21].

Malting

The barley samples (BRS Caue and Elis, > 2.2-mm screen) were malted following two germination time programs: 64 h and 96 h. For the both programs, steeping was comprised of two cycles of 4 h of immersion and air rest at 20 °C in Schmidt-Seeger® [14]. Germination lasted 64 h (reduced time) and 96 h (usual time) at 20°C. Moisture content (m/m) of the grain was monitored and adjusted daily ~ 46%. The kilning program was as follows: 16 h and 30 min at 50°C, 1 h at 60°C, 1 h at 70°C and 2h and 30 min at 80°C.

β-Glucanase addition and malt analyses

Prior to mashing the malt germinated at 64 h, the commercial endo-1.3(4) β-Glucanase (EC 3.2.1.6, CAS no. 62213-14-3, AB Enzymes, Darmstadt, Germany) was added. The β-Glucanase solution (5.0 mL) was sprinkled at the concentrations: 0, 25, 50 and 100 mg.kg^{-1} of malt (BRS-Caue and Elis). The malt moisture content (EBC 4.2) and friability (EBC 4.15) were analyzed according to EBC [22]. The β-Glucanase activity was analyzed using Azo-Barley-Glucan essay kit (Megazyme Ltda., Irlanda) as described by McCLEARY and SHAMEER [23] at 590 nm (Hewlett Packard, model 8453).

Mashing

Wort was prepared (EBC 4.2) and analyzed according methods described in EBC[23]: wort extract (EBC 4.5.1), pH (EBC 8.17), cooking color (EBC 4.19), β-Glucan concentration (EBC 4.16.3) and diastase power (EBC 4.12.1). Wort viscosity was measured according to MEBAK 4.1.4.4 [20].

Pilot-scale brewing

The beer samples were brewed from BRS-Caue malt samples germinated at 64 and 96h and added of commercial β-Glucanase (0, 25 and 50 $mg.kg^{-1}$). Each milled malt samples (3 kg) were mashed with 10 L of drinking water (chlorine free) at 40°C, 30 min at 50°C and 20 min at 60°C. Then, the temperature was increased 1 °C/min until 72°C and kept for one hour and 15 min at 76°C. The wort was diluted with 12 L of hot water (80°C), totaling 18 L per malt sample. Magnum (5 g) and Mittelfriih (10 g) hops were added. The boiled wort was then transferred to the whirlpool vessel and rested for 15 min. The wort was cooled at 23°C and rehydrated yeast *Saccharomyces carlsbergensis* (10 g, Diamond Lager Yeast) added to brew wort. Fermentation was conducted for 12 days at 12.5°C. To improve carbonation, natural lemon juice (15 mL) and 50 g of sucrose were added to the 600 mL green beer bottle. Maturation was performed in amber glass bottles for 7 days at 17°C. The bottled beers were then used for further beer characterization.

Beer analyses

The beer physical-chemical analyses were performed based on standards set by Adolfo Lutz Institute [24]. Samples were decarbonated at 20 and 25°C. The parameters measured were: alcohol content (247/IV), real extract (248/IV), pH (017/IV), total acidity (234/IV), reducing sugars (239/IV) and soluble solids (315/IV). Microbiological analyses consisted of the enumeration and identification of potential pathogens according to International Standard Organization Procedures (ISO) for the quantification of *Staphylococcus aureus*, mesophilics at 30°C, total and thermotolerant coliforms, yeasts and moulds [25,26,27].

Sensory evaluation

Ethical clearance approval for this study was granted by the UNIR (Federal University of Rondonia, Brazil) Research Ethics CEP/UNIR, (717442217.1.0000.5300). Descriptive sensory analysis of the beers was developed by the trained sensory panel [28]. The descriptive terminology was also developed based on the Kelly's Repertory Grid method [29]. The description terms of attribute were: color, gloss, effervescence, body, turbidity, formation of foam, stability of foam, aroma, taste and overall assessment. Reference material for the sensory perception was applied during training. Twenty-five panelists were selected considering the ability to discriminate samples and repeatability in attribute description. Four beer samples were numbered at 10°C were served simultaneously in glass (40 mL each). Evaluators drank water to avoid cross-contamination between the beer samples. Evaluation forms were presented in horizontal 9 cm structural intensity scale (from 0 "less" to 9 "much").

Statistical analyses

Barley, malt, wort and beer assessments data were subjected to an analysis of variance (ANOVA) and Tukey's test for the means comparisons STATISTICA software, version 7.0 [30]. Individual performance of sensorial analysis considered 2 factors, sample and assessor [28].

RESULTS

Table 1 presents the results of barley quality analyses.

Table 1. BRS-Caue and Elis barley cultivar characterization

Cultivar	Moisture (%)	Germinative energy (%)	Protein (%)	β glucan (%)	Classification* (%)
BRS-Caue	11.93±0.05[a]	97±0.82[a]	11.20±0.08[a]	3.72±0.53[a]	92.27±0.33[b]
BRS-Elis	10.47±0.05[b]	98±1.70[a]	10.33±0.05[b]	3.37±0.36[a]	93.20±0.08[a]

* 2.8 + 2.5 mm. Mean values in the same column followed by different superscript letters, for barley moisture, germinative energy, protein, β-Glucan and Classification, are significantly different ($p \leq 0.05$).

The Figure 1 show β-Glucan concentration in BRS-Caue and Elis malt cultivars germinated for 64h after the application of different commercial β-Glucanase concentrations.

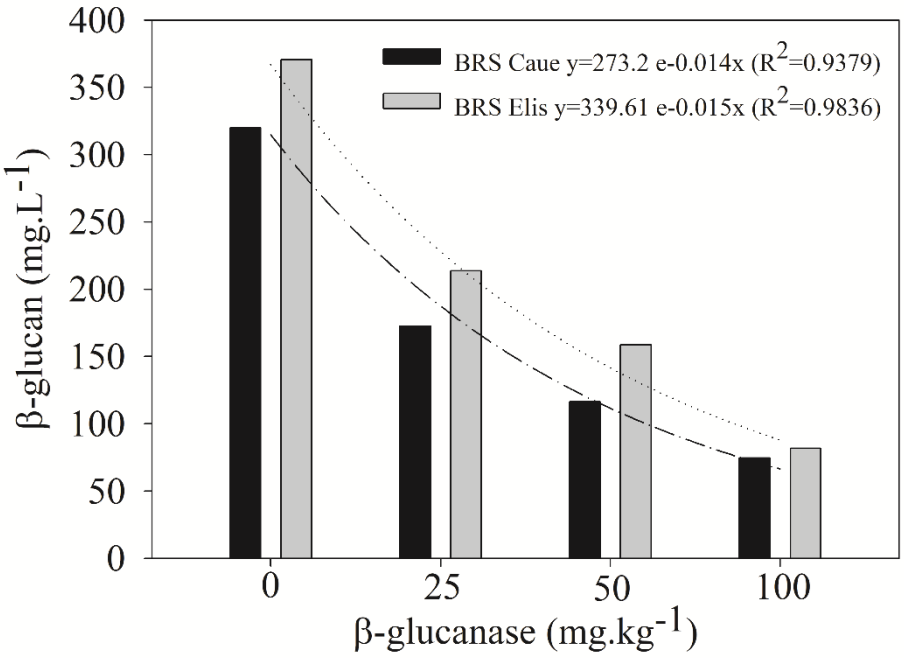

Figure 1. β-Glucan concentration in BRS-Caue and Elis malt cultivars germinated for 64h after the application of different commercial β-Glucanase concentrations.

Table 2 presents the physical-chemical characteristics of malt produced from barley germinated for 96 h and 64 h added with β-glucanase at concentrations 0, 25, 50 and 100 $mg.kg^{-1}$ (BRS-Caue and Elis).

Table 2. Physical-chemical characterization of malt produced from cultivars BRS Caue and Elis

Treatment	Germination Time (h) / β-glucanase (mg.kg^{-1})				
	96 / 0	**64 / 0**	**64 / 25**	**64 / 50**	**64 / 100**
			Moisture (%)		
BRS Caue	4.17±0.05^{d}	4.80±0.00^{c}	5.47±0.05^{a}	5.23±0.05^{b}	5.20±0.00^{b}
BRS Elis	4.23±0.05^{d}	4.73±0.05^{c}	5.27±0.00^{a}	5.47±0.05^{a}	5.13±0.05^{a}
			Friability (%)		
BRS Caue	85.0±0.86^{a}	70.80±0.73^{b}	68.6±0.83^{b}	69.96±0.94^{b}	68.70±1.36^{b}
BRS Elis	87.9±0.57^{a}	73.30±0.57^{b}	71.1±0.59^{c}	71.87±0.54^{bc}	71.63±0.37^{bc}
			β-Glucanase (U.kg^{-1})		
BRS Caue	471.0±22.5^{a}	317.7±27.4^{c}	339.3±13.3^{bc}	342.7±6.60^{bc}	396.3±19.1^{b}
BRS Elis	462.3±10.6^{a}	332.3±16.8^{b}	344.3±29.3^{b}	350.0±12.0^{b}	380.7±17.6^{b}

Mean values in the same column followed by different superscript letters, for malt moisture, friability and β-Glucanase, are significantly different ($p \leq 0.05$).

Table 3 presents the analyses of wort produced from malt germinated for 96 h and 64 h added with β-Glucanase at concentrations 0, 25, 50 and 100 mg.kg^{-1} (BRS-Caue and Elis cultivars).

Table 3. Physical-chemical characterization of wort produced from cultivars BRS Caue and Elis

Treatment	Germination Time (h) / β-glucanase (mg.kg^{-1})				
	96 / 0	**64 / 0**	**64 / 25**	**64 / 50**	**64 / 100**
			Color (EBC)		
BRS Caue	6.13±0.12^{a}	5.60±0.08$^{b.c}$	5.50±0.00^{c}	5.90±0.14^{ab}	5.57±0.05^{c}
BRS Elis	6.20±0.14^{a}	5.67±0.05^{b}	5.87±0.12^{b}	5.70±0.00^{b}	5.67±0.05^{b}
			Extract (%)		
BRS Caue	80.90±0.08^{c}	81.40±0.08^{b}	81.40±0.08^{b}	82.13±0.21^{a}	81.53±0.05^{b}
BRS Elis	81.63±0.05^{b}	82.10±0.16^{a}	82.27±0.05^{a}	82.23±0.05^{a}	82.47±0.19^{a}
			pH		
BRS Caue	5.97±0.02^{a}	5.84±0.01^{c}	5.94±0.01^{ab}	5.93±0.01^{ab}	5.91±0.03^{b}
BRS Elis	5.83±0.02^{e}	5.87±0.00^{d}	5.93±0.01^{c}	6.04±0.00^{a}	5.99±0.01^{b}
			Diastatic power (ºWK)		
BRS Caue	304.3±3.86^{a}	277.7±6.13^{bc}	282.3±6.13^{bc}	268.7±2.87^{c}	286.0±5.89^{b}
BRS Elis	281.0±0.82^{a}	229.7±2.87^{b}	236.3±1.89^{b}	242.7±18.37^{b}	252.0±5,89^{b}
			Viscosity (mPa.s)		
BRS Caue	1.45±0.01^{c}	1.57±0.01^{a}	1.55±0.00^{a}	1.51±0.00^{b}	1.52±0.00^{b}
BRS Elis	1.47±0.01^{d}	1.59±0.00^{a}	1.55±0.00^{b}	1.52±0.00^{c}	1.51±0.00^{c}

Mean values in the same column followed by different superscript letters, for wort color, extract, pH, diastatic power and viscosity, are significantly different ($p \leq 0.05$).

Table 4 show the physical-chemical characteristics of beer produced from malt (BRS-Caue) germinated for 64 h and 96 h added with 0, 25 and 50 $mg.kg^{-1}$ of commercial β-Glucanase.

Table 4. Physical-chemical features of beer produced with BRS-Caue malt germinated for 96 and 64 h and added with commercial β-Glucanase

Treatment	**Germination Time (h) / β-glucanase ($mg.kg^{-1}$)**			
Analyses	**96 / 0**	**64 / 0**	**64 / 25**	**64 / 50**
acidity (% m/v)	$0.18±0.01^{c}$	$0.17±0.01^{d}$	$0.25±0.01^{a}$	$0.24±0.01^{b}$
pH	$4.26±0.03^{c}$	$4.35±0.02^{b}$	$4.48±0.05^{a}$	$4.51±0.05^{a}$
SS* (ºBrix)	$7.00±0.51^{bc}$	$7.05±0.20^{b}$	$8.40±0.11^{a}$	$6.48±0.52^{c}$
Alcohol (%)	$5.15±0.1^{b}$	$4.32±0.1^{c}$	$6.87±0.1^{a}$	$5.12±0.1^{b}$
Reducing sugar ($g.L^{-1}$)	$1.51±0,01^{c}$	$1.28±0,02^{d}$	$2.92±0,03^{a}$	$2.51±0,02^{b}$
Real Extract (%)	$5.01±0,16^{bc}$	$5.17±0,16^{ab}$	$5.27±0,12^{a}$	$4.97±0,08^{c}$

*SS: soluble solids. Mean values in the same line followed by different superscript letters, for beer acidity, pH, soluble solids, alcohol content, sugar, real extract, are significantly different ($p \leq 0.05$).

Tables 5 and 6 presents the analyses of variance of the sensory analyses and resulted of the sensory analysis of the developed beers and its comparison with the conventional formulation, respectively.

Table 5. Variance analyses of judges' individual performance in the beer sensory analyses

Efect	**Test**	**Valor**	**F**	**Efect df**	**Error df**	**p**
Assessor	Wilks	0.186728	1.1500	30	339.0914	0.167657
Sample	Wilks	0.029056	13.781	230	576.5266	0.000000

Table 6. Sensory analysis of the developed beers and its comparison with the conventional formulation

Sensorial	**Beer samples**			
attributes	**96 / 0**	**64 / 0**	**64 / 25**	**64 / 50**
Body	7.17^{a}	5.93^{b}	5.73^{b}	6.92^{a}
Turbidity	0.87^{a}	1.43^{b}	1.44^{b}	0.94^{a}
Foam formation	7.71^{a}	5.95^{b}	6.09^{b}	7.63^{a}
Foam stability	7.84^{a}	5.23^{b}	5.34^{b}	7.74^{a}
Aroma	7.52^{a}	5.80^{b}	6.59^{b}	4.43^{c}
Taste	6.94^{a}	4.20^{b}	2.67^{c}	7.33^{a}
Color	5.92^{a}	5.92^{a}	5.78^{a}	5.92^{a}
Gloss	5.78^{a}	6.13^{a}	5.62^{a}	6.16^{a}
Effervescence	7.76^{a}	7.72^{a}	7.80^{a}	8.04^{a}
Overall avaliation	8.52^{a}	8.26^{a}	8.07^{a}	8.47^{a}

Means followed by different letters on the same line differ statistically by the Tukey test at a 95% confidence level ($p \leq 0.05$).

DISCUSSION

The BRS Caue and Elis cultivar presented good quality for the malt processing. The protein content was lower than 12%, moisture was lower than 13% and germination power was higher than 95% [31,32]. The barley grains naturally presented about 3 to 11% of β-Glucans [33]. Environmental and genetic factors affect the final β-Glucan content in barley [34]. The analyses of ten Brazilian barley cultivars (IAC) showed β-Glucan content ranged between 2.04 to 9.68% [35]. For malt production, the appropriate β-Glucan content must be 3.0 - 4.5% [36]. The BRS Caue and Elis cultivars (2013 crop) are the results of genetic improvement [37]. And these cultivars presented β-Glucan content between 3.72% and 3.37%, respectively, as shown in Table 1.

The β-Glucan concentration ≤ 178 $mg.L^{-1}$ is the recommended maximum limit for brewing malt [38]. The BRS-Caue and Elis cultivars germinated for 64 h without enzyme addition presented high β-Glucan concentrations in wort (320.0 and 370.7 mg/L, respectively). On the other hand, the addition of 100 $mg.kg^{-1}$ of commercial β-Glucanase reduced by 76.67% and 77.96% the β-Glucan content (74.67 and 81.67 $mg.L^{-1}$) in BRS-Caue and Elis, respectively. These values at 64 h did not differ statistically ($p>0.05$) from the values detected for cultivars germinated for 96 h.

The concentration of commercial β-Glucanase necessary to reach 178 $mg.L^{-1}$, was calculated based on the equations Figure 1. The addition of 31.39 $mg.kg^{-1}$ in the Caue (R^2= 0.9836) malt cultivar, and of 44.13 $mg.kg^{-1}$ in the BRS-Elis (R^2= 0.9379) would be enough to conduct to acceptable brewing process.

In a malting program of 120 h with germination temperature at 22°C (1st day) to 17°C (2nd day), Brazilian barley cultivars N721 and N740 presented β-Glucan content of 112 and 214 $mg.L^{-1}$, respectively [39]. Although cultivars N721 and N740 had presented high β-Glucanase activity (> 800.0 $U.kg^{-1}$). The β-Glucan contend presented by BRS Caue and Elis cultivars (96 h, Table 3) revealed higher hydrolysis of β-Glucan than N721 with 198 mg. L^{-1} for 96 h and 112 $mg.L^{-1}$ for 120 h of germination [39]. Once higher temperatures in the maceration/germination stage lead to a better water absorption, the temperature may affect β-Glucan degradation [14]. Poor barley germination can affect the quality required for beer production.

The moisture content recommended for brewing malt varies from 4% to 5%. The moisture content > 5% limits product storage. The aspersion of 5 mL of enzyme solution in malt samples (1 kg) increased the moisture content. All samples were dried by the same process and the enzymatic solution was applied just after drying. The moisture content ranged from 4.17% to 4.80% in the malt samples not aspersed (96/0 and 64/0) and from 5.13 to 5.47% in samples which the commercial β-Glucanase was aspersed (Table 2). All the analyses were performed up to 3 days after processing to avoid deterioration due to moisture during storage.

The sample with the shortest germination time affected the grain friability. During the germination stage, the enzymatic complex is activated and hydrolyses the grain cell wall, increasing grain friability [39]. The acceptable malt friability must be higher than 85% [40,41]. Both cultivars germinated for 96 h reached adequate friability degree. But the same was not

observed in samples germinated for 64 h, that shown friability of 68.6% to 73.30%, as shown in Table 2.

Genetic and environmental factors such as soil, weather, temperature and growing region can affect the enzymatic complex expression and consequently, the enzymatic activity. The analyses of eighteen barley genotypes used in the Brazilian beer industry showed variation of β-Glucanase activity from 187.02 to 518.40 $U.kg^{-1}$ [42]. The BRS Caue and Elis cultivars (crop 2013), were developed through genetic improvement. These cultivars presented β-Glucanase activity of 332.3 $U.kg^{-1}$ (BRS Elisa 64/0) and 471.0 $U.kg^{-1}$ (BRS - Caue 96/0). The lowest β-glucanase activity ($p<0.05$) was detected in the BRS – Caue sample germinated for 64 h without enzyme addition. Although the samples germinated for 96 h showed wort with better β-Glucanase activity ($p<0.05$) than samples germinated for 64 h and added with the commercial enzyme (25 to 100 $mg.kg^{-1}$), the last one required shorter germination time (Table 2).

Germination time is important for the endogen β-glucanase expression in the grain. The β-Glucan concentration decrease was correlated to the concentration β-Glucanase added in the malt (Figure 1). Samples germinated for 64 h did not show significant differences ($p>0.05$) in β-glucanase activity between different added enzymes concentration (25, 50 or 100 $mg.kg^{-1}$). This difference is due the differences in the analyses. β-Glucanase activity was analyzed direct in the malt using the azo barley β-Glucan method [22], which do not require a mashing step. The mashing step during β-Glucans analyses [23] allowed enzymes to be activated, including added β-Glucanase, resulting in lower β-Glucans content.

The endogenous and exogenous enzymes (commercial) during mashing led to a decrease in β-Glucan and viscosity parameters.

Germination time is important to reducing sugars content as well as Maillard reaction substrates, and beer color. The longest germination time (96 h) led to higher wort cooking color values (6.13 and 6.20 EBC) when compared to samples germinated for 64 h (5.60 and 5.67 EBC) in both cultivars (Table 3). It is already known that the wort cooking color directly affects the beer color [43].

The values of extract or the fine milling extract (FME) are very important during selection of a malt cultivar, values > 80.0% are recommended for brewing malt [44]. Extract consumption by the embryo is demanding for grain transformation into a new plant. Germination time 64 h was enough to get FME values adequate for BRS-Caue and Elis, as shown in Table 3.

The diastatic power expresses the amount of produced enzyme and the activity during malting. These enzymes are crucial for reducing sugar production, which are essential for yeast energetic metabolism in beer production. Wort presenting diastatic power higher than 200 ^{0}WK is considered suitable for brewing [45]. All wort samples presented diastatic power values > 200 ^{0}WK. Samples germinated for 96 h showed higher diastatic power (281.0 and 304.3 ^{0}WK) ($p<0.05$), than 64 h germinated samples (229.7 and 286.0 ^{0}WK) for both cultivars (Table 3).

The wort pH value ranged from 5.83 to 6.04 for BRS-Caue and Elis, respectively. These values are close to those recommended for brewer's malt – from 5.9 to 6.0 [41]. These pH

values were adequate for the commercial β-Glucanase activity, the supplier recommended a pH range between of 4.0 to 7.0 (AB-Enzyme).

The viscosity of the wort depends on the β-Glucan content [46,10]. High viscosity affects the rheological properties of wort and can cause high turbidity and filtration issues during brewing [47,10]. Samples germinated for 96 h and 64 h added with the commercial enzyme showed viscosity values classified from very good (≤1.53 mPa.s) to good (1.53 to 1.57 mPa.s). The samples germinated for 96 h resulted in wort with better viscosity ≤1.47 mPa.s (Table 3). On the other hand, samples germinated for 64 h without enzyme addition (64/0) presented viscosity classified just as satisfactory (1.58 to 1.61 mPa.s) [46,10].

Some studies investigated de production of beer directly from barley, without malting process [48,49,50]. Although the possibility of producing beer with a mixture containing up to 50% malt and barley was already studied [13], however the consumer acceptability of beer produced with unmalted barley is uncertain. These studies are from 1970s – 1980s. Currently, the chance of getting a good product using raw barley would be greater, considering the genetic improvement of barley.

Goode *et al* [9] evaluated a β-Glucanase of *Bacillus subtilis* (Bioglucanase B10L) in unmalted barley. This commercial β-Glucanase reduced the β-Glucan content and showed little impact on mash filtration. Concentrations from 0.5 to 20.0 BG $U.g^{-1}$ reduced wort viscosity from 1.80 mPa.s, to 1.79 to 1.74 mPa.s of unmalted barley. The wort color ranged from 5.16 to 5.49 EBC and the extract, from 79.3% to 80.5% in unmalted barley added with 20.0 BG $U.g^{-1}$ of Bioglucanase B10L [9]. The barley cultivar (Optic, Irish, harvest 2001) used by these authors presented β-glucan content (2.84%) lower than that in BRS-Caue and Elis, whose barley presented 3.72% and 3.37% of β-Glucans before malting (Table1).

The enzymatic formulation OndeaPro® (Novozymes), composed of α-amylase, β-glucanase, xylanase, proteinase, pullulase and lipase was applied in raw barley [10]. They observed a high wort viscosity (2.09 mPa.s). Although the beer presented a good-foam low FAN concentration and diacetyl effect was observed using this formulation. In the raw barley, a concentration of 500 $mg.kg^{-1}$ of OndeaPro® was required to obtain beer with organoleptic quality similar to beer produced with malted barley [10]. In our work, the partial malting (64 h germination) of BRS-Caue and Elis required β-Glucanase concentrations approximately 10 to 20 times lower the applied by these authors. Malting stage elimination reduces process costs and quickly fulfills the demand for raw material in the brewing industry. But, cost evaluations show that the adjunct prices, as enzyme addition, must be considered.

Muller & Methner [11], compared the conventional malting process [51] and the process under different maceration conditions at germination time 73-74 h. This modification in the process allowed more energy eficiency and better malting yield. Significant cost savings of € 1.27 to 4.87 Euros per malt ton were obtained when compared to the conventional method [11]. In our work the germination time was reduced from 96 to 64 h. Even though use of commercial β-Glucanase represents an additional cost, the difference of 32 h in the process must offset the expenses, since it was possible to produce a good quality malt to meet the high demand of the brewing industry.

Beer produced using malt germinated for 64 h and added of 50 $mg.kg^{-1}$ commercial β-Glucanase (64 /50, Table 4) was that presented most similar to the conventional sample

96/00 ($p>0.05$). These two samples did not differ in alcohol, soluble solids and real extract content. The acceptable value of beer pH is about 4.3 to 4.6 [10]. The pH of the beer samples ranged from 4.26 (96/00) to 4.51 (64/50) (Table 4). Beer elaborated with 100% raw barley added with the enzyme OndeaPro® resulted in higher pH values from 4.7 to 4.72 [10]. The malt germination is an important step to produce beer with appropriate pH values.

The highest ethanol production (6.87%) was observed in the sample whose soluble solids (SS) content was higher (64 /25, Table 4). The content of SS of the samples ranged from 6.48ºBrix (64/50) to 8.40. (64/25). The high SS is a result of a syrup of sucrose and lemon juice addition. The syrup is added to improve the carbonation process [52]. The added sucrose was fermented and resulted in the high ethanol production found in the sample (64/25, Table 4).

All beer samples (96/00, 64/00, 64/25 and 64/50) presented microbiological counting lower that 1 Log UFC.mL^{-1} in mesophilic, *Staphylococcus aureus*, molds and yeasts, total and thermotolerant coliforms. Thus, the craft beer samples presented proper microbiological quality for consumption [53]. Then, the beer samples were evaluated by sensory analyses in order to verify consumers' perception about the tested formulations.

The individual performance evaluation considered two factors (sample and taster), and $p = 0.1676$ value ($p > 0.05$). This confirms the reproducibility and repeatability between the final scores of panelists, according to the results of the variance of the sensorial analyzes shown in Table 5.

The BRS-Caue wort presented viscosity ranged from 1.45 to 1.57 mPa.s (Table 3). These parameter have direct influence on turbidity. The highest turbidity was visually detected by tasters in the sample germinated for 64 h and 50 mg.kg^{-1} of enzyme (Table 6).

Beer foam is one of the most important sensory quality parameters, since it requires good formation, texture, stability and adherence to the glass [54]. Foam stability is related to higher β-Glucan and coagulable nitrogen content in beer [10]. The best scores for formation and stability were observed in the 96/00 and 64/50 beer formulations. The scores on these two samples were very close, except for the aroma, the 64/50 was the less aromatic. However, the aroma did not negatively affect the overall evaluation of the 64/00 formulation, since there was no sensorial difference for this attribute among all the different samples analyzed.

A concentration of 31.39 mg.kg^{-1} of commercial β-Glucanase would be enough to reach β-Glucan content =178 mg.L^{-1} (Figure 1). But the statistical treatment of the sensorial results showed that the addition of a higher concentration of β-Glucanase (50 mg.kg^{-1}) and the reduction of the germination time from 96 to 64 hours did not negatively interfere in the sensorial characteristics of the beer, ensuring a good consumer acceptance. On the other hand, beer produced with malt germinated for 64 h without enzyme addition presented moderate color, gloss and body, low aroma content, foam formation and stability, and poor taste. Thus, according to Garcia-Villalba *et al* [55] and Fumi *et al* [56] this formulation did not present sensorial characteristics expected for a beer, since for these authors beer taste must be suitable for the type of beer and is characterized by aroma and palatefulness, the sparkle and the bitter taste.

CONCLUSION

In this work we showed that an acceleration of malting process is possible, reducing germination time to 64 h and adding β -Glucanase. Previous characterization of barley cultivars is important to preview the concentration necessary of commercial enzyme to achieve the quality demanded by the brewers. But the analysis of the final product determines adjusts in the β -Glucanase necessary to produce beer with good acceptance by the consumer. Considering the cultivars currently used commercially in Brazil, the 50 $mg.kg^{-1}$ is enough to produce a good quality malt, reducing germination time from 96 h to 64 h. The reduction of 32 h in the process of germination of the brewing malt shown in this work represents an increase in the production capacity, considering the high malt demands of the breweries in the country.

Conflicts of Interest: "The authors declare no conflict of interest."

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