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Optimization of Fermentable Sugars Production from Stale Bread using Response Surface Methodology

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ABSTRACT

Background: Bread is the major waste product worldwide. It contains a considerable amount of starch, which is easily converted into sugars using enzymes. The aim of this study was to produce fermentable sugars from stale bread using barley malt as substitute of commercial enzymes.

Methods: Response surface methodology based on Box-Behnken design was applied to optimize the parameters of enzymatic hydrolysis such as malt content (1.25, 2.5 and 3.75%), hydrolysis temperature (55, 62.5 and 70°C) and hydrolysis time (1, 1.5 and 2 h) to maximize fermentable sugars yield.

Result: This study showed that malt content and hydrolysis temperature had a significant effect on fermentable sugars yield that increased with the increase in these two parameters. In contrast, hydrolysis time had no significant effect on fermentable sugars yield. The optimal parameters were 2.75% for malt content, 64.41°C for hydrolysis temperature and 1.31 h for hydrolysis time. At these optimal conditions, the fermentable sugars yield was at maximum reaching 63.21%. The results of this study suggested that barley malt could potentially be used to substitute commercial enzymes.

Key words: Box-behnken design, Fermentable sugars, Hydrolysis parameters, Optimization, Stale bread.

INTRODUCTION

Food waste and loss has become a global problem in recent years. According to FAO (2013), every year, a third of the total amount of food produced for human use, around 1.3 billion tons, is lost or wasted. A significant part of wasted food is cereals and bakery by-products (Xue *et al*, 2017).

Bread is a staple food in many cultures around the world and is the most widely consumed floury product. However, the amount of bread wasted each day around the world throughout in life cycle, from production to distribution, is expected to be hundreds of tons, presenting both economic and environmental concerns (Gül *et al*., 2003; Verni *et al*., 2020). Despite the use of a small portion of bread waste for animal feed, the majority ends up in landfills.

Bread is an important source of energy due to its high content of easily digestible starch (39.3 to 42.7 g/100 g), making it a potential bio-resource for the production of fermentable sugars that are precursors to valuable bio-products through the fermentation process (Mckevith, 2004; Sükrü Demirci *et al*., 2017). As a result, bread waste provides an important fact as a potential carbon source for innovative industrial processes and several alternative pathways to utilize bread waste have been developed (Narisetty *et al*., 2021). The production of nutrient rich hydrolysate from bread waste involves the use of commercial enzymes that can be very expensive (Melikoglu and Webb, 2013).

Among cereals, barley possesses the highest diastasic activity when it is properly germinated. The most important physiological processes associated with the germination phase are the synthesis of amylases, proteases and other endogenous hydrolytic enzymes (Gebeyaw, 2021; Serna-Saldivar, 2012; Baranwal, 2017).

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The objective of this study was to investigate the effect of malted barley as substitute for commercial enzyme for the production of fermentable sugars from stale bread, as well as to optimize the hydrolysis conditions using response surface methodology.

MATERIALS AND METHODS

The experiment was conducted at LAPAPEZA laboratory, Institute of Veterinary Sciences and Agricultural Sciences, University of Batna 1 in March 2022.

Stale bread (without mold spoilage sign) was obtained from local bakeries in Collo (Skikda province, Algeria) as shown in Fig 1. It was sundried on aluminum trays for 7 days and grounded using laboratory hammer mill. The obtained stale bread powder (SBP) with particles size below 1 mm was stored in hermetic plastic bottles at 4°C. SBP has 6.56± 0.2% of moisture content, 58.56±0.7% of starch and 1.37± 0.01% of total sugars determined in triplicate according to AACC method 44-15A (AACC, 2000), Ewers polarimetric method whereas the starch is released from the sample by boiling in dilute hydrochloric acid HCl (ISO, 1997) and Anthrone assay (Laurentin *et al*, 2003) respectively.

Barley grains were purchased from local market (Collo, Algeria), cleaned, washed and dried.

A sample of 1 kg barley grains were soaked in water (1:3 w/v) for 72 h at ambient temperature (20°C) with continuous water replacement every 12 hours. Soaked barley grains were then placed in a sprouter at 30°C for 4 days. The sprouter contained a thin layer of 5 mm thickness of hydrophilic cotton moisturized with distilled water. The green malt was obtained when the acrospires reached about 5 mm and the rootlets 10 mm. Green malt was then laid into trays and dried for a full day in air oven at 40°C. Rootlets, acrospires and not germinated grains were discarded and the obtained malt was kept in opaque glass bottles at room temperature.

SBP was added to distilled water at the ratio of 25% (w/v) and pH was adjusted to 5.5 using HCL or NAOH (2M). The mixture was maintained at 80°C for 2 h to enable starch gelatinization. After cooling the suspension to the suitable temperature (55, 62.5 and 70°C), the malt was added in ratio of 1.25, 2.5 and 3.75% (w/v), then the enzymatic hydrolysis of starch was carried out for 1, 1.5 and 2 h. the resulted fermentable sugars were determined using DNS method (Miller, 1959). Fig 2 summarized the different steps of fermentable sugars production.

Response surface methodology was used to investigate the effect of hydrolysis parameters on fermentable sugars yield (FSY). In this study, 3 factor, 3 levels Box-Behnken experimental design (Box and Behnken, 1960) with three replicates at the center point was applied.

The actual variables were malt content, hydrolysis temperature and hydrolysis time coded as X_1, X_2 and X_3 respectively at three levels for each variable as follows: 1.25,

Fig 1: Stale bread collected.

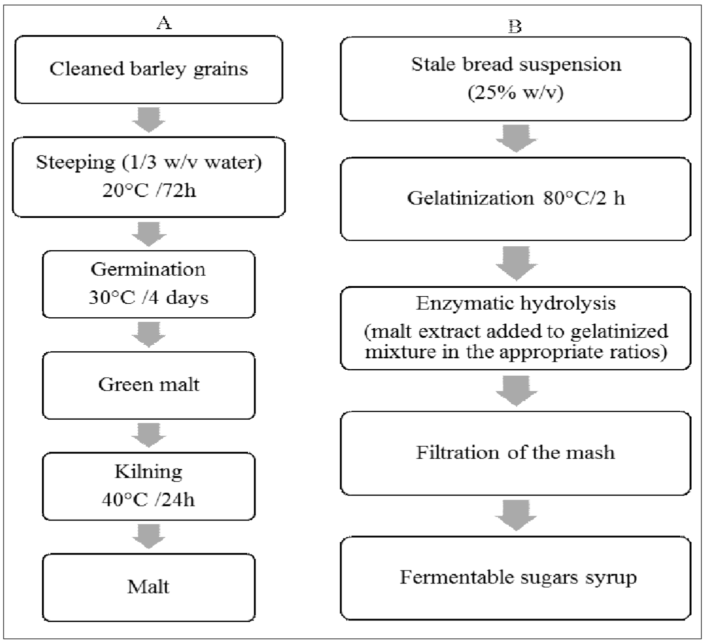


Fig 2: Diagram of malt preparation and fermentable sugars production.

2.5 and 3.75% for malt content, 55, 62.5 and 70°C for hydrolysis temperature and 1, 1.5 and 2 h for hydrolysis time, while the response was FSY coded as Y. The order of experiments was fully randomized (Table1).

A polynomial second degree quadratic equation for this model is represented by equation 1:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad \ldots (1)$$

Where,

Y = Predicted response (FSY).

b_0 = Average value of the response at the center point of the design.

b_1, b_2, b_3 = Linear terms.

b_{12}, b_{13} and b_{23} = Interaction terms.

b_{11}, b_{22} and b_{33} = Quadratic terms.

The optimum values of the variables were obtained by analyzing three-dimensional plots and desirability function. After optimization, experiments were conducted to validate the predicted FSY under the optimum hydrolysis conditions predicted by the statistical model.

Design Expert software (Version, 11.0, Stat-Ease. USA) was employed for experimental design, data analysis and model building. The coefficient of determination (R^2) was used to validate the model and an F-test ascertained its statistical significance. Models were considered significant at p values <0.05.

RESULTS AND DISCUSSION

Model adequacy

F-test value of the regression model was 14.47 with p-value of 0.0045 including that the model is statistically significant. A lack of fit p-value greater than 0.05 further confirmed the adequacy of model fitting (Table 2) and a satisfactory value of coefficient of determination (R^2=0.963) indicates that 96.3% of the variability in the response could be explained by the statistical model.

Effect of processing variables on fermentable sugars yield

The results of the different experiments are presented in Table 3.

The mathematical model was expressed by equation 2:

$$FSY= -1204.29 + 16.64X_1 + 36.88X_2 + 85.71X_3 + 0.113X_1X_2 + 1.748X_1X_3 - 1.143X_2X_3 - 4.757X_1X_1 - 0.277X_2X_2 - 6.64X_3X_3 \quad \ldots (2)$$

Malt content (X_1) showed a positive significant effect on FSY. This increased with the increase of malt content up to 61.79% (Fig 3 A and B). This result could be explained by the proportional relation between enzyme concentration and the obtained hydrolysates (Robinson, 2015). Further increase in malt content resulted in a decreased in FSY, which may be due to the feedback inhibition of produced fermentable sugars or the presence of enzyme inhibitors since wheat flour contains endogenous amylase inhibitors as reported by Hammer (1995). Ebrahimi *et al* (2008) showed that the increase in amylase activity due the increase in temperature did not increase the degree of solids dissolution.

Hydrolysis temperature (X_2) had a great positive effect on FSY; from 55°C to 64°C, there was an increase in FSY and beyond 64°C, FSY decreased (Fig 3 A and C). This finding is related to the optimal temperature of amylolytic stage. According to Evans *et al* (2005), the optimal temperature of enzymatic hydrolysis ranged from 60 to 65°C.

Fig 3B and C showed that hydrolysis time had no significant effect (p>0.05) on FSY, Olsen (2008), Kuddus (2019) and Sirohi *et al* (2020) have reported similar findings.

Optimal conditions for fermentable sugars production

From the model predictions showed in Fig 4, the optimal conditions to obtain the highest FSY were determined as

Table 1: Box-Behnken experimental design with coded and real values of hydrolysis variables.

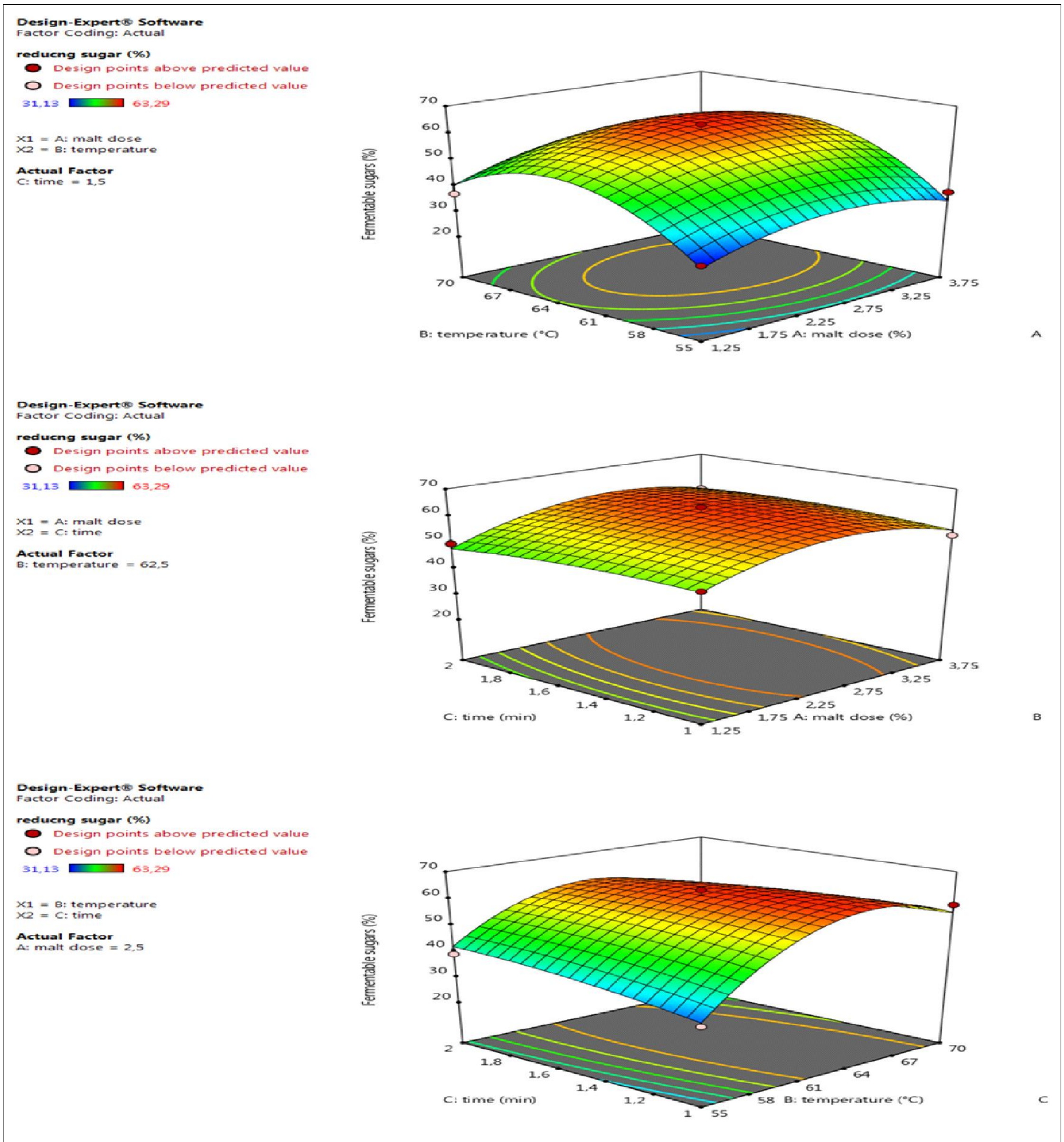
	Coded values			Actual values		
Run	X_1	X_2	X_3	Malt content (%)	Hydrolysis temperature (°C)	Hydrolysis time (h)
1	-1	-1	0	1.25	55	1.5
2	+1	0	-1	3.75	62.5	1
3	0	0	0	2.5	62.5	1.5
4	+1	0	+1	3.75	62.5	2
5	+1	-1	0	3.75	55	1.5
6	-1	+1	0	1.25	70	1.5
7	+1	+1	0	3.75	70	1.5
8	0	0	0	2.5	62.5	1.5
9	0	-1	-1	2.5	55	1
10	0	-1	+1	2.5	55	2
11	-1	0	-1	1.25	62.5	1
12	0	0	0	2.5	62.5	1.5
13	0	+1	-1	2.5	70	1
14	-1	0	+1	1.25	62.5	2
15	0	+1	+1	2.5	70	2

Table 2: Significance of the model regression coefficients.

Source	df	F-value	p-value
Model	9	14.47	0.0045
X_1-Malt content	1	6.96	0.0460
X_2-Hydrolysis temperature	1	25.80	0.0038
X_3-Hydrolysis time	1	0.0880	0.7787
X_1X_2	1	0.3875	0.5609
X_1X_3	1	0.4136	0.5485
X_2X_3	1	6.36	0.0530
X_1^2	1	17.67	0.0085
X_2^2	1	77.69	0.0003
X_3^2	1	0.8343	0.4029
Lack of fit	3	5.48	0.1583

follows: 2.75% for malt content, 64.41°C for hydrolysis temperature and 1.31 h for hydrolysis time with a desirability of 0.97.

After hydrolysis of stale bread powder under the optimal conditions, the FSY was 63.21±1.1% that was not significantly different from the predicted value of 62.49%. This value was lower than 97.04% obtained by Yilmaz *et al.* (2021) and 59.8% reported by Sadaf *et al* (2021) using commercial enzymes. However, our result is close to the outcomes of Nwaga *et al*. (2008) and Torabi *et al*. (2021) who reported a yield of 67.8% and 69.8% respectively. In contrast, it was higher than that obtained by Hudečková *et al*. (2017) and Mihajlovski *et al*. (2020) with 7.75% of total sugars and 1.98% of reducing sugars respectively.



(A); Malt content and temperature. (B); Malt content and time. (C); Temperature and time.

Fig 3: 3D plots of the combined effects of two variables on fermentable sugars yield.

Table 3: Fermentable sugars content according to variables variation.

Run	Malt content (%)	Hydrolysis temperature (°C)	Hydrolysis time (h)	Fermentable sugars (%)
1	1.25	55	1.5	31.13
2	3.75	62.5	1	52.83
3	2.5	62.5	1.5	60.85
4	3.75	62.5	2	55.85
5	3.75	55	1.5	37.58
6	1.25	70	1.5	36.93
7	3.75	70	1.5	47.61
8	2.5	62.5	1.5	63.29
9	2.5	55	1	32.73
10	2.5	55	2	39.04
11	1.25	62.5	1	50.90
12	2.5	62.5	1.5	59.85
13	2.5	70	1	57.79
14	1.25	62.5	2	49.55
15	2.5	70	2	46.96

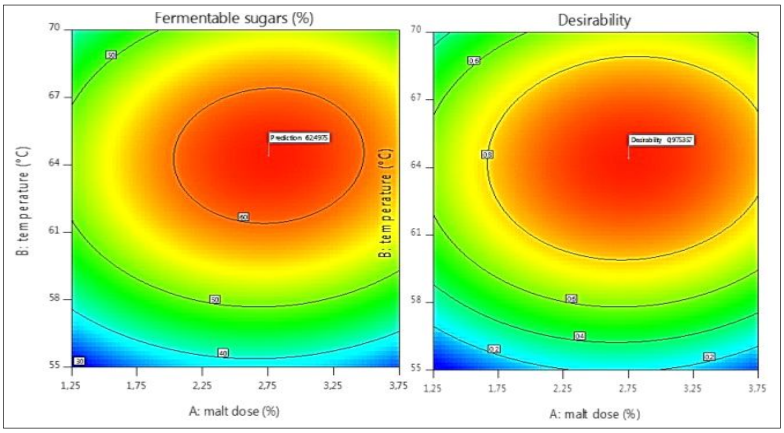


Fig 4: Plots of optimization of hydrolysis parameters.

CONCLUSION

The effect of hydrolysis parameters on fermentable sugars yield from stale bread was studied using response surface methodology based on a Box-Behnken design. Both malt content and hydrolysis temperature highlighted a significant effect on fermentable sugars yield.

This study presented the possibility of using stale bread as bio-resource for the production of fermentable sugars (63.21%) suitable as an added-value product by enzymatic hydrolysis with 2.75% of malted barley (instead of commercial enzyme) at 64.41°C during 1.31 h. Response surface methodology successfully optimized the hydrolysis conditions studied and then maximized the level of fermentable sugars.

Conflict of interest: None.

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