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PRODUCTION OF LACTIC ACID FROM BREWER'S SPENT GRAIN BY USING DIFFERENT NITROGEN SOURCES AND DIFFERENT *LACTOBACILLUS PENTOSUS* STRAINS

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ABSTRACT

Brewer's spent grain (BSG), which is released as a by-product from brewing process, has attracted the attention of scientific research as a low-cost raw material for biotechnological applications. In this study, BSG was used as fermentation medium for production of lactic acid by using different *Lb. pentosus* strains *(Lactobacillus pentosus O157, Lactobacillus pentosus O18, Lactobacillus pentosus O19).* Initially, BSG was diluted with 1.25% of H_2SO_4, a solid/liquid ratio (1:8 g/g) for hydrolysis of the medium containing hemicellulose sugars. Lactic acid production conditions by using *Lactobacillus pentosus* strains were optimized as: pH 6.0, 96 hours at 31°C and 20.0 % of xylose concentration. The maximum amount and yield for lactic acid (22.4 g/L; 13.80 %) was produced with a strain of *Lactobacillus pentosus O18*, respectively**.** In the second part of the study, different nitrogen sources (yeast extract, corn steep water, peptone, urea and tryptone) were used for production of lactic acid by using *Lactobacillus pentosus O18* strain. In order to meet the complex organic matter needs of lactic acid bacteria, different nitrogen sources were calculated on the basis of equivalent nitrogen (10 g/L of yeast extract) amount. Among used sources the highest yield was obtained with yeast extract followed by peptone.

Keywords: lactic acid; fermentation; brewer's spent grain

INTRODUCTION

For the low cost in industrial production, it is an advantageous practice of using some materials such as molasses, rice husk, wheat bran, vegetable and fruit residues and brewer's spent grain that are economically and easily supplied. Additionally they are ecologically advantageous since these waste products are coming as by-products from industrial and agricultural production. In recent years among this food waste brewer's spent grain take more interest.

Beer is a popular alcoholic drink produced by fermentation of the aqueous liquid extract of malted barley by using hops (**Chen *et al.*, 2017**). Malting and brewing are the two basic processes of beer production. Brewing, which is one of these two processes, consists of four stages in itself: wort production that includes mashing and boiling, fermentation, maturation and filtration/stabilization (**Wunderlich *et al.*, 2009**). Mashing is the process of mixing malt with hot water to obtain soluble malt, also known as wort, by breaking down proteins and starch by enzymes. After this stage, wort and brewer's spent grain (BSG) are separated from each other. BSG is the most important valuable by-product of beer production **(Proaño *et al.*, 2020).**

The use of biomass with lignocellulosic composition for the production of high value-added products is increasing significantly. BSG's global production is estimated at 180 million tons per year, most of it being discarded or used as a low-value animal feed (**Rojas-Chamorro *et al.*, 2020**). Due to it's composition, it is a rich by-product containing valuable components. More than 0.5 million BSG occur annually from beer production (**Mussatto *et al.*, 2009).** For production of 1 L beer, 200 grams of BSG is originated **(Akermann *et al.*, 2020).** This covers approximately 80.0 % of waste produced during beer production.

Since BSG contains cellulose 16.8 (g/g), hemicellulose 28.4 (g/g), lignin 27.8 (g/g) and other compounds 27.0 (g/g) could be used in the food industry as a fermentation medium, especially lactic acid production **(Mussatto *et al.*, 2009**). Some pre-treatments are applied to BSG so that it can be utilized by microorganisms. Some compounds are produced during fermentation by releasing structural carbohydrates known as "BSG hydrolysates" from enzymatically, chemically or physically pretreated BSG. Since BSG has a high sugar content, it has been stated that especially ethanol, butanol and lactic acid could be produced by fermentation process **(Akermann *et al.*, 2020).** BSG is a highly nutritional substance due to it is cellulose, hemicellulose, lignin and high protein contents. The most common monosaccharides in BSG are xylose, arabinose and glucose **(Mussatto, 2009; Patrignani, 2021).** There are also vitamins and minerals. The main vitamins found in BSG are biotin, choline, folic acid, niacin, riboflavin and minerals namely magnesium, calcium cobalt, potassium, manganese, sodium and sulphur (**Khidzir *et al.*, 2010).**

BSG is a lignocellulosic biomass composed of fiber (cellulose and hemicellulose), protein and lignin. The fiber content of BSG is 50% based on dry weight. Protein ratio can also reach up to 30.0%. In addition, hemicellulose in the content of BSG is found up to 40% on dry weight basis and became its main component. Due to its high fiber and protein content, this waste by-product has become a preferred raw material for many foods and non-foods applications (**Mussatto, 2009; Patrignani, 2021; Kieran, 2016).**

Even BSG is rich valuable compounds, it is generally used as animal feed because it contains essential amino acids for animal nutrition (70.0%), some for storage (20.0%), and a small amount is used for biogas plant (10.0%) **(Bianco *et al.* 2020).** So it is of prominent interest for the production and development of food products due to its nutrient content, low cost and abundant availability. Additionally, BSG is becoming progressively important as a source of health-promoting bioactive ingredients. Alternative uses of BSG has become evident not only from the evaluation of this by-product, but also from recycling and reuse of industrial waste and by-products **(Kieran *et al.*, 2016)**. Regarding the food industry, BSG could be used in human and animal nutrition, for production of phenolic acids, xylitol, arabitol, pullulan, bioethanol, enzymes, absorbents and some other compounds, especially lactic acid **(Mussatto, 2013; Tišma, 2018).**

In some studies have been reported that some cultures as *Lactobacillus delbrueckii*, *Lactobacillus pentosus* and *Lactobacillus rhamnosus* could be used for production of lactic acid by using BSG **(Chetrariu and Dabija, 2020).** Lactic acid bacteria can turn hemicellulosic sugars into food additive ingredients found in food waste, such as brewer's spent grain. Complex polymeric components such as cellulose, hemicellulose and lignin must be hydrolyzed and destroyed before this conversion **(Bustos *et al.*, 2004).**In a study related to this topic, 5.4 g/L L-lactic acid was produced using 0.73 g/g glucose by using *Lactobacillus delbrueckii* from brewer's spent grain **(Mussatto *et al.*, 2007).**

Lactic acid bacteria have the ability to produce organoleptically active metabolites as well as lactic acid. These metabolites include organic acids (such as acetic acid and formic acid), esters (such as ethyl acetate) and a wide variety of higher alcohols, aldehydes, ketones, phenolic and heterocyclic compounds **(Dysvik *et al*,. 2019).**

The vast majority of lactic acid bacteria require very different variety of growing factors containing fatty acids, amino acids, vitamins, pyrimidines and purines for their growth and biological activity. Therefore, the substrate composition with which the strain interacts and the nutritional elements needed by the strain substantially affect the wide-ranging performance of the fermentation. By using *L. rhamnosus* was produced large amount of lactic acid L-(+)-LA in all fermentations (98.0 %) models. In a study related to this topic the maximum lactic acid yield

(96.0 %) and volumetric productivity (0.52 g/L· h^{-1}) were achieved when yeast extract was added in the ratio of 2.0 % **(Pejin *et al.*, 2015).** The maximum L-(+)-LA concentration, yield, and volumetric lactic acid productivity during fermentation were reached with the decreasing sugar concentration of 5.4 % and yeast extract substance of 5.0 % in BSG hydrolysate **(Pejin *et al*., 2017).**
In another study, thin stillage (5.0–50.0 %) and glucose addition of brewer's spent grain hydrolysate during batch and fed-batch L-(+)-LA fermentation was investigated. The thin stillage addition considerably increased the free amino nitrogen concentration. A positive correlation was determined between L-(+)-LA concentration and free amino nitrogen. During fed-batch fermentation the highest L-(+)-LA volumetric productivity, yield and concentration 48.02 g/L, 87.8 % and 0.96 g/L h^{-1} were determined, respectively **(Pejin *et al.*, 2019).**
Numerous carbohydrates and nitrogenous substances can be selected as substrates for the production of lactic acid. Substrate selection made and based on features such as yield of high lactic acid, very little by-product output, optimum biomass production, high fermentation rate, less pretreatment, low cost and easy availability. Substrate selection for lactic acid fermentation must be done depending on substrate price, requirement of pretreatment, microorganism and desired product purity **(Moldes *et al.*, 2006).**
Lactic acid has been produced chemically for many years, as well as by fermentation. However, there is a need for low-cost production by fermentation. The use of waste materials from food production as a un expensive carbohydrate source could be provide as a new way for high efficiency production of lactic acid Results demonstrated that different lactic acid bacteria provide different quantities of lactic acid. Additionally different carbohydrate sources effected in a different way on concentration and yield of produced lactic acid. So, there is a need to evaluate more specifically the strain differentiation and suitable additional carbohydrate sources for production of high yield lactic acid by using brewer's spent grain.
So, in this study, the brewer's spent grain obtained during beer production. It was prepared as lignocellulosic substances for fermentation by different *Lb. pentosus* strains. Based on the studies carried out to increase the yield of lactic acid production, different nitrogen sources (yeast extract, corn steep water, peptone, urea and tryptone) were evaluated during fermentation and the best one was determined.

MATERIAL AND METHODS

Preparation of brewer's spent grain hydrolysate

The brewer's spent grain (BSG) is a complex material that included a lot of valuable compounds. Among them in free form are found cellulose, hemicellulose, protein, and lipids. Except these components the brewer's spent grain included some soluble compounds that are in adhered form. These are mainly sugar molecules. In order to liberate them into the solution some pretreatment are required.
As material was used brewer's spent grain (BSG) kindly supplied from Tuborg A.Ş (Turkey). Brewer's spent grain was dried at 65°C for 24-48 hours. After completing the drying process it was grinded to particle size <50 µm. So, before using it as substrate during lactic acid fermentation it was subjected to some preliminary treatment. Then hydrolyses was carried out with H_2SO_4 at 121°C at 121°C for 17 min. **(Mussatto *et al.*, 2006).** After application of acid hydrolysis, two main phases were obtained: solid and liquid part. The brewer's spent grain liquid fraction was used as fermentation media.
In order to propagate the growing of *Lactobacillus pentosus* strains in the media different nitrogen sources were used: yeast extract, corn steep water, peptone, urea and tryptone. Xylose, arabinose and glucose from brewer's spent grain were used as the carbon source.
The amounts of nitrogen sources used in production media are presented in Table 1. The calculations were made for each nitrogen source as an equivalent to the amount of nitrogen present in 10 g/L of yeast extract.

Table 1 Nitrogen sources and their amounts used for development of production media for development of *Lactobacillus pentosus* strains.

Nitrogen Source	Nitrogen (%)	Amount (g/L)
Yeast extract	9.8	10
Corn steep water	6.44	15.2
Peptone	14	7
Urea	47	2.08
Tryptone	12.7	7.71

The amounts of carbon sources present in production media are presented in Table 2.

The medium was concentrated in a hot water bath at 20 °C to bring the sugar content to the desired level. The last xylose content was 20 g/L, the arabinose content was 10.26 g/L and the glucose content was 3.9 g/L.
The pH of the fermentation medium was adjusted to 6.0 by NaOH (Sigma-Aldrich). $CaCO_3$ was used to buffer the pH value. Sterilization of media was fulfilled at 121 °C for 15 minutes.

Table 2 Carbon sources and their amounts used for development of production media for development of *Lactobacillus pentosus* strains.

Carbon sources	Amount (g/L)	The amount after adjustment
Glucose	3.48	3.9
Arabinose	8.98	10.26
Xylose	17.5	20

Microorganism and inoculum cultivation

As cultures were used different *Lactobacillus pentosus* strains. Cultures were kindly supplied from Department of Food Engineering at Pamukkale University. Three different strains were used: *Lactobacillus pentosus O157, Lactobacillus pentosus O18 and Lactobacillus pentosus O19.*
The *Lactobacillus pentosus* cultures were incubated at 37 °C in tubes containing 10 ml MRS broth for 24 h. After this procedure, 1 ml of this cultures were transferred to a new tube containing 10 ml of MRS broth and incubated at the same conditions. After separations, the cultures were incubated as % 2 (v/v).

Fermentation conditions

After pretreated of brewer's spent grain by acid hydrolysis, obtained liquid part was used for fermentation. Hemicellulosidic sugars remaining in the liquid part were used as fermentation medium for lactic acid production. Prepared media of brewer's spent grain (hydrolyzed and adjusted carbon sources) was sterilized (121 °C, 15 min) and enriched with nitrogen sources (121 °C, 15 min). Fermentations were run at 250 ml Erlenmeyer containing 150ml media by using shaking incubator (160 rpm) with adjusted temperate carried out at 37 °C for 5 days. The inoculations (%2 w/v) were done for each *Lactobacillus pentosus* at the same time. Samples were taken through 12 hours intervals and stored at +4 °C in dark glass bottles. After fermentation, obtained fermented media were centrifuged at 4000 g for 20 minutes.

Yield parameters

The effective yield of lactic acid, which is formed as a result of fermentation, is common in percentage of sugar found at the beginning.

Effective efficiency, % = P / S_o
P: Lactic acid, at the end of fermentation, g/L
So: initial carbohydrates, g/L
Conversion yield is defined as the percentage ratio of lactic acid produced to the amount of sugar consumed in fermentation. This value shows how much of the substrate used by bacteria in fermentation is used for lactic acid production. Since the substrate is spent on biomass formation as well as the product, this term in which the product formed per spent substrate is calculated becomes important.

Conversion service, % = P / S_o-S
P: Lactic acid formed at the end of fermentation, g/L
So: initial carbohydrates, g/L
S: Sugar remaining after use at the end of fermentation, g/L
Volumetric efficiency, defined as the amount of lactic acid in unit time, indicates the formation of lactic acid.

Efficiency (g/L/h) = Pt / t
Pt: Concentration of lactic acid in the medium at a given t time
t: time (hour)

Analytical methods

Determination of sugar content

All HPLC analyses in this study were conducted using an HPLC (High Performance Liquid Chromatography- Agilent 1200) system.
The sugar content of the pretreated brewer's spent grain was determined by HPLC. An HPLC equipped with a refractive index detector (RID-10A) and a Biorad Aminex HPX-87H column were used for the determinations. The HPLC conditions were acetonitrile-water (75:25) as mobile phase, flow rate of 0.6 mL/min, column temperature of 45 °C, and injected volume of 20 µL. Glucose, arabinose and xylose standard solutions were prepared at a concentration of 2000 ppm and standard curves were drawn using concentration rates of 5, 10, 15, 20 mg/L**.**

Determination the amount of lactic acid

Lactic acid concentration was determined by high-pressure liquid chromatography (HPLC), using C18 column equipped with HPLC-UV-DAD detector. The conditions for HPLC analyses were done according to **Musatto et al. (2007).** The HPLC conditions were acetonitrile-water (75:25) as mobile phase, particle size (5.0 µL), flow rate of 0.7 mL/min, column temperature of 25 °C, and injected

volume of 10 µL. The lactic acid standards were prepared at a concentration of 1000 ppm and the standard curve was drawn using concentration rates of 5, 10, 15, 20 mg/L.
Samples were analyzed after passing 0.45 µm membrane filter (Sartorius syringe tip filter PTFE). As mobile solution was used 0,01M H_2S0_4 which was degassed in an ultrasonic water bath (Grant) for 15 minutes after filtration (Whatman No 1).

Statistical evaluation

Significant differences between averages were obtained at the 95% significance level. By using a Post-Hoc test, the least significant differences (LSD) test was performed.

RESULTS AND DISCUSSION

The brewer's spent grain, as a by-product of the beer industry, has been pretreated and prepared as lignocellulosic substance for fermentation by *Lb. pentosus* strains. Based on the lack of studies carried out to increase the yield in lactic acid production by different *Lb. pentosus* strains and enriched nutrition media, different nitrogen sources (yeast extract, corn steep water, peptone, urea and tryptone) were added to the fermentation medium in presence of different *Lb. pentosus* strains and their effects were examined.
The main reason for using brewer's spent grain for fermentation by *Lactobacillus pentosus* for lactic acid production was related to the high content of hemicellulosidic substances that could be fermented. So, the main purpose was to evaluate the valorization of BSG during production of lactic acid by different *Lb. pentosus* strains and addition of different nitrogen sources.

The effects of sugar content in brewer's spent grain hydrolysate on lactic acid fermentation

Some microorganisms, such as lactic acid bacteria, could turn hemicellulosic sugars into valuable food ingredients. Among them, *Levilactobacillus brevis, Lactococcus lactis, Lactiplantibacillus plantarum* and other lactic acid strains performed extremely successful metabolic productions **(Wang *et al.*, 2021).** BSG, a by-product of the brewing industry, has pretreated and fermented to produce lactic acid production. As starter culture were used different *Lb. pentosus* strains. In Figure 1 was demonstrated the kinetic profile of lactic acid fermentation done by using BSG.

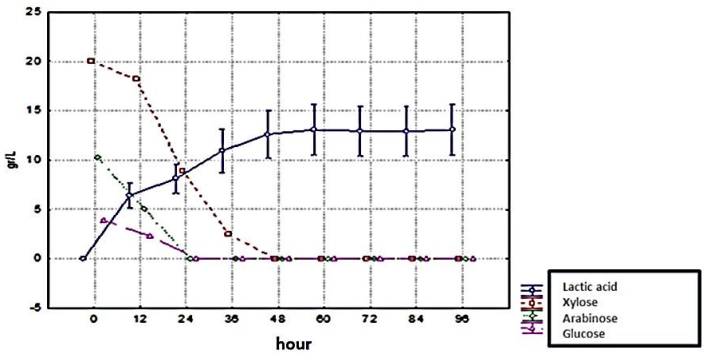

Figure 1 Kinetic profile of lactic acid fermentation

As seen in figure 1, the average and maximum values of lactic acid were obtained as 10 g/L and 22.4 g/L, respectively. The highest value of lactic acid production (22.4 g/L) was reached at 48 hours. In the same period, there was a decrease in xylose, arabinose and glucose values. These results demonstrate that optimum temperature, time for maximum lactic acid is 48h. In the study which 5.4g/L L-lactic acid was produced, 0.73 g/g glucose was consumed and *Lactobacillus delbrueckii* was used as starter culture **(Mussatto *et al.*, 2007).**
It was stated that *L. rhamnosus* could produce a large amount of L-(+)-LA (98.0 %) during fermentation. The maximum L-(+)-LA yield (96.0 %) and volumetric productivity (0.52 g/L· h^{-1}) were achieved when yeast extract was added in the ratio of 2.0 % **(Moldes *et al. 2006*).** The maximum L-(+)-LA concentration, yield and volumetric productivity were reached with the decreasing of sugar concentration up to 5.4 % and yeast extract substance of 5.0 % in BSG hydrolysate **(Pejin *et al.*, 2015).** In fed-batch fermentation the highest L-(+)-LA volumetric productivity 48.02 g/L, yield 87.8 % and 0.96 g/L h^{-1} concentration were determined **(Radosavljević *et al.*, 2017).**
In other study related to lactic acid production by using BSG and *Lactobacillus rhamnosus* ATCC 7469 was determined the effect of extract and sugar consumption during lactic acid fermentation. The result demonstrated that pH had significantly reduced L-(+)-LA content, yield and volumetric efficiency by reducing consumption of sugar. The highest L-(+)-LA yield and volumetric productivity were obtained by reducing the amount of sugar to 54 g/L. The content of L-(+)-LA (39.38 g/L), cell viability of *L. rhamnosus* (9.67 log CFU / mL), yield of L-(+)-LA (91.29 %) and volumetric productivity (1.69 g/L/h), was achieved by reduction of sugar content of 54 g/L and yeast extract content of 50 g/L **(Mussatto and Roberto, 2005) .**
Moldes *et al.* (2016) have demonstrated that *Lb. pentosus* could be used for production lactic acid in presence of different lignocellulosic substances obtained from agricultural waste. In a study the highest lactic acid concentration was obtained in case of production by using barley bark (33 g/L). The highest efficiency of 0.76 g/g was achieved through using of pentoses obtained from grape flakes.

The effects of different Lactobacillus pentosus strains on lactic acid fermentation

In this study was conducted the experiment related to the evolution of the performance of different *Lactobacillus pentosus strains* on lactic acid fermentation. Three cultures strains were used: *Lactobacillus pentosus O157, Lactobacillus pentosus O18* and *Lactobacillus pentosus O19*. The highest lactic acid yield was obtained with the strain named *Lactobacillus pentosus O18* (22.4 g/L 48 hours). The results are visible in Figure 2.

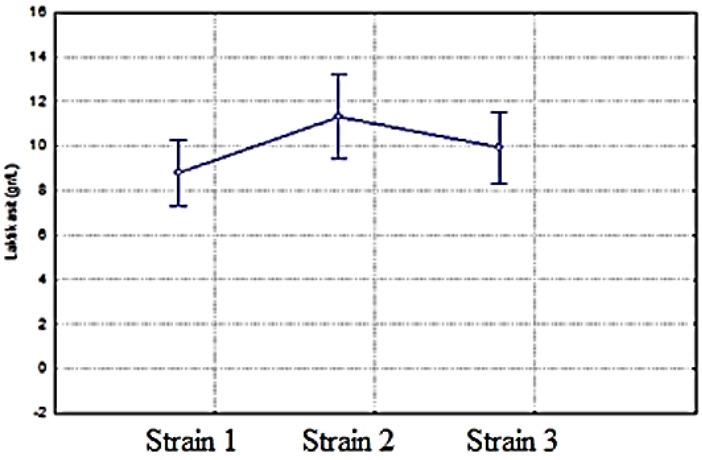

Figure 2 Graphical display of the amount of lactic acid obtained in trials with different strains. Strain 1; *Lactobacillus pentosus O157*; Strain 2; *Lactobacillus pentosus O18*; Strain 3; *Lactobacillus pentosus O19*

As shown in Figure 2, the highest value of lactic acid content was obtained with Strain 2 :(*Lactobacillus pentosus O18*), followed by Strain 3: (*Lactobacillus pentosus O19*) and Strain 1: (*Lactobacillus pentosus O157*). The differences among strains were determined as statistically significant ($p < 0.05$). The yield values of produced lactic acid by using three different strains were calculated and presented in Table 3.

Table 3 Yield values obtained during lactic acid fermentation done by different *Lactobacillus pentosus* strains in presence of brewer's spent grain

Microorganism	Lactic acid (g/L)	Sugar (g/L)	Remaining sugar (g/L)	Effective efficiency (%)	Conversion efficiency (%)	Efficiency (g/L/h)
Lactobacillus pentosus O157	13.6	34.16	7.62	39.81	51.24	0.82
Lactobacillus pentosus O18	22.4	34.16	5.21	65.57	77.35	1.36
Lactobacillus pentosus O19	15.5	34.16	7.21	45.37	57.51	0.94

As demonstrated in Table 3, the best result was obtained with *Lactobacillus pentosus O18 (*strain 2). By using this lactic acid bacteria 65.57 % effective yield and 77.35 % conversion efficiency was obtained. At the end of fermentation 22.4 g/L lactic acid was reached.

Musatto *et al.* (2008) produced 5.4 g/L lactic acid by using *L. debruecki.* In this study brewer's spent grain which contained 50.0% glucose, and no additional nutrients was evaluated. The performance of production 5.4 g/L lactic acid inoculated with *L.debruecki.* In this production, the efficiency of glucose

consumption was determined as 73.0%. Moreover, it was determined that the pH decreases effects glucose consumption and lactic acid concentration.
Garde *et al*. (2002) have produced lactic acid by using *L. brevis* and *L.pentosus* from hemicellulosidic hydrolysate obtained from wheat shell . In a study the amount of 11-12 g / L fermentable sugar was obtained in hydrolysate. While the yield by using *L.brevis* was 51.0 % and the yield by using *L. pentosus* was 61.0 %. By using both microorganisms the yield increased up to 95.0 %.
Our data related to the consumption of carbohydrates during lactic acid production by using *Lactobacillus pentosus O18* are exposed by Table 4.

Table 4 Data related to consumption of carbohydrates during lactic acid production

Time(hour)	Xylose (g/L)	Arabinose(g/L)	Glucose (g/L)
0.	20	10.26	3.9
12.	18.2	5.14	2.3
24.	8.9	2.4	1.2
36.	2.5	2.4	1.2
48.	1.6	2.4	1.2
60.	1.6	2.4	1.2
72.	1.6	2.4	1.2

As seen in Table 4, concertation of xylose reached 1.6 g/ L at 48h and become stable after this time. The results related to arabinose and glucose are similar. Their values become stable after 24h of fermentation.

The effect of different nitrogen sources addition on lactic acid fermentation

Yeast extract, corn steep water, peptone, tryptone, urea were used as different nitrogen sources. The effects of these nitrogen sources on lactic acid production were examined. The distribution of lactic acid production according to different nitrogen sources is given in Figure 3. Considering the distribution, clusters at the highest point were obtained by using yeast extract. The results obtained were found statistically significant ($p < 0.05$).

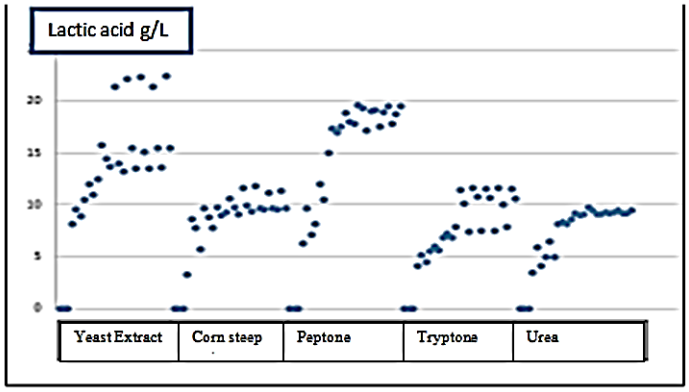

Figure 3 Lactic acid concentrations by using different nitrogen sources

Nitrogen source is an important parameter in the production of lactic acid. Yeast extract addition has been found as the best additive for lactic acid production. Lactic acid production efficiency could be increased depending on changes such as nitrogen sources, carbon sources, temperature, fermentation type and time, pH, neutralization agents and aeration **(Toptaş *et al*., 2012).**
According to **Hu *et al.* (2016)** the lactic acid produced by using different nitrogen sources (yeast extract, tryptone, beef extract, acid hydrolyzed casein, or corn steep powder) was evaluated, the yields of lactic acid were 0.94, 0.47, 0.47, 0.61 and 0.87 g/g, respectively. The productivity of lactic acid was 0.65, 0.33, 0.42, 0.37 0.60 g/L/h respectively. The medium containing yeast extract has been chosen as the most suitable nitrogen source.
This finding supports our results that even by using different *Lb. pentosus* strains the most suitable nitrogen sources was determined yeast extract component.

The effect of yeast extract addition on lactic acid fermentation

The yeast extract was added to the medium used during fermentation to obtain suitable medium. The effect of different *Lb. pentosus* strains (*Lactobacillus pentosus O157, Lactobacillus pentosus O18, Lactobacillus pentosus O19)* in case of using yeast extract were presented in Figure 4.

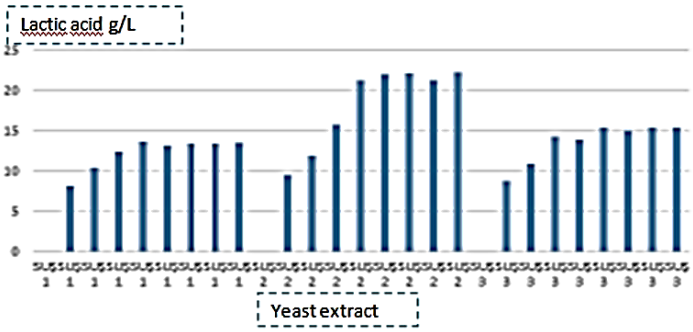

Figure 4 Lactic acid concentrations obtained as a result of using different strains during fermentation Strain 1 (*Lactobacillus pentosus O157*), Strain 2 (*Lactobacillus pentosus O18*) and Strain 3 (*Lactobacillus pentosus O19*) in presence of yeast extract

As can be seen from Figure 4 the highest value of lactic acid (22.4 g/L) was obtained by using *Lactobacillus pentosus O 18* strain in presence of yeast extract. The least significant differences (LSD) analyses were used to determine the relationship among samples treated with yeast extract and different strains during fermentation Obtained differences were determined as statistically significant ($p < 0.05$).

CONCLUSIONS

The brewer's spent grain has been used as lignocellulosic substances for lactic acid fermentation by different *Lb. pentosus* (*Lactobacillus pentosus O157, Lactobacillus pentosus O18, and Lactobacillus pentosus O19)* and different nitrogen sources (yeast extract, corn water, peptone, tryptone and urea).
The highest amount of lactic acid (22,4 g/L) was produced with *Lactobacillus pentosus O18*. The highest yield (13.80 %) was reached by *Lactobacillus pentosus O18*. Five different sources of nitrogen (yeast extract, corn water, peptone, tryptone and urea) were used for fermentation medium. The highest yield was obtained with yeast extract and the second one with peptone. The impact on production of other nitrogen sources were found to be close to each other. The result demonstrated that BSG enriched with yeast extract and subjected to lactic acid fermentation by *Lactobacillus pentosus O18* could provide high yield of lactic acid.

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