

Biotechnological Production of Xylitol from Banana Peel and Its Impact on Physicochemical Properties of Rusks

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ABSTRACT

Xylitol is the first rare sugar that has global market due to having beneficial health properties and being an alternative to current conventional sweeteners. Biotechnological production of xylitol by *Candida tropicalis* DSM 7524 as an alternative to chemical method for the fermentation of xylose to xylitol was studied. Banana peel was used as a substrate for xylitol production. Hydrolysate was detoxified by neutralization, activated charcoal treatment and vacuum evaporation. Effect of pH was tested for *C. tropicalis* at three different levels and pH value of 2.5 was found to be the best; producing 24.7 g L⁻¹ xylitol. Rusks were prepared by replacing sucrose with xylitol at different levels. Physicochemical analysis of rusks at different intervals of storage i.e. 0, 10, 20 and 30 days was carried out. Hardness decreased significantly ($P < 0.05$) from the treatment having 100% sucrose (4,950.6 g) to 100% xylitol (3,090.3 g) upon replacing sucrose with xylitol, while fracturability value increased from 71.90 to 74.26 mm for the treatment containing 100% xylitol. Color value and water activity of rusks increased significantly with the replacement of sucrose with xylitol. The increase in moisture content and decreasing trend in other parameters with storage were observed in rusks. Xylitol has low calorific value as compared to sucrose so it can be incorporated into dietetic foods which may help in controlling sugar level in diabetic patients.

Keywords: Banana peel, Biotechnology, Rusk, Texture, Xylitol.

INTRODUCTION

Xylitol known as birch sugar is obtained from the reduction of xylose. It is naturally found in the fiber of many fruits and vegetables including berries, corn husks, oats and mushrooms having sweetness level similar to that of sucrose. It has 40% fewer calories and 75% fewer carbohydrates (Emodi, 1978). It is metabolized and absorbed very slowly as compared to sucrose in the human body. One-third of the xylitol consumed by the body is absorbed in liver, the remaining two-thirds find their way to intestinal tract, where xylitol is attacked by the intestinal bacteria and is broken down into short chain fatty acids (Pepper and Olinger, 1988). Xylitol

metabolizes independent of insulin, so it can be used as a sugar substitute to treat diabetic patients (Makinen, 1978).

Xylitol is currently being produced from lignocellulosic materials of agricultural wastes. Lignocellulosic materials are available abundantly and are renewable to serve the purpose of the substrate for xylitol production. These lignocellulosics obtained from agricultural wastes include plant parts (seeds, stalks) and processing by-products (distiller's grain, corn solubles) (Smith *et al.*, 1987). Lignocellulosics are the major structural components of the woody and non-woody plants. Cellulose, hemicellulose and lignin, are the major components of lignocellulosics and vary with plant species (Malherbe and Cloete, 2003). Banana peels

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are composed of 6-12% lignin, 7.6-9.6% cellulose and 6.4-9.4% hemicellulose (Debabandya *et al.*, 2010); corn cobs have 45% cellulose, 35% hemicellulose and 15% lignin while wheat straw and rice straw have 30%, 32% cellulose, 50%, 24% hemicellulose and 15%, 18% lignin, respectively (Howard *et al.*, 2003).

Basically, Pakistan is an agricultural country; agriculture is the largest sector of the economy of Pakistan and it is usually divided into four main sub-sectors: crops, livestock, forestry and fisheries. The livelihood of 44.7% of the total employable labor force is dependent on agriculture and it contributes up to 21.8% to the GDP of Pakistan.

Banana ranks second among fruit production after citrus, contributing to about 16% of the world's total fruit production. It is fourth on the list of the developing world's most important food crops, after rice, wheat and maize (Anon, 2002). The total production of banana in Pakistan during 2008-09 was 150 thousand tons. Banana peel is 18-20% of the fruit, considering waste. It is also a good source of lignocellulosic materials and contains 91% organic matter out of which 59% is carbohydrate (Anhwange, 2008). Banana peel hemicellulose consists of *L*-arabinose (52.5%), *D*-xylose (25.6%) and *D*-galactose (21.6%) (Whistler, 1998). Due to the significant production of banana in Pakistan, it is imperative to pay attention to utilizing banana peels, currently ending up in the waste stream, which may prove to be a good source of xylose and convert it into a value added product; xylitol.

In baked goods, sweeteners provide sweetness, texture, humectancy (Salminen and Halikainen, 1990) and increase shelf life (Hett and Butterill, 1999). Xylitol finds its application in confectionary industry; especially sugar free products and non-cariogenic chewing gums (Makinen *et al.*, 1995). It also finds its uses in diabetic and dietetic foods. It is extensively used as an ingredient in chewing gums, candy, soft

drinks, ice cream and oral hygiene products (Aminoff *et al.*, 1978).

This research work was planned to utilize waste banana peels for the production of xylitol and its utilization in rusks to determine its impact on physico-chemical properties.

MATERIALS AND METHODS

Procurement of Raw Material

Banana, wheat flour and yeast were purchased locally from local market of Faisalabad. Analytical grade chemicals were purchased from Sigma-Aldrich Chemie GmbH, Germany.

Chemical Analysis of Banana Peel

Banana peel was analyzed for moisture, crude protein, crude fat, crude fiber, nitrogen free extract and total ash content according to methods described in AOAC (2000).

Determination of Insoluble Dietary Fibers
Insoluble dietary fibers were determined by the Van Soest and Wine's method (1968).

Preparation of Banana Peel Hydrolysate

Banana peels were oven dried at a temperature of 105°C for 24 hours, ground in a mixer down to fine particles and then passed through a mesh screen. The ground material was mixed with 1% H₂SO₄ at a ratio of 1 g of biomass to 10 mL of acid solution and autoclaved for 1 hour at 121°C and the aqueous solution was separated. The hydrolysate was neutralized using Ca(OH)₂ and precipitates were removed by centrifugation. The recovered liquid was treated with active charcoal to remove inhibitory substances (Sreenivas *et al.*, 2006). Activated carbon treatment of the hydrolysate was performed in an orbital

shaker at 200 rpm for 1 hour and temperature was adjusted to 30°C. Activated carbon (10 g 100 mL⁻¹) was used for the purpose of removing inhibitors (Pandey *et al.*, 2000).

Microorganism and Culture Conditions

Candida tropicalis DSM 7524 was procured commercially and grown on a medium having the following composition; Yeast Nitrogen Base 6.7, D-xylose 20 and agar 20 g L⁻¹.

Inoculum Preparation

The inoculum (25 mL) was prepared in 100 mL Erlenmyer flasks having the following media components (NH₄)₂SO₄ (5 g L⁻¹), MgSO₄·7H₂O (0.5 g L⁻¹), KH₂PO₄ (1 g L⁻¹), CaCl₂·2H₂O (0.1 g L⁻¹), yeast extract (1 g L⁻¹) and D-xylose (30 g L⁻¹) (Silva and Afschar, 1994).

Fermentation of Hydrolysate

Banana peel hydrolysate was supplemented with the following nutrients (g L⁻¹); yeast extract 5; peptone 10; K₂HPO₄ 0.5; KH₂PO₄ 0.5; MgSO₄ - 7H₂O 0.5 and ammonium sulfate 2, and was inoculated with the inoculum in a 1,000 mL Erlenmeyer flask in orbital shaker (IOC 402 XX1.C, Sanyo, UK) at 30°C at 200 rev min⁻¹ for a period of 100 hours at pH levels of 2.5, 3.0 and 3.5.

Treatment with Charcoal

Fermentation broth was treated with activated charcoal in order to remove the color and vacuum filtration was carried out to remove the charcoal particles. Afterwards fermentation broth was subjected to vacuum evaporation for 3 fold concentrations in a rotary evaporator developed by EYELA (Affleck, 2000).

Freeze Drying

The concentrated solution was subject to freeze drying at -60°C under low pressure for the crystallization in a freeze drier manufactured by Christ® (Alpha 1-4 D).

Analytical Procedure

Xylitol content was determined by gas chromatography using an FID Detector. The injector temperature was 280°C; the detector temperature was 300°C. A capillary column DB-1 (length 30 m, internal diameter 0.25 mm, film thickness 0.2 μm) was used. The oven temperatures were programmed as follows: starting at 180°C, then increasing to 200°C at the rate of 1.5 °C min⁻¹ for 4 minutes and then start from 260°C and increased to 290°C at the rate of 10 °C min⁻¹. Helium (He) was used as the carrier gas at a constant flow rate of 30 mL min⁻¹ and pressure was maintained at 150 psi. The standards from Sigma-Aldrich and Applichem were used for quantitative calibration. All determinations were executed by the internal standard method. Phenyl β-D-glucopyranoside was used as an internal standard. Data was acquired and processed with the HP-Chemstation software (Winterova *et al.*, 2008).

Preparation of Rusks

Rusks were prepared from commercial flour with sucrose and xylitol in the ratio of 100:0 (T₀), 75:25 (T₁), 50:50 (T₂), 25:75 (T₃) and 0:100 (T₄), respectively. Rusks were prepared according to method followed by Yaseen (2000) with certain modifications. The recipe followed was flour (500 g), sugar (150 g), yeast (7.5 g), salt (7.5 g), oil (25 g), ghee (50 g) and one egg. The ingredients were weighed accurately. Water and yeast were mixed in a separate pan and then transferred to the kneader containing flour, sugar, shortening and salt. After homogeneous mixing, the dough was placed



in a proofer for 15 minutes to activate the yeast. After the said time, it was again transferred to the mixer and oil was added and mixing was continued until dough became somewhat elastic. The dough was divided into dough balls (50 g), transferred to pans and received proofing time of 15 minutes. Afterwards, loaves were baked in a baking oven for 10-12 minutes at 218°C. Loaves were cooled down and cut into two pieces and again baked until the required color was obtained.

Physical Analysis of Rusks

Rusks were analyzed for texture analysis with the help of texture analyzer (Mod.TA-XT2 Stable Microsystems, Surrey, UK) with a 5kg load cell according to method presented by Piga *et al.* (2005). Color value was determined with the help of color meter (Color Test-II; Neuhaus Neotec) as described by Stekelenburg and Labots (2007). The color meter was calibrated using standards (54 CTn for dark and 151 CTn for light). Water activity was determined by using an electronic hygropalm water activity meter (Model Aw-Win, Rotronic, equipped with a Karl-Fast probe) as described by Rocha and Morais (2003). Physical analysis of rusks was performed at 10 day intervals during storage period of 30 days.

Chemical Analysis of Rusks

The rusks were analyzed for moisture, crude protein, crude fat, crude fiber, nitrogen free extract (NFE) and ash content at 10 day intervals during storage period of 30 days according to the methods described in AOAC (2000).

Sensory Evaluation of Rusks

Rusks were evaluated for taste test by a panel of five judges by using the scoring

method as described by Land and Shepherd (1988).

Statistical Analysis

Data were statistically analyzed using the analysis of variance technique (Steel *et al.*, 1997). Duncan's multiple range test was applied to assess the difference between means. Significance was defined at $p < 0.05$. Each experiment was repeated at least three times, and the values are given as means.

RESULTS AND DISCUSSION

Chemical Analysis of Banana Peel

Results regarding chemical composition of banana peel indicate that it contained moisture 6.57%, crude protein 1.2%, crude fat 1.67%, crude fiber 30.48%, ash content 10.42% and nitrogen free extract 49.66%. The results are in close agreement with the findings of Anhwange (2008).

Xylitol Production

Insoluble dietary fibers, hemicellulose, cellulose and lignin present in banana peel are shown in Figure 1. The data showed that there was a good concentration of insoluble dietary fibers present in the banana peel. It contains 21.4% NDF, 13.6% ADF, 7.04%

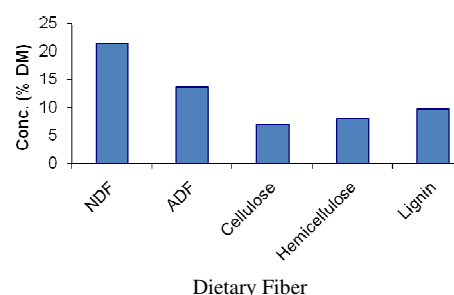


Figure 1. Dietary fiber composition of banana peel.

cellulose, 7.94% hemicellulose and 9.70% lignin on dry weight basis. Thomas *et al.* (2007) reported that banana peel contains 26.1% NDF, 19.7% ADF, 7.6% cellulose, 8.4% hemicellulose and 12.1% lignin. The difference in results with the present study might be due to varietal difference and climatic factors.

After determining the insoluble dietary fiber components, the hydrolysate was analyzed for the presence of sugar components and results illustrated that peel hydrolysate contains total sugars 25.5%, reducing sugars 12.6% and non-reducing sugar 12.9% as presented in (Figure 2-a).

Denis *et al.* (2002) found that banana peel contained 26.2% total sugars and 13.2% reducing sugars.

Banana peel hydrolysate was analyzed for *L*-arabinose, xylose, *D*-galactose, glucose and phenolic compounds and results (Figure 2-b) are close to the findings of Whistler (1998), who reported that banana peels contain 52.5% *L*-arabinose, 25.6% *D*-xylose and 21.6% *D*-galactose.

The hydrolysate obtained after dilute acid hydrolysis also contained low concentrations of non-carbohydrate compounds (phenolic compounds, acetic acid), which hinder the xylose to xylitol bioconversion by the

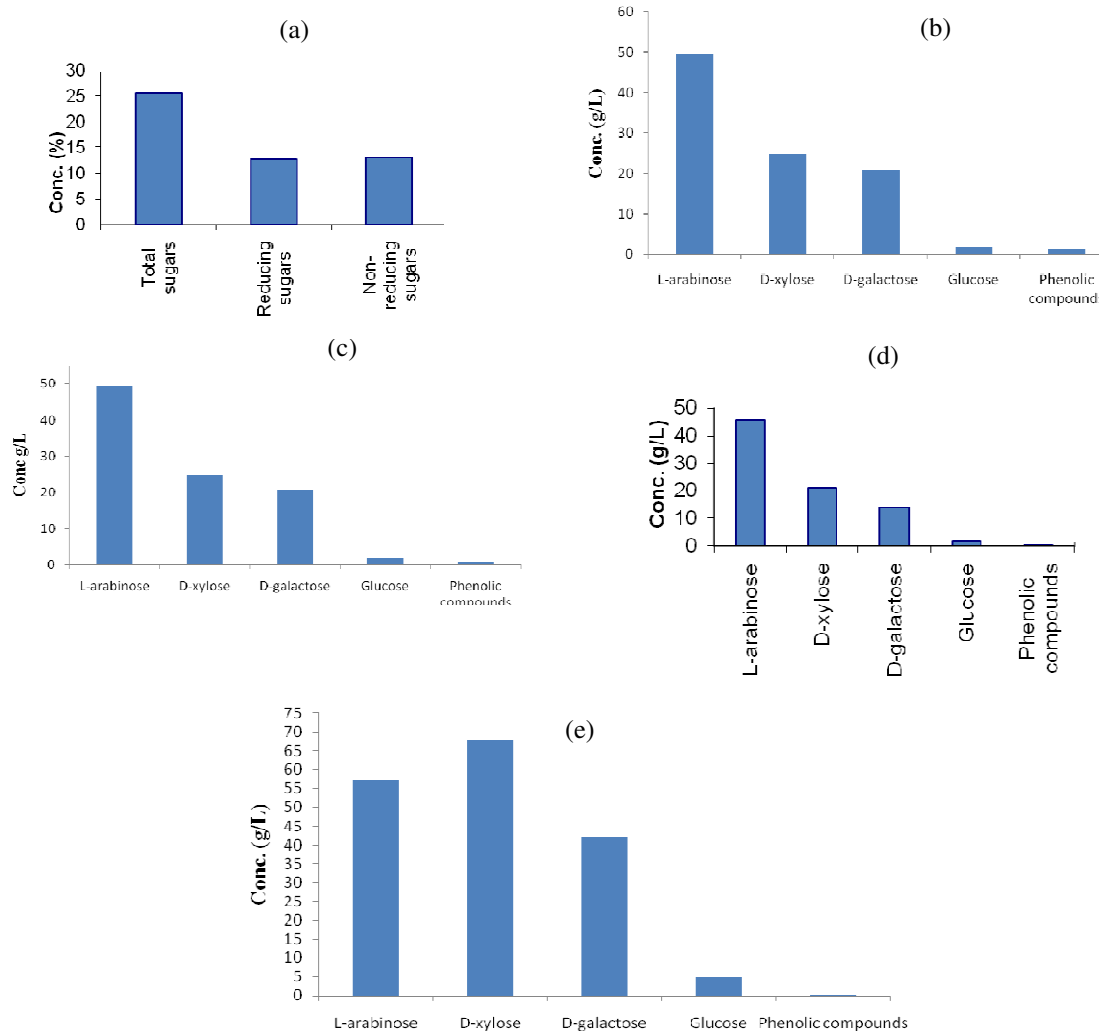


Figure 2. Sugar composition of banana peel after acid hydrolysis (a), Composition of banana peel after acid hydrolysis (b), Composition of banana peel hydrolysate after neutralization (c), Composition of banana peel hydrolysate after charcoal treatment (d), Composition of banana peel hydrolysate after vacuum evaporation (e).



yeasts (Canilha, 2005). Concentration of these compounds depends upon the concentration of acid used and time given for the treatment. To overcome the depletion of these compounds, neutralization was carried out using $\text{Ca}(\text{OH})_2$. Analysis for sugars revealed that neutralization has non-significant ($P < 0.05$) effects on sugars, *L*-arabinose 49.53 g L^{-1} , *D*-xylose 24.65 g L^{-1} ,

D-galactose 20.75 g L^{-1} and glucose 1.90 g L^{-1} while phenolic compounds decreased significantly ($P < 0.05$), as indicated in (Figure 2-c).

After neutralization, the hydrolysate was treated with charcoal in order to minimize the inhibitory substances. Due to absorbance power of charcoal, sugar concentrations decreased in the hydrolysate *L*-arabinose 45.34 g L^{-1} , *D*-xylose 21.08 g L^{-1} , *D*-galactose 14.15 g L^{-1} , glucose 1.72 g L^{-1} and phenolic compounds 0.35 g L^{-1} as indicated by the (Figure 2-d). Canilha (2005) observed similar trends in wheat straw for glucose, xylose and arabinose from 26.56, 61.22 and 9.51 to 21.20, 49.77 and 8.28 g L^{-1} , respectively after detoxification with charcoal.

In order to increase the concentration of sugars in the hydrolysate, it was vacuum evaporated in a rotary evaporator. As a result, sugars were increased significantly ($P < 0.05$); *L*-arabinose 57.35 , *D*-xylose 67.8 , *D*-galactose 42.04 and glucose 4.71 g L^{-1} , as shown in (Figure 2-e). Fermentation was carried out at three different levels of pH 2.5, 3.0 and 3.5 keeping the temperature constant and xylitol produced by fermentation was crystallized by freeze drying. Xylitol production was quantified by GC-MS and it was noted that *Candida tropicalis* yields the best results at pH 2.5 with a yield of 24.57 g L^{-1} as presented in Figure 3. Similar results were reported by Silva and Afschar (1994) and Mazaheri and Nikkhah (2002).

Chemical Analysis of Rusks

Chemical studies of rusks showed that during the whole storage, there were significant ($P < 0.05$) changes in the moisture content and non-significant ($P < 0.05$) changes were observed in fat, ash, protein,

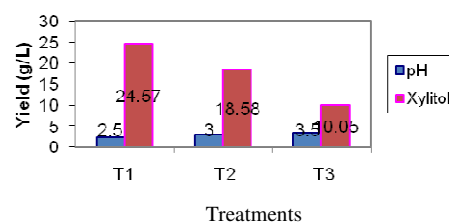


Figure 3. Production of xylitol at different treatments. Fermentation of banana peel hydrolysate at different pH level T1= 2.5, T2= 3.0 and T3= 3.5

fiber and nitrogen free extract (NFE) (Tables 1 and 2). There was a significant ($P < 0.05$) change in moisture contents ranging from T_0 (2.62) having the lowest score to T_4 (2.83) having the highest score.

Results pertaining to physical and taste tests of rusks are presented in the Tables 3 and 4. The results of texture profile analysis indicated that hardness decreased significantly ($P < 0.05$) from $4,950.6 \text{ g}$ (T_0) to $3,090.3 \text{ g}$ (T_4). The progressive decrease in the hardness during the whole storage period was due to increase in moisture content of rusks since xylitol is hygroscopic in nature. This is favored by less gluten development and less crystallization properties of the xylitol. Olinger and Velasco (1996) investigated cookies with polyols and reported that cookies are softer when prepared with polyols. Zarina et al. (2010) reported similar results for cookies prepared with the incorporation of xylitol.

Fracturability represents the crispiness of a product. A product having lower value is crispier than a product having higher value. Results indicated that fracturability values increased from T_0 (71.90 mm) to T_4 (74.26 mm). This showed that the treatment having more hardness (firmness) is crispier and vice versa. Fracturability is significantly ($P < 0.05$) affected by storage as shown in Table 4. The minimum value was obtained at 0 day (73.34 mm) and gradually increased to 73.84 mm after 30 days of storage. Higher values indicate less fracturability and decrease in crispiness. This significant ($P < 0.05$) change

Table 1. Effect of different treatments on the means of proximate composition of rusks ^a.

Treatments	Moisture (%)	Fat (%)	Fiber (%)	Protein (%)	Ash (%)	NFE (%)
T ₀	2.62 ^c	11.585	0.36	7.546	1.473	76.508
T ₁	2.71 ^{bc}	11.563	0.356	7.528	1.459	76.470
T ₂	2.76 ^{ab}	11.561	0.354	7.495	1.444	76.428
T ₃	2.78 ^{ab}	11.555	0.358	7.426	1.443	76.447
T ₄	2.83 ^a	11.550	0.356	7.519	1.424	76.281

^a Data with different letters in each column differ significantly according to DMR test at $P < 0.05$.

Table 2. Effect of storage period on the means of proximate composition of rusks ^a.

Storage (days)	Moisture (%)	Fat (%)	Fiber (%)	Protein (%)	Ash (%)	NFE (%)
0	2.68 ^b	11.577	0.359	7.504	1.457	76.495
10	2.73 ^{ab}	11.562	0.358	7.505	1.451	76.457
20	2.75 ^{ab}	11.560	0.354	7.508	1.447	76.403
30	2.81 ^a	11.553	0.351	7.490	1.441	76.352

^a Data with different letters in each column differ significantly according to DMR test at $P < 0.05$.

can be associated with the increase in moisture content. As xylitol is hygroscopic, it absorbs moisture and the product loses its crispiness during storage. Zarina *et al.* (2010) results bolster the findings of this study. Razavi *et al.* (2009) also concluded that physical properties are dependent on moisture content.

The results for color analysis are presented in the Table 3. The values are indicative of the lightness of the samples. Lower color values indicate a darker surface color. Xylitol is chemically inert due to lack of an active carbonyl group. It cannot participate in browning reactions. Therefore, there is no caramelization during heating, as is typical of sugars (Hyvonen and Espo, 1981; Olinger and Pepper, 2001). Since xylitol does not form Millard reaction, the color of rusks prepared with xylitol is lighter (187.75 CTn)

than those made from sucrose (173 CTn). Zoulias *et al.* (2000) and Zarina *et al.* (2010) observed similar trends for polyol. Storage (Table 4) has non-significant ($P < 0.05$) effect on the color values of the rusks.

Water activity is the ratio of vapor pressure of water in food to the vapor pressure of pure water. Water activity indicates the shelf stability of the product and freshness. Effects of different treatments on the water activity of rusks are presented in Table 3. Xylitol exerts higher osmotic pressure thus provides lower water activity in the product as shown by T₄ (0.16) when compared with T₀ (0.24). Bond and Dunning (2006) reported that owing to lower water activity, xylitol gives higher shelf stability to the product than sucrose. Storage (Table 4) has a significant ($P < 0.05$) effect on the water activity, which is due to hygroscopic

Table 3. Effect of different treatments on the means of physical characteristics of rusks ^a.

Treatments	Hardness (g)	Fracturability (mm)	Color (CTn)	Water activity (a _w)	Taste
T ₀	4950.6 ^a	71.90 ^c	173.00 ^d	0.24 ^a	7.20 ^{bc}
T ₁	4816.8 ^b	73.76 ^b	174.08 ^d	0.22 ^b	7.50 ^{ab}
T ₂	3755.3 ^c	73.81 ^b	177.83 ^c	0.20 ^c	7.40 ^{abc}
T ₃	3840.1 ^c	74.09 ^a	182.42 ^b	0.17 ^d	7.70 ^a
T ₄	3090.3 ^d	74.26 ^a	187.75 ^a	0.16 ^c	7.80 ^a

^a Data with different letters in each column differ significantly according to DMR test at $P < 0.05$.

**Table 4.** Effect of storage on the means of physical characteristics of rusks ^a.

Storage (days)	Hardness (g)	Fracturability (mm)	Color (CTn)	Water activity (a _w)
0	4586.6 ^a	73.34 ^b	178.93	0.15 ^d
10	4388.6 ^b	73.38 ^b	178.73	0.20 ^c
20	3903.4 ^c	73.70 ^a	180.00	0.22 ^b
30	3483.7 ^d	73.84 ^a	178.40	0.22 ^a

^a Data with different letters in each column differ significantly according to DMR test at $P < 0.05$

nature of xylitol. Taste is an important dimension of the total product quality and is evaluated by human senses. In the present study, all treatments containing xylitol were good for taste (Table 3). Treatment T₄ and T₃ obtained maximum taste scores by the judges followed by T₁, T₂ and T₀.

CONCLUSIONS

Banana peel proved to be a good source for producing xylose. Xylose can be used as a substrate for xylitol production by acid hydrolysis of banana peel. Detoxification of peel hydrolysate by neutralization, charcoal treatment and vacuum evaporation increased the xylitol yield. Xylitol can be used to replace sugars in different products such as bakery and confectionary products without affecting their physico-chemical characteristics and shelf stability. The product prepared by replacement of xylitol was shelf stable for 30 days.

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تولید بیوتکنولوژیک زایلیتول از پوست موز و تاثیر آن بر خصوصیات نان سوخاری

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چکیده

زایلیتول نخستین قند نادری است که به سبب دارا بودن خواص مفید برای سلامتی به عنوان جایگزین شیرین کننده‌های رایج بازار جهانی پیدا کرده است. تولید بیوتکنولوژیک زایلیتول با استفاده از *Candida tropicalis* DSM 7524 به عنوان روش جایگزین برای تخمیر زایلوز به زایلیتول مورد مطالعه قرار گرفت. پوست موز به عنوان بستر تولید زایلیتول استفاده شد. آبکافتة حاصل با استفاده از خنثی سازی، تیمار با ذغال فعال و تبخیر در خلاء سم زدایی گردید. اثر pH بر *C. tropicalis* در سه سطح pH بررسی شد و pH ۲/۵ بهترین شناخته شد که موجب تولید ۲۴/۷ g/L زایلیتول شد. نان سوخاری با جایگزینی ساکروز با زایلیتول در مقادیر متفاوت تهیه گردید. آنالیز فیزیکی-شیمیایی در فواصل زمانی متفاوت نگهداری صفر، ۱۰، ۲۰ و ۳۰ روز انجام گردید. سختی به طور معنی داری ($p < 0.05$) از تیمار حاوی ۱۰۰٪ ساکروز (۴۹۵۰/۶ گرم) تا تیمار حاوی ۱۰۰٪ زایلیتول (۳۰۹۰/۳ گرم) کاهش یافت در حالی که مقدار خردشوندگی از ۷۱/۹۰ mm به ۷۴/۲۶ mm در تیمار حاوی ۱۰۰٪ زایلیتول افزایش یافت. مقادیر رنگ و فعالیت آب در نان سوخاری با جایگزینی ساکروز با زایلیتول به طور معناداری افزایش یافتند. افزایش مقدار رطوبت و روند کاهشی پارامترهای دیگر با افزایش زمان نگهداری نیز در نان های سوخاری مشاهده شدند. زایلیتول ارزش انرژی زایی کمتری در مقایسه با ساکروز دارد و بنابراین می تواند در غذاهای رژیمی مورد استفاده قرار گیرد و به این ترتیب به کنترل قند خون بیماران دیابتی کمک کند.