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Optimization of leucrose production by dextransucrase from *Streptococcus mutans* and its application as an adipogenesis regulator

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ABSTRACT

Leucrose is a sucrose isomer which has an α-1,5-linkage, and slowly hydrolyzed to glucose and fructose by small intestinal α-glucosidases. Leucrose can be produced by an isomerization reaction of dextransucrase on a sucrose substrate. In this study, the recombinant dextransucrase from *Streptococcus mutans* (*Sm*DS) was applied to optimize the reaction conditions for leucrose production. With a substrate mixture of 0.5 M sucrose + 1.0 M fructose, the greatest yield (ca. 24.5%) of leucrose was obtained by *Sm*DS treatment at 30 °C for 120 h. When preadipocyte 3T3-L1 cells were treated with leucrose, this disaccharide inhibited intracellular lipid accumulation in a dose-dependent manner and significantly suppressed mRNA levels of major adipogenic genes, including CCAT/enhancer-binding protein α (*C/EBPα*), peroxisome proliferator-activated receptor-γ (*PPARγ*), fatty acid synthase (*FAS*), and sterol regulatory element-binding protein-1C (*SREBP-1C*). Phosphorylation of PI3 kinase/Akt/mTOR was also reduced with leucrose treatment. These results suggest that leucrose has a potential in regulating adipogenesis.

1. Introduction

Dextransucrase (E.C.2.4.1.5, DS) is a type of glycosyltransferases with the systematic class name of 1,6-α-D-glucan 6-α-D-glucosyltransferase. DS mainly catalyzes the synthesis of linear α-1,6-linked glucans by transferring glucose from sucrose while liberating fructose (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005) to acceptor molecules such as sucrose, isomaltose, leucrose, and dextran (Koepsell et al., 1953). Due to the minor activity of DS, leucrose [D-glucopyranosyl-α-(1 −5)-D-fructopyranose] can be produced via isomerization on sucrose molecules, and it has half the sweetness of sucrose (Bailey & Bourne, 1959). In nature, leucrose is present as a component of honey and pollen with a high resistance to acid hydrolysis (Bailey & Bourne, 1959), and it has a slow degradation rate to monosaccharides (glucose and fructose) by small intestinal mucosal α-glucosidases (Lee et al., 2016). Interestingly, leucrose cannot be utilized by *Streptococcus mutans* in human oral cavities, and it has non-pathogenic, non-toxic, and non-transgenic properties (Elias, Benecke, & Schwengers, 1996). Thus, leucrose has great potential to substitute sucrose as a functional sweetener in the food industry (Schwengers & Benecke, 1987).

Obesity, defined as excess fat for a particular body size, is currently the leading metabolic disease affecting humans and has become a global public health issue (Wang et al., 2014). Obesity is mainly caused by hyperplasia (increased cell numbers) and hypertrophy (increased cell mass), and it has been emphasized that control of adipogenesis is important for maintaining normal adipocyte functions (Min et al., 2013; Spalding et al., 2008). Adipogenesis is the process by which preadipocytes differentiate into mature adipocytes, and it is initiated with the activation of CCAT/enhancer-binding proteins (*C/EBPs*) and the peroxisome proliferator-activated receptor-γ (*PPARγ*). These transcription factors represent early and central regulators of adipogenesis (Min et al., 2013; Rosen & MacDougald, 2006; Tang & Lane, 2012). In

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response to the up-regulation of *C/EBPα* and *PPARγ*, downstream adipocyte-specific genes, such as the sterol regulatory element-binding protein-1C (*SREBP-1C*) and fatty acid synthase (*FAS*), are activated to promote adipocyte differentiation (Rosen & MacDougald, 2006). In previous studies, high concentrations of glucose were shown to promote adipogenic differentiation of muscle-derived stem cells and to up-regulate gene expression of *SREBP-1C* and *PPARγ* (Aguiari et al., 2008). PI3K/Akt/mTOR signaling has also been associated with adipogenesis and is triggered via the activation of insulin receptors and tyrosine kinases (Kim & Chen, 2004; Kim et al., 2012; Wu et al., 2017).

Recently, high intake of carbohydrates, and sucrose in particular, has been identified as a major cause of metabolic diseases such as type 2 diabetes, dyslipidemia, non-alcoholic fatty liver disease, and obesity (Keyhani-Nejad et al., 2015). Thus, there is a growing demand for natural sweeteners with a low calorie count and glycemic index (GI) to substitute sucrose due to sugar-induced obesity and diabetes (Malik, Popkin, Bray, Després, & Hu, 2010). There is also high interest among researchers to produce functional sweeteners via enzymatic modifications of carbohydrate molecules due to its eco-friendly and substrate-specific properties. In this study, we investigated the optimal reaction conditions of *Sm*DS to improve the production yield of leucrose as well the effect of leucrose on adipogenesis. The results from this research can be applied to produce a high-value sweetener and functional food ingredients for the food industry.

2. Materials and methods

2.1. Materials

Glucose, fructose, and sucrose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Leucrose was purchased from Carbosynth Ltd. (Berkshire, UK). Other chemicals used in this research were of analytical grade.

2.2. Preparation of dextransucrase from Streptococcus mutans (SmDS)

Recombinant *Escherichia coli* BL21 cells containing the dextransucrase gene were cultured in a Luria-Bertani (LB) medium containing kanamycin (200 μg/mL) at 37 °C, and dextransucrase was induced by isopropyl-β-D-thiogalactioside (IPTG) at 16 °C for 18 h (Robeson, Barletta, & Curtiss, 1983). The cells were harvested by centrifugation (5500*g* at 4 °C for 20 min), and the precipitant was re-suspended in 50 mM Tris-HCl (pH 7.0). The cell extract was disrupted by the Sonic Dismembrator (Model 100; Fisher Scientific, Pittsburgh, PA), and the supernatant was obtained by centrifugation (10,000*g* at 4 °C for 30 min). The recombinant protein was purified through nickel-nitrilotriacetic acid (Ni-NTA) affinity column chromatography (Qiagen, Hilden, Germany).

2.3. Determination of enzyme activity

Enzyme activity assay was carried out in a sodium acetate buffer (20 mM, pH 5.5) with sucrose (10%, w/v) as a substrate at 25 °C for 30 min. One unit (U) of *Sm*DS activity was defined as 1 μmol of fructose production from 10% sucrose (w/v) per minute at 25 °C (Girard & Legoy, 1999). The amount of reducing sugars (mainly fructose) liberated from sucrose by the *Sm*DS reaction was measured by the dinitrosalicylic acid (DNS) method using fructose as a standard (Sumner & Howell, 1935).

2.4. Determination of leucrose production at various reaction conditions

To optimize the high-yield leucrose production, various reaction conditions such as substrate concentrations (0.5, 1.0, 1.5, and 2.0 M), pHs (5.0, 5.5, and 6.0), temperatures (20, 25, and 30 °C), and fructose concentrations (0.1, 0.5, 1.0, 1.5, and 2.0 M) as an acceptor molecule were examined with *Sm*DS (0.5 $U/mL_{reactant}$). A high-performance anion-exchange chromatography (HPAEC) equipped with an ED40 electrochemical detector (Thermo Scientific, Sunnyvale, CA) was used to determine the amount of leucrose, and the injected sample (25 μL) was chromatographically separated by the CarboPac™ PA1 analytical (4 × 250 mm) and guard columns (4 × 50 mm, Thermo Scientific) after filtration through a nylon membrane filter (0.22 μm, Anaqua Chemicals Supply Inc., Houston, TX). The eluent (150 mM NaOH) was isocratically flowed at 1.0 mL/min for 15 min (Kim et al., 2017), and the peaks in the chromatogram was quantified with glucose, fructose, sucrose, and leucrose as standard materials.

2.5. Cell culture and differentiation of 3T3-L1 preadipocytes

For this study, 3T3-L1 preadipocytes were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's Modified Eagles Medium (DMEM, Welgene, Korea) supplemented with 10% bovine calf serum (BCS), 1.5 g/L sodium bicarbonate, 100 U/mL of penicillin, and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA). The cells were seeded in 6-well plates and maintained at 37 °C in humidified 5% CO_2 for two days until they reached confluence. Differentiation was induced by incubating the cells with DMEM without glucose that was supplemented with 10% fetal bovine serum (FBS), 500 μM IBMX, 1 μM dexamethasone, and 10 μg/mL insulin. After an additional two days, the medium was replaced with DMEM without glucose that was only supplemented with 10% FBS and 1 μg/mL insulin. The cells were then treated with 25 mM glucose (Glc) or varying concentrations of leucrose representing 50%, 75%, or 100% of the total glucose concentration (L50, L75, and L100, respectively) for 8 days.

2.6. Cell viability

Cell viability was analyzed with the MTT assay kit (Sigma-Aldrich Chemical Co., St. Louis, MO) as described previously (M. Kim et al., 2014). Briefly, 3T3-L1 preadipocytes were seeded into 96-well plates and maintained at 37 °C and 5% CO_2. After 24 h, the cells were treated with 25 mM glucose or 50%, 75%, or 100% of leucrose. After 24 h and 48 h, 0.5 mg/mL MTT solution was added to each well. After 3 h, the formazan crystals that developed were dissolved with the addition of 200 uL dimethyl sulfoxide (DMSO), and absorbance values at 570 nm were recorded with a microplate reader (Molecular Devices, Sunnyvale, CA, USA). The results of these assays are presented as the percentage of viable cells compared to the control cells.

2.7. Oil red O staining

To detect adipocyte differentiation, Oil red O staining (Sigma-Aldrich) was performed as previously described (Park et al., 2016). Briefly, 3T3-L1 cells were seeded in 6-well plates and treated with 25 mM glucose or 50%, 75%, or 100% leucrose for 8 days. The cells were washed twice with phosphate-buffered saline (PBS) and then fixed with formaldehyde for 1 h. The cells were subsequently washed with 10% isopropanol for 5 min and stained with Oil red O solution for 30 min. After the stained cells were gently washed with distilled water, cell morphology for each was imaged and photographed. Finally, isopropanol (100%) was added to each well to dissolve the stained lipid droplets and absorbance values at 492 nm were recorded to quantify lipid accumulation.

2.8. Quantitative real-time PCR

Total RNA was isolated using a TRIzol reagent (Invitrogen) and cDNA was synthesized by reverse transcription with a RevertAid First Strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania). Quantitative real-time PCR was performed by using a Rotor-Gene SYBR Green PCR

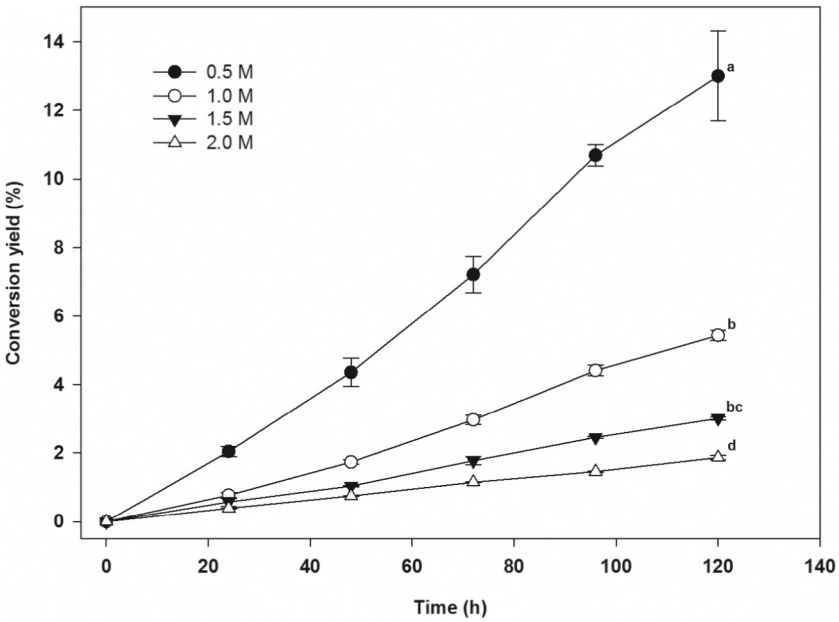

Fig. 1. Production yield (%) of leucrose from different concentrations of sucrose by *Sm*DS at pH 5.5 and 25 °C. Means with different letters at 120 h are significantly different (significance level, $p < .05$). Error bars indicate standard deviation.

kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The cycles included: 95°Cfor 15 min, followed by 40 cycles of 94 °C for 15 s, 55 °C for 30 s, and 70 °C for 30 s. The primers used for real-time PCR included the following: mouse *PPARγ*, 5′-GAGCACTTC ACAAGAAATTACC-3′(forward) and 5′-GAACTCCATAGTGGAAGCCT-3′(reverse); *C/EBPα*, 5′-CCAAGAAGTCGGTGGACAAGA-3′(forward) and 5′-CGGTC ATTGTCACTGGTCAACT-3′ (reverse); *FAS,* 5′-CTTCGC CACTCTACCATGG-3′ (forward) and 5′-TTCCACACCCATGAGCGAGT-3′ (reverse); *SREBP-1C*, 5′-TAGAGCATTCCCC CAGGTG-3′ (forward) and 5′-GGTACGGGCCACAAGAAGTA-3′ (reverse); *GAPDH*, 5′-AACTTTGG CATTGTGGAAGG-3′ (forward) and 5′-TGTGAGGGAGATGCTCAGTG-3′ (reverse). All mRNA levels were normalized to internal control, *GAPDH*.

2.9. Western blotting analysis

To obtain extracts for Western blotting analysis, cells were washed twice with cold PBS and were then incubated on ice with a Pro-prep buffer (iNtRON Biotechnology Inc, Seongnam, Korea) supplemented with a phosphatase inhibitor cocktail. After 20 min, the cells were mechanically harvested and centrifugated at 12,000*g* for 15 min at 4 °C. Total protein concentrations were determined with a Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA). Lysates were then separated with 10% dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were subsequently blocked with 5% bovine serum albumin (BSA) or 5% skimmed milk in a TBS buffer containing Tween-20 for 1 h at room temperature before being incubated with primary antibodies recognizing phosphorylated PI3 kinase, PI3 kinase, phosphorylated Akt, Akt, phosphorylated mTOR, and mTOR (Cell Signaling Technology, Danvers, MA). After the membranes were washed and incubated with the appropriate secondary IgG-conjugated horseradish peroxidase antibodies (Santa Cruz Biotechnology, Dallas, TX), bound antibodies were visualized with an enhanced chemiluminescence detection reagent (Animal Genetics, Inc., Suwon, Korea). Detection of β-actin (Abcam, Cambridge, UK) was performed as a loading control.

2.10. Statistical analysis

All measurements were made in triplicate. The analysis of variance (ANOVA) was used to statistically analyze the experimental results, followed by Tukey's multiple comparison test. All statistical analyses were carried out with SAS software (version 9.4, SAS institute, Cary, NC).

3. Results and discussion

3.1. Optimization of leucrose production by enzymatic bioprocess

The production yield of leucrose by *Sm*DS was dependent on the concentrations (0.5, 1.0, 1.5, and 2.0 M) of sucrose as the substrate (Fig. 1). The greatest amount of leucrose (22.26 ± 2.24 mg/mL) and conversion yield (13.01 ± 1.31%) were obtained at 0.5 M sucrose, which was almost 2.5 times higher than that of 1.0 M sucrose. The result clearly showed that lower substrate concentration was preferred to obtain a larger amount of leucrose via the isomerization of *Sm*DS. A higher concentration of sucrose substrate leads to a decrease in water activity and thus limits the mobility of sucrose and enzyme molecules in the solution, which reduces the enzyme accessibility to the substrate (Monsan & Combes, 1984).

The amount (mg/mL) of leucrose produced by the *Sm*DS reaction was found to be closely dependent on the pH (5.0, 5.5, or 6.0) and temperature (20, 25, or 30 °C) (Table 1). The greatest production amount (mg/mL) and conversion yield (%) among various pH and temperatures was obtained at pH 6.0 and 30 °C, respectively. Previous studies showed that the optimal reaction conditions of leucrose production by *Sm*DS was measured at around 25 °C and pH 5.5 (Böker, Jördening, & Buchholz, 1994; Reh, Noll-Borchers, & Buchholz, 1996; Reischwitz, Reh, & Buchholz, 1995), which were indirectly determined by quantifying the amount of reduced sugars by DNS assay instead of

Table 1
Production amount and conversion yield of leucrose by dextransucrase from *Streptococcus mutants* at various reaction conditions with 0.5 M sucrose.

Reaction conditions		Production amount (mg/mL)	Conversion yield (%)
pH	5.0	19.04 ± 1.40	11.13 ± 0.82
	5.5	19.54 ± 1.66	11.42 ± 0.97
	6.0	28.99 ± 7.99	17.26 ± 4.22
Temperature (°C)	20	19.57 ± 1.45	11.43 ± 0.85
	25	18.07 ± 0.33	10.56 ± 0.19
	30	30.38 ± 1.33	17.75 ± 0.78

*Leucrose conversion yield (%) = (the production amount of leucrose)/(initial concentration of sucrose) × 100.

Table 2
Production amount and conversion yield of leucrose by the presence of fructose as an acceptor molecule with 0.5 M sucrose at optimized reaction conditions (pH 6.0 and 30 °C).

Fructose (M)	Production amount (mg/mL)	Conversion yield (%)
0.0	22.26 ± 2.24	13.01 ± 1.31
0.1	24.30 ± 1.07	14.20 ± 0.62
0.5	29.77 ± 0.21	17.39 ± 0.13
1.0	47.65 ± 1.12	27.84 ± 0.66
1.5	38.37 ± 0.55	22.42 ± 0.32
2.0	38.77 ± 1.58	22.65 ± 0.93

Leucrose conversion yield (%) = (the production amount of leucrose)/(initial concentration of sucrose) × 100.

leucrose. In this study, we directly calculated the amount of products via HPAEC analysis with different concentrations of leucrose (> 98% purity) as a standard material. The result clearly showed that the optimal conditions for leucrose production were 30 °C and pH 6.0, respectively.

Table 2 showed the production yield of leucrose from sucrose with *Sm*DS, which was dependent on the fructose concentrations (0.1, 0.5, 1.0, 1.5, and 2.0 M) as an acceptor molecule. Previous studies showed that the isomerization of sucrose to turanose (D-glucopyranosyl-α-(1–5)-D-fructopyranose) can be produced by amylosucrase, and that this reaction is promoted by the presence of fructose as an acceptor molecule (Park et al., 2016; Wang et al., 2012). Therefore, this study also provides an additional acceptor molecule to accelerate the isomerization reaction of dextransucrase. As the concentration of fructose increased up to 1.0 M, the amount of produced leucrose also increased. However, the amount of leucrose produced decreased as concentrations of fructose rose above 1.0 M. Perhaps the decrease in the production yield at high concentrations of fructose could be due to the increase in the viscosity of the reactant, which could have limited the accessibility of molecules to dextransucrase (Tanriseven & Robyt, 1993). The highest production amount (47.65 ± 1.12 mg/mL) and yield (27.84 ± 0.66%) of leucrose was obtained at 0.5 M sucrose with 1.0 M fructose, which was almost 2.4 times higher than a sucrose-only solution. This result clearly confirmed that the presence of fructose as an acceptor molecule promotes the isomerization of *Sm*DS leucrose production.

Fig. 2 showed that the production yield of leucrose obviously increased in proportion to the *Sm*DS amount, indicating that the reaction time can be regulated by controlling the enzyme amount. Also, the result confirmed the production yield (%) of leucrose at 120 h was 2.4 times significantly ($p < .05$) higher in the optimal reaction conditions (sucrose: 0.5 M, temperature (°C): 30, pH: 6.0, and fructose (M): 1.0) compared to previous reaction conditions at 25 °C with pH 5.5 (Fig. 3).

3.2. Effect of leucrose on lipid accumulation in adipocytes

Various concentrations of leucrose were applied to 3T3-L1 preadipocytes and cell viability was assessed. No significant effect on cell viability was observed at either of the 24 or 48 h time points, suggesting that leucrose does not induce cytotoxicity on 3T3-L1 cells (Fig. 4A). However, leucrose treatment did attenuate the increase in lipid accumulation that was induced by 25 mM glucose ($p < .001$) in a dose-dependent manner as evidenced by the Oil red O staining and intracellular TG concentration analyses that were performed (Fig. 4B). Repeated exposure to high glucose has been proposed as increasing the risk of abnormal metabolic consequences in different cell types. Previous studies where 25 mM glucose in culture media induced adipocyte differentiation and lipid accumulation in 3T3-L1 cells and mesenchymal stem cells (Aguiari et al., 2008; Palacios-Ortega, Varela-Guruceaga, Martinez, de Miguel, & Milagro, 2016). However, in the present study, glucose was replaced with 50%, 75%, and 100% leucrose reduced lipid content at 17%, 34%, and 68%, respectively ($p < .001$ for all) compared to the Glc group. Taken together, these results indicate that leucrose is able to inhibit adipogenesis in 3T3-L1 preadipocytes by decreasing lipid accumulation in the cytoplasm while not inducing cytotoxicity.

3.3. Effect of leucrose on the expression of various adipogenic genes and signal transduction pathways

To investigate whether changes in gene expression are involved in mediating the observed anti-adipogenic effects of leucrose in 3T3-L1 cells, mRNA expressions levels of *C/EBPα*, *PPARγ*, *SREBP-1C*, and *FAS* were detected (Fig. 5A). All four genes were highly up-regulated in the Glc group compared to the non-treated Ctrl group ($p < .001$). In contrast, the levels of all four genes were significantly down-regulated in each of the leucrose treatment groups. Moreover, the L100 group exhibited a recovery of gene expression levels to those of the Ctrl group.

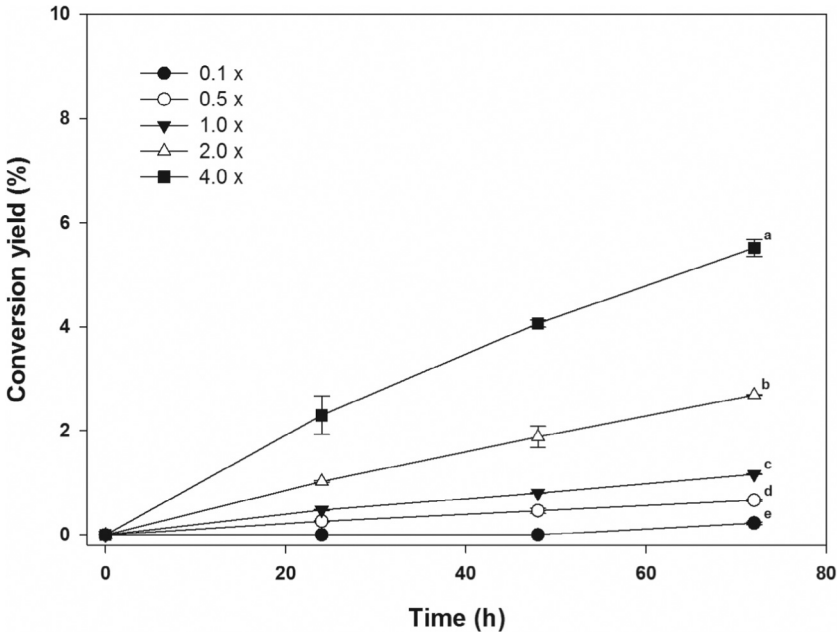

Fig. 2. Production yield (%) of leucrose depending on the amount of enzyme [0.1× (●), 0.5× (○), 1.0× (▼), 2.0× (□) and 4.0× (■)]. Enzyme concentration of 1× represents 0.5 $U/mL_{reactant}$ of dextransucrase. Means with different letters at 72 h are significantly different (significance level, $p < .05$). Error bars indicate standard deviation.

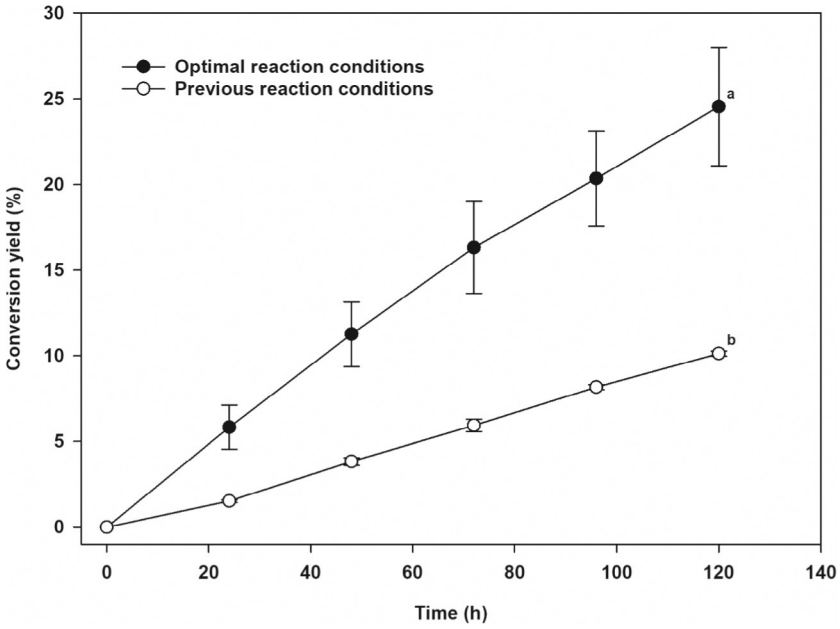

Fig. 3. Production yield of leucrose under the determined optimal reaction [sucrose: 0.5 M, temperature (°C): 30, pH: 6.0, and fructose (M): 1.0] conditions and previous reaction conditions [sucrose: 0.5 M, temperature (°C): 25, pH: 5.5, and fructose (M): 0.0]. Means with different letters at 120 h are significantly different (significance level, $p < .05$). Error bars indicate standard deviation.

C/EBPs and PPARγ are major regulators of adipogenesis (Aguiari et al., 2008; Min et al., 2013; Rosen & MacDougald, 2006). C/EBPα is an important transcription factor in early adipogenesis. Following its activation, C/EBPα activates PPARγ and they work together to promote the expression of other adipogenic genes, including *FAS*, to maintain a differentiated state during adipocyte maturation (Rosen & MacDougald, 2006; Tang & Lane, 2012). Both *C/EBPα* and *PPARγ* need to be activated to achieve terminal adipocyte differentiation, while early differentiation involves the activation of *SREBP-1C* and *FAS* (Rosen & MacDougald, 2006). SREBC-1C and its downstream lipid

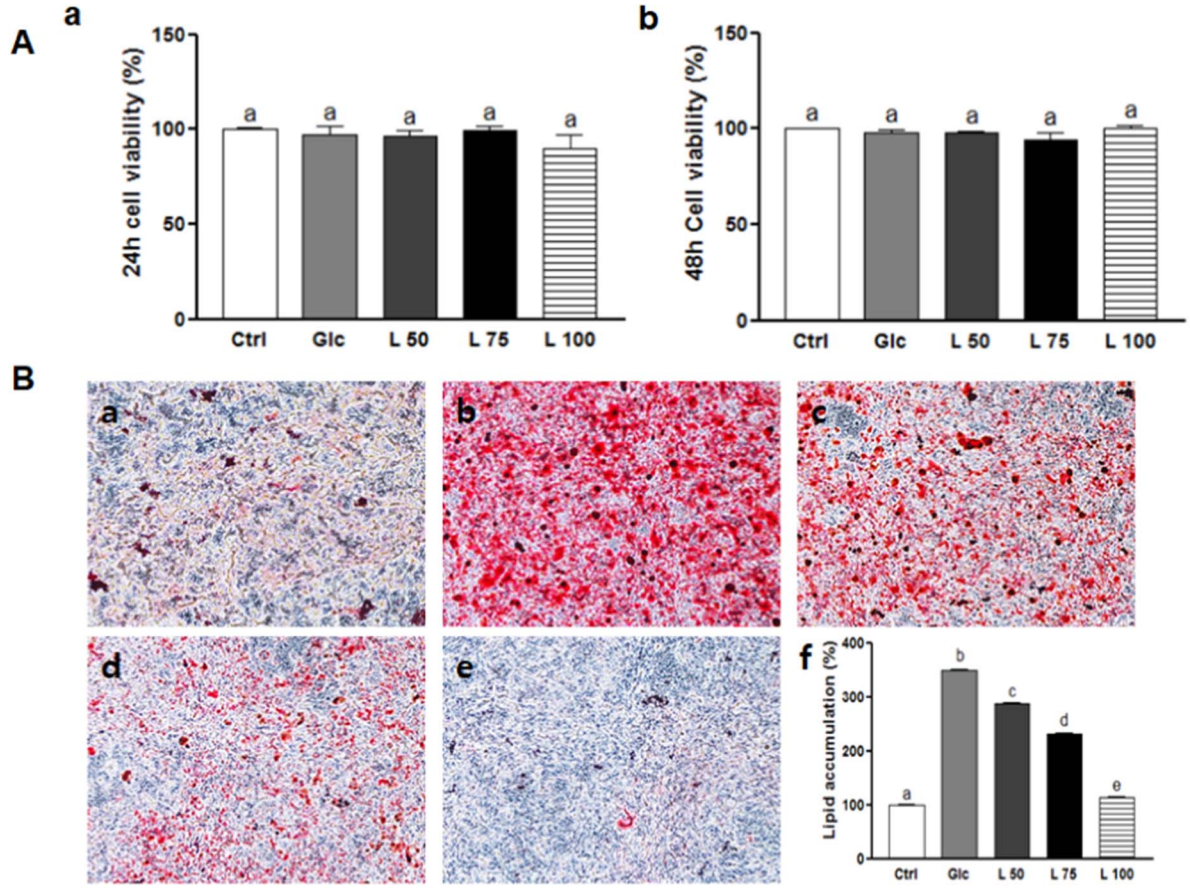

Fig. 4. Leucrose suppressed lipid accumulation in 3T3-L1 preadipocytes. (A) Cell viability of 3T3-L1 preadipocytes was assessed in MTT assays following treatment with leucrose for 24 h (a) and 48 h (b). (B) Intracellular lipid accumulation was examined with Oil red O stainings (a, Ctrl; b, Glc; c, L50; d, L75; e, L100). Quantification of these data are shown in (f) and the data are presented as the mean ± standard error the mean (SEM). Different letters for each bar means the values are significantly different from each other ($p < .001$). Ctrl, control; Glc, 25 mM glucose; L 50, L 75, and L 100; leucrose replaced 50%, 75%, and 100% of the 25 mM glucose in the cell media, respectively.

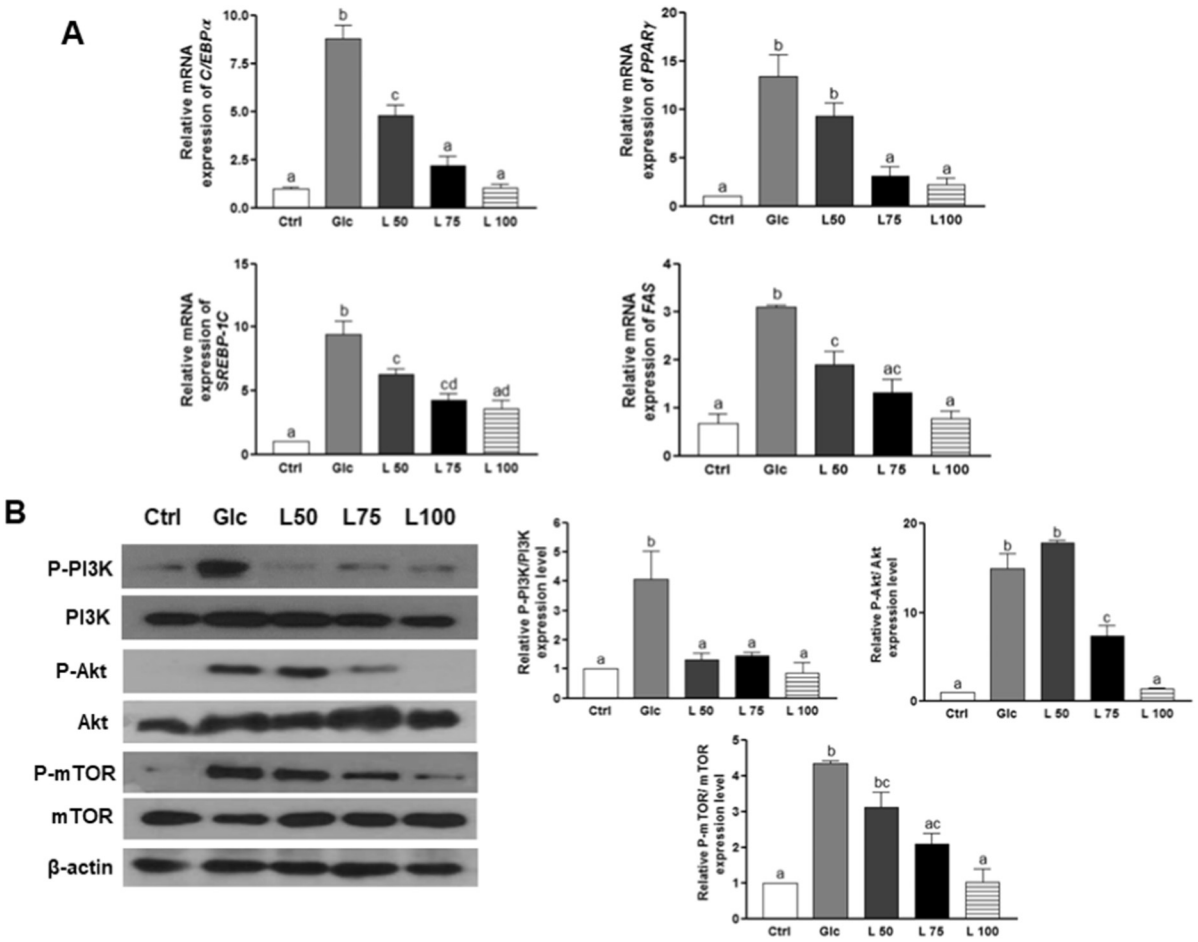

Fig. 5. Leucrose down-regulated the mRNA levels of adipogenic genes and regulated PI3K/Akt/mTOR signaling in 3T3-L1 cells. (A) mRNA expression levels of adipogenic genes, (a) *C/EBPα*, (b) *PPARγ*, (c) *SREBP-1C*, and (d) *FAS* were detected by quantitative real-time PCR. The expression of *GAPDH* was applied as an internal control. (B) Phosphorylation of PI3K, Akt, and mTOR were analyzed by a Western blotting assay. Representative blots are shown (left panel) and quantification of the levels are shown (right panel). The data are presented as the mean ± standard error the mean (SEM). Different letters for each bar means the values are significantly different from each other ($p < .001$). Ctrl, control; Glc, 25 mM glucose; L 50, L 75, and L 100; leucrose replaced 50%, 75%, and 100% of the 25 mM glucose in the cell media, respectively.

synthesis-related enzyme, FAS, also play important roles in adipocyte maturation (H. Kim, Hiraishi, Tsuchiya, & Sakamoto, 2010). In the present study, mRNA expressions levels of both *FAS* and *SREBP-1C* decreased with leucrose treatment.

The anti-adipogenic effect of dietary carbohydrates has previously been demonstrated (Lim et al., 2015; Park et al., 2016). For example, D-xylose has been shown to suppress adipogenesis and regulate lipid metabolism by regulating *C/EBPα* and *SREBP-1*, as well as *FAS* and *PPARγ*, in high fat diet-induced obese mice (Lim et al., 2015). Similarly, another sucrose isomer, turanose, has been reported to inhibit adipogenesis by regulating the expression of *SREBP-1C*, *PPARγ*, and *FAS* in 3T3-L1 cells (Kim et al., 2014). Taken together, these results indicate that leucrose is able to inhibit the expression of adipogenic genes in the early and terminal phases of adipogenesis. Furthermore, despite the fact that these results presented here derive from an *in vitro* model, the capacity for leucrose to inhibit lipid accumulation and down-regulate several adipogenic genes is predicted to mediate an anti-obesity effect *in vivo* as well. Correspondingly, another isoform of sucrose, palatinose, has been shown to reduce the accumulation of hepatic fat and improve glucose metabolism and oxidation in muscle tissue (Keyhani-Nejad et al., 2015).

It has been hypothesized that cross-talk occurs between signal transduction pathways that are induced by high glucose levels during adipogenesis. The PI3K/Akt signaling cascade and mTOR are two major signal transduction pathways that play important roles in adipogenesis (Kim & Chen, 2004; Kim et al., 2012; Wu et al., 2017). In the present study, phosphorylation of PI3K/Akt and mTOR significantly increased in the Glc group, and these levels were reduced with leucrose treatment (Fig. 5B). PI3K/Akt signaling contributes to the early phase of adipogenesis and is a major target for controlling metabolic diseases, including obesity and diabetes (Zhong et al., 2015). In addition, the Akt signaling cascade leads to the activation of C/EBPα and PPARγ by regulating FOXO1 and GSK3β during 3T3-L1 cell differentiation (Wu et al., 2017; Zhang et al., 2009). Correspondingly, in the absence of Akt, and following the knock-down of Akt, mouse embryonic fibroblasts and 3T3-L1 cells are unable to differentiate into mature adipocytes. Conversely, overexpression of Akt has been shown to increase glucose uptake and adipocyte differentiation in 3T3-L1 cells (Baudry, Yang, & Hemmings, 2006; Xu & Liao, 2004; Zhang et al., 2009).

4. Conclusion

This study investigated the optimal reaction conditions of leucrose, a novel sweetener, by enzymatic conversion technology to increase the production yield. Our research identified the optimal reaction conditions for improved yield, which can be applied for mass production of leucrose by the food industry. Furthermore, the present study clearly demonstrated that leucrose has the potential to serve as an alternative to sucrose and inhibit adipogenesis as a functional sweetener.

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