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Production of Glucose Syrup through Enzymatic Hydrolysis of Flint and Floury Corn Flour Mixtures and Evaluating its Properties as Cost-Effective Syrup

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ABSTRACT

Corn starch derivatives, including glucose syrups, are nowadays widely used in food industry. Glucose syrup is used in food industry, not only due to its sweetening power and nutritional value, but also for its functional properties (moisture stabilization, softening ability, improving texture and preventing sucrose crystallization). Floury (soft) corn (*Zea mays var. amyalcea)* is usually used to produce glucose syrup, but the most imported corn in Iran is flint or hard corn (*Zea mays var. indurate*) which is all the year round available and consequently, using both corn flour types would be inevitable. Therefore, the purpose of this study was to investigate the effect of using flint (hard) and floury (soft) corn flour to produce glucose syrup. Four treatments including hard flour + soft flour in four ratios of 30% + 70%, 50% + 50%, 70% + 30% and 100% soft flour as control were prepared and the physicochemical and organoleptic properties of the produced syrups were evaluated. According to the obtained results, using flint corn flour affected physicochemical and organoleptic properties of the samples ($P<0.05$). Increasing the ratio of flint corn flour had significantly decreased DE (dextrose equivalent), soluble solids and pH of glucose syrups ($P<0.05$). Also induced the increasing of color parameters and sulfated ash values of the produced syrups. However, cost estimates indicated a reduction in the cost of raw materials and consequently a general reduction in production costs by replacing hard corn flour. It can be concluded from the results that hard flour can be used on all surfaces, but the best treatment was 50% replacement level or a bit more, in the production which had a good effect on the properties of glucose syrup and showed more similarity with the control sample. All of these, along with being cost effective, appeared this treatment to have the potential of supposing as a sugar substitute in food industry.

1- Introduction

Starch, as the most abundant stored carbohydrate in plants, is the only raw material needed for industrial and mass production of various types of sweeteners called glucose syrups. Hydrolyzed products from different starch sources are identical in chemical, physical and organoleptic properties [1]. As a result, starch hydrolysis products are manufactured from a wide range of raw materials worldwide. The most important sources of starch in the world are including corn, wheat, rice, potatoes, tapioca and sago. The type of starch source and its purity affect the process of producing glucose syrups [2].

In industrial production, corn starch is the main raw material for glucose and fructose syrup production in the United States and many other parts of the world [3]. Corn is one of the tropical cereals and belongs to the family of monocotyledon. It is considered to be the most high-yielding grain in the world and is ranked second in wheat and rice production. The ripe corn is composed of crust, bud and endosperm. The endosperm also consists of two main parts: flour endosperm (floury or soft) and glass endosperm (flint or hard) [4].The high yield of corn production makes this plant source of primary source for starch extraction and glucose syrup production. Due to the limitations of sugar cane and sugar beet cultivation in the world and the high fluctuations in sugar prices and its disadvantages, the tendency to consume these sweeteners has been increased [4,5]. High fructose corn syrup (HFCS) first was prepared by hydrolysis of corn starch into glucose by glucoamylase and alpha-amylase followed by glucose isomerization into fructose. The final syrup was a mixture of glucose, fructose and larger saccharides [5].

The benefits of using corn syrup in different food formulations include its similarity to regular sugar, increasing the intensity of other flavors due to rapid detection by taste buds [6[, maintaining freshness and increasing the product shelf life due to moisture control and reduced microbial growth [1], soft texture preservation of baking products due to preservation of moisture and crystallization resistance, development of taste and brown color in baking products [7], better preservation of color in products such as ketchup or strawberry based products, maintaining structural stability over a wide range of temperature and acidity [8], frozen products flexibility maintenance due to low freezing point, increased dough fermentation [4] and reduce teeth damage compared to sugar [7]. Starch hydrolysis can be accomplished using either acid or enzyme or both. Hydrolysis involves the breakdown of oxygen and the incorporation of its elements into the remaining portions. Therefore, any broken joint will increase the weight of the solids [9]. The enzymatic method has been considered the dominant process because of better control of process conditions and the resulting products. The range of products from acid hydrolysis to starch is limited to DE (dextrose equivalent) from 30 to 55 (sulfuric acid is cheaper and non-corrosive than hydrochloric acid, but the sulfate and calcium residues in the syrup are inappropriate so more hydrochloric acid is used) [10].

On the other hand, enzymatic hydrolysis of starch can be obtained in DE 95 or more [11]. Due to the disadvantages of using acid hydrolysis in production of glucose syrups, use of enzymatic methods for the production of glucose syrups is preferable and considered more efficient [12]. Amylases are one of the most important enzymes used in the industry. These enzymes hydrolyze starch molecules into smaller polymers composed of glucose units. The most common and most important forms of industrial amylase are alpha-amylases that are derived from various sources such as plants, animals and microorganisms [2]. Alpha-amylase (dextrin synthase enzyme) hydrolyzes the glucosidic linkage from the inner parts of the starch chain randomly. Therefore, these enzymes are called endo-enzymes. These enzymes act as non-transporter chains and produce low molecular weight reducing sugars, which are mainly maltose, maltotriose and maltotetraose [13].

One of the common and effective enzymes used in starch enzymatic hydrolysis is glucoamylase, which directly can converts starch into glucose. In this process, the glucoside bands would be attacked. At high concentrations of glucose, the monomeric glucose units may combine to form some of the oligosaccharides called the reversal phenomenon. Return products are similar to products formed during the acid hydrolysis of starch [13]. Various starch sources have been investigated to produce glucose and fructose syrups including modified corn and rice starch [14], cassava starch [15], sago starch [16], millet and sorghum [17].

Although most of the corn used in the flour industry is floury (soft) corn (*Zea mays var. amyalcea)*, but most imported corn in Iran is flint (hard) corn (*Zea mays var. indurate*) which is available all year round, so using both types of corn would be inevitable. The size of floury corn kernel is very small. Most of its endosperm contains soft starch and hard endosperm contains only a thin outer layer. Due to its high starch content, it is used in starch and alcohol factories. As the seeds dry, they tend to shrink evenly and often do not become serrated. It is mostly grown in Peru, Bolivia and Mexico. 10 to 12% of world corn production is of this type. The whole endosperm of flint corn is in the center of the grain. The outer part of the seed is covered with a hard, thick glassy layer of endosperm. Grain shape is spherical, smooth and shiny and its color is creamy white to yellow-orange. Seed growth period is between 80 to 100 days. This corn has a high growth rate and early ripening and is mostly cultivated in Italy, India, France and Argentina. It accounts for 15% of world corn production [14,18].

The flour used in this study is a by-product of dry mill which reduces water and wastewater consumption. In terms of innovation, there has been no research on the use of flour extraction and glucose syrup production from different varieties of corn and it was corn flour variety which has always been considered. Therefore the main subjective of this study was the comparison of physicochemical and organoleptic properties of glucose syrups obtained from different corn flours and evaluating feasibility of flint (hard) corn flour replacement with soft corn flour to produce glucose syrups.

2- Materials and Methods

2-1-Materials

Samples of flint (hard) corn (*Zea mays var. indurate*) flour (Brazilian corn flour) & floury (soft) corn (*Zea mays var. amyalcea)* flour (Russian corn flour) were obtained from *Gandomkoub* Company, Iran. All chemicals used were of analytical grade and obtained from Merck Co., Germany. The commercially available enzymes used to produce glucose syrup were as follows:

Glucoamylase (EC 3.2.1.3) from *Aspergillus niger* (Dextrozyme GA 1.5X, Novozymes, Denmark) (specific activity: 180 U/ml) at operating conditions (pH: 3.5-5 and temperature: 55-65 °C) and Alpha-amylase (EC 3.2.1.1) from *Bacillus licheniformis* (LIQMAX HT, ORBA, Turkey) (specific activity: 55 U/mg) at operating conditions (pH: 6-7 and temperature: 95-105 °C) both of industrial grade.

2-2-Methods

2-2-1- Corn Flour Mixture Preparation

Treatments evaluated in this study were 4 individual mixtures including: 30% flint (hard) corn flour + 70% floury (soft) corn flour as treatment 1, 50% flint (hard) corn flour + 50% floury (soft) corn flour as treatment 2, 70% flint (hard) corn flour + 30% floury (soft) corn as treatment 3 and 100% floury (soft) corn flour as control.

2-2-2-Glucose Syrups Formulation

Calculated amounts of raw materials (w/w and percentage amounts) in preparation of glucose syrup formulations for 4 individual treatments were presented in Table 1 and 2, respectively.

2-2-3-Preparation of Glucose Syrup

Preparation of glucose syrups was carried out according to the method described previously [19] with some modifications. The main procedure using different individual treatments and individual formulations (Table 1 and 2) with temperature and pH control is presented in Fig. 1 as a schematic diagram.

Corn flour was first hydrolyzed by alpha-amylase (LIQMAX HT) and short chains of polysaccharides were produced. Second, glucoamylase (Dextrozyme GA 1.5X) broke down the sugar chains more easily. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 μmolmin^{-1} of reducing sugar as glucose at the optimal pH and temperature. The obtained syrups were purified through two separate steps.

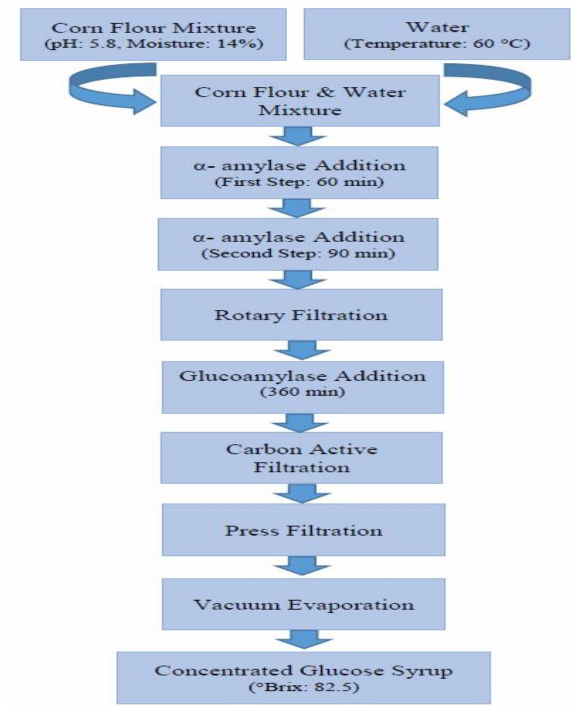

Fig 1 Schematic of Glucose Syrup production with temperature & pH control. (Rendo´n-Villalobos, et al., 2011).

Table 1 Raw material calculated amounts (w/w) in glucose syrup formulations.

Samples	Floury (soft) corn flour (kg)	Flint (hard) corn flour (kg)	α-amylase (mL)	Glucoamylase (mL)	Water (L)
Control	5	0	2.0	0.2	15
1	3.5	1.5	2.0	0.2	15
2	2.5	2.5	2.0	0.2	15
3	1.5	3.5	2.0	0.2	15

Table 2 Raw material calculated amounts (%) in glucose syrup formulations.

Samples	Mixtures of Corn Flour (flint corn + floury corn)	Variable formulation components % (w/w)		Fixed formulation components % (w/w)		
		Floury (soft) corn flour	Flint (hard) corn flour	α-amylase	Glucoamylase	Water
Control	0 + 100	25	0	0.009	0.0009	74.99
1	30 + 70	17.5	7.5	0.009	0.0009	74.99
2	50 + 50	12.5	12.5	0.009	0.0009	74.99
3	70 + 30	7.5	17.5	0.009	0.0009	74.99

2-2-4- pH Measurement

The pH values of the samples were evaluated according to the mentioned method [20]. The pH meter and electrodes (691-Metrohm, Switzerland) were standardized with pH 4 and 7 buffers according to the manufacturer's instructions. Specified amount of sample was placed in a clean beaker. Samples were diluted as specified and stirred at a rate sufficient to produce a small vortex at the liquid surface. The standardized electrode was immersed in the sample. The pH value was observed and recorded to the nearest 0.1 pH unit, after a stable reading was achieved. In order to measure corn flour pH values, the sample weight (g) / Purified Water (mL) ratio was 20 / 100. This ratio for syrups was 100 / 100 (g / mL).

2-2-5 -Measuring Moisture Content

In order to measure moisture content of flour samples, the conventional method of moisture measurement [21] was used. The clean aluminum pans were placed in the 105 °C oven for two hours and cooled to a constant weight after 15 minutes of placement in the desiccator. After weighing, 10 g of the samples was weighed and transferred to the oven of 105 °C. After 4 hours, the pans were removed and cooled in the desiccator. The moisture content of samples based on wet-weight basis was calculated using the following equation (1):

$$\text{Moisture content (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

Where, W_1 is weight of container with pan; W_2 is weight of container with pan and sample before drying and W_3 is weight of container with pan and sample after drying.

2-2-6- Determination of Crude Total Fat Content

Determination of total fat content of flour samples was carried out according to AOAC Method [22] as the *Soxhlet* extraction method. The final fat content was calculated according to the following equation (2):

$$\text{\% Crude fat} = \frac{(W2-W1)}{S} \times 100$$

Where, W_1 is weight of empty flask (g), W_2 is weight of flask and extracted fat (g) and S is weight of sample.

2-2-7- Determination of Dextrose Equivalent (DE) Values

Dextrose equivalent is a measure of the amount reducing sugars present in a sugar product, expressed as a percentage on a dry basis relative to dextrose. The dextrose equivalent gives an indication of the average degree of polymerisation (DP) for starch sugars. The dextrose equivalent values (DE) of the liquefied syrups were evaluated through measuring reducing sugars quantity (as glucose) by the *Lane and Eynon* procedure [23].

The amount of glucose in the sample was determined by comparison with a known glucose standard (0.2 mg mL^{-1}). The amount of glucose as a percentage of the total carbohydrate (w / w) in the sample, which is also known as the DE and the measuring of starch hydrolysis, was calculated according to the following equation (3):

$$\text{Reducing Sugars (\%)} = \frac{\text{\% Reducing Sugars}}{\text{Total Solids}} \times 100$$

2-2-8- Measurement of Soluble Solids (°Brix)

Measuring soluble solids by refractometric method determines the concentration by weighing sucrose in the solution that has the same refractive index (n) as the solution analyzed. Refractometer reading of solutions was performed at 20°C. Samples (approximately 50 g) were blended with 200 mL of deionized water using a blender. The total soluble solids were determined using a digital refractometer (PAL-a; ATAGO; Japan) and the ºBrix value calculated using a dilution factor, adapted from the previously described method [21].

2-2-9- Determination of Sulfated Ash Content

Determination of Ash Content was performed according to the procedure described by the AOAC Assay [22]. Accurately, to the nearest 0.0001 g, the specified amount of the sample weighted into a pre-heated, cooled silica dish. 5 mL of sulfuric acid solution was added and mixed by swirling with a glass rod. The dish was placed in a forced air oven at 105°C overnight (16 hours). Then it was removed from oven and heated gently over an open flame on a hot plate until sample was thoroughly carbonized. After that, it was placed in a muffle furnace at specified temperature for specified time until ash was free from carbon. The sample was cooled in a desiccator and weighed. The total sulfated ash content was calculated according to the following equation (4):

$$\text{\% Ash } (d_s b) = \frac{\text{Ash Wt.}(g)\times 100}{\text{Sample Wt.}(g)\times \text{total solid dry basis Wt.}(\%)/100} \times 100$$

2-2-10- Measurement of Colorimetric Parameters

The reflectance of surface color for all samples analyzed using the Minolta Chroma meter (CHROMA METER CR-400, Konica Minolta, SENSING INC., Japan) based on the standard CIELAB color system (L* is the value on the white/black axis (lightness), a* is the value on the red/green axis (red to green) and b* is the value on the yellow/ blue axis (yellow to blue).
A standard white calibration plate was employed to calibrate the device following the manufacturer's procedure. ΔE* which indicates the size of color difference and the total color change, was defined according to equation 5 [24]:

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

2-2-11- Sensory Evaluations

The sensory assessment of the obtained syrups was evaluated and scored by 60 panelists who were selected among the non-smoking and healthy staff members of Zar Macaron Company. They were males and females aged between 25 and 55. They were made to understand the different quality attributes chosen for the sensory evaluation, the score chart and the way of scoring. For sensory evaluation, the samples were assessed on the basis of organoleptic properties including flavor, aroma, appearance and general acceptance characteristics, ranging from 1 (dislike) to 5 (like). A 5-point hedonic scale was used to aid the ease of scoring and to evaluate how much the judges liked the mentioned characteristics. They were advised to sniff three times in order to judge about the aroma and then to taste and swallow the sample. The evaluators were also asked to rinse their mouth and drink water after testing any of the previous samples to omit the effect of their previous evaluations on the consecutive one. The Randomized Complete Block Design was applied for the sensory evaluation [25].

2-2-12- Statistical Analysis

Treatments evaluated in this study were 4 individual mixtures including: 30% flint (hard) corn flour + 70% floury (soft) corn flour as treatment 1, 50% flint (hard) corn flour + 50% floury (soft) corn flour as treatment 2, 70% flint (hard) corn flour + 30% floury (soft) corn as treatment 3 and 100% floury (soft) corn flour as control. For all individual samples, the experiments were performed at least in triplicate via a completely randomized design. The results were correlated according to the nonparametric *Kolmogorov Smirnov* test (K-S Test). Analysis of the results (which were subjected to ANOVA one-way analysis of variance) was done using SPSS statistical software version 22 at probability value of 5% ($P<0.05$). The obtained results were expressed as means ± standard error and the mean significant difference was assessed using Duncan's multiple range tests.

3- Results and Discussion

3-1 -Corn Flour Mixtures Properties

Samples of flint (hard) corn (*Zea mays var. indurate*) flour (Brazilian corn flour) and floury (soft) corn (*Zea mays var. amyalcea)* flour (Russian corn flour) obtained from *Gandomkoub* Company, Iran. The physicochemical characterization of individual flint (hard) and floury (soft) corns were as follows, respectively: Ash (1.7 ± 0.16 %, 1.7 ± 0.16 %), Protein (10.3 ± 0.33 %, 10.7 ± 0.28 %), Crude fiber (2.2 ± 0.19 %, 2.2 ± 0.19 %), Fat (5.0 ± 0.35 %, 5.4 ± 0.38 %), Moisture content (10.5 ± 0.23 %, 9.6 ± 0.14 %) & Carbohydrate (70.3 ± 0.46 %, 70.4 ± 0.40 %).
Physicochemical Properties of corn flour mixtures according to individual treatments and analysis of variance and evaluating of corn flour replacement effect are presented in Tables 3 and 4, respectively. The results showed that different amounts of hard corn flour replacement had a significant effect on fat content, moisture content and pH ($P<0.05$). Comparing different amounts of replacement showed that the addition of flint (hard) corn flour increased the crude fat content. As the percentage of hard corn flour increases, the amount of fat would be increased too. The highest and lowest fat contents were observed at 2.47% and 1.5%, respectively, in 70% flint corn flour treatments and the control.

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Table 3 Physicochemical Properties of corn flour mixtures according to individual treatments.

Treatment	Mixtures of Corn Flour (flint corn + floury corn)	Total Fat Content (%)	pH	Moisture Content (%)
Control	0 + 100	1.56 ± 0.43[c]	5.69 ± 0.13[c]	12.41 ± 0.33[b]
1	30 + 70	1.78 ± 0.23[bc]	5.78 ± 0.08[bc]	12.85 ± 0.45[ab]
2	50 + 50	2.09 ± 0.19[ab]	5.84 ± 0.11[ab]	13.22 ± 0.39[a]
3	70 + 30	2.47 ± 0.34[a]	5.89 ± 0.05[a]	13.44 ± 0.29[a]

* Reported values are the means ± standard deviation of three replicates.
Different letters in each column represent significant differences in mean (P<0.05).

Table 4 Analysis of variance. Effect of corn flour replacement on mean physicochemical parameters of flour treatments.

Variable	Source	Degree of freedom	sum of squares	average of squares	Test statistics	Significant levels observed
pH	Between groups	3	0.06	0.02	8.6	0.007*
	Within groups	8	0.018	0.002		
	Total	11	0.078			
Moisture Content	Between groups	3	1.411	0.470	4.8	0.034*
	Within groups	8	0.783	0.098		
	Total	11	2.194			
Total Fat Content	Between groups	3	1.609	0.536	6.775	0.014*
	Within groups	8	0.633	0.079		
	Total	11	2.242			

* Significance at the 5% level.

Generally, the amount of fat in hard corn flour is higher than that of soft corn flour [26]. The fat content in hard corn flour is about 2 to 2.5 %, while the amount of soft corn flour is 1.2 to 1.5 %. Typically, the maximum amount of fat in corn flour is 1% [8]. Fatty substances (lipids) in cereal starches (such as corn flour) are mainly fatty acids. In corn flour granules, there is at least part of the amylose and lipid in the form of the amylose-lipid complex. High levels of lipid in cereal starch can cause adverse effects [27]. The pH values of the treatments were also affected by hard corn flour, so that the corn flour changed the pH value. The highest pH was observed with 5.89 in the sample with 70% hard corn flour and the lowest with 5.69 in control treatment.

Generally, the pH values of corn flour is at the range of 4.5 to 7.0 [8] and in the present study all treatments were within the permissible range. The moisture content in the treatments was also affected by the replacement of hard corn flour. The addition of hard corn flour increased the moisture content of the treatments. The highest and lowest moisture content were observed in 70% hard corn flour (13.44%) and control (100% soft corn flour) treatments (12.41%), respectively. Typically, the maximum moisture content of corn flour was 14% [26], which was within the permissible range for all treatments in the present study. Hygroscopic or absorbent material is said to absorb moisture from the environment. The rate of moisture uptake depends on both the relative humidity of the surrounding air and the temperature [26].

Essentially all products made from starch hydrolysates have the potential to be hygroscopic, but the lower the DE, the slower the rate of moisture absorption. Because the moisture content is higher On the other hand, since the water absorption, as well as the percentage of water absorbed by simple sugars, is higher and the moisture content decreases with increasing DE, so that as well as the DE decreases, the moisture content in the flour would be increased [8].

Starch affects moisture retention, and the amount of moisture absorbed by starch granules depends on the relative humidity and the atmospheric temperature at which they are stored. Normal starches have 10-20% moisture under normal conditions [10]. Corn syrup contains simple glucose and fructose sugars that have the ability to absorb moisture. This may also be due to the higher retention of water by simple sugars (glucose and fructose) than sucrose [7]. Glucose syrup has a higher water holding capacity due to its lower molecular weight reducing sugars [1].

Some researchers [17] investigated the preparation of glucose syrup by enzymatic hydrolysis of corn flour, millet and sorghum. Their results showed that there was no significant difference in the amount of fat in corn flour, millet and sorghum. The moisture content of corn flour, millet and sorghum were 6.7%, 5.3% and 6.6%, respectively. According to them, the discrepancy observed between these three types of cereals may be due to differences in the variety or physicochemical properties of the cereals [16].

Regardless of the source of the plant from which the starch is extracted, the differences in the lengths of the amylose and amylopectin chains, their molecular weight and the presence of other compounds such as fat and protein cause important differences in the performance and properties of starches from their sources [3].

Overall, according to the results, fat content, pH and moisture content of the floury (soft) corn flour (control treatment) was lower than those of the others, although showed no significant difference with 30% flint (hard) corn flour treatment (P>0.05). But at higher concentrations, these amounts of decreased significantly (P<0.05). Therefore, the use of flint (hard) corn flour instead of soft corn flour increased the pH, moisture and fat content of the treatments.

3-2- Glucose Syrups Characteristics

Physicochemical Properties of the standard and the obtained glucose syrups according to individual treatments are presented in Tables 5-8 and also depicted in Figures 2-5.

Table 5 Standard physicochemical properties of glucose syrup*.

	Characterization	Acceptable Range
1	Taste	Has its own sweetness
2	Aroma	Has a special smell
3	Color	Colorless to bright yellow
4	Appearance	Clear and Transparent
5	External Materials	Negative
6	Dextrose Equivalent (total solid dry basis, wt. %)	20 (minimum)
7	pH	5 ± 0.5
8	°Brix	78 (minimum)
9	Sulfated Ash (total solid dry basis, wt. %)	0.7 (maximum)
10	Sulfur anhydride (mL/kg)	20 (maximum)
11	Starch	Negative

(Data Base: The Institute of Standards and Industrial Research of Iran, No. 621, 2009).

3-2-1-Glucose Syrups Dextrose Equivalent Values

Table 6 presents the effect of corn substitution on dextrose equivalent of glucose syrups. Results showed that dextrose equivalent was significantly affected by different amounts of hard corn flour (P<0.05). According to the statistical analysis, there was no significant difference between DE of control glucose syrup and treatment with 30% hard corn flour (P>0.05), but was significantly higher than those of in treatments containing 50 and 70% hard corn flour. The highest amount of glucose syrup DE was observed in control and the lowest related to the treatment containing 70% hard corn flour. According to flint flour replacement results, the addition of hard corn flour reduced the dextrose equivalent of glucose syrups. The results of this analysis corresponded to the standard values provided and were within acceptable limits.

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Table 6 Physicochemical Properties of produced glucose syrups according to individual treatments.

Treatment	Mixtures of Corn Flour (flint corn + floury corn)	pH	Dextrose Equivalent (DE) (%)
Control	0 + 100	5.07 ± 0.11^{a}	42.51 ± 0.39^{a}
1	30 + 70	4.98 ± 0.05^{ab}	42.11 ± 0.25^{ab}
2	50 + 50	4.91 ± 0.03^{b}	41.65 ± 0.12^{b}
3	70 + 30	4.90 ± 0.02^{b}	40.75 ± 0.28^{c}

* Reported values are the means ± standard deviation of three replicates.
Different letters in each column represent significant differences in mean (P<0.05).

Typically, the amount of dextrose equivalent in hard flour was lower than that of soft corn flour [10], so that the use of flint corn flour instead of soft corn flour in the production of glucose syrup reduced the dextrose equivalent. Regarding to this, the lowest amount of dextrose equivalent was 40.75 in the sample with 70% hard corn flour and the highest was observed with 42.51 in control (100% soft corn flour). Dextrose equivalent is a measure of total reducing sugars, calculated as dextrose, expressed as a percentage of dry solids. Starch has zero DE. Dextrose, the end product of hydrolysis or starch breakage, has DE 100. Therefore, DE can be considered as a scale and indicator for measuring the degree of hydrolysis that a product can endure [10].

Glucoamylase hydrolyzes 1, 4 as well as 1, 6-alpha-linkages in the amylopectin part of liquefied starch [29]. Glucoamylase is an exo-amylase, which cleaves 1,4-α-glycosidic bonds from the non-reducing end of the glycosidic chains releasing d-glucose, thus increasing the content of fermentable carbohydrates and reducing the non-fermentable dextrin [30]. On the other hand, the use of α-amylase for dextrose production facilitates industrial production of this product and reduces production cost [10].

The benefits of α-amylase are include reducing by-products, increasing dry matter content, rapidly reducing viscosity during hydrolysis, reducing dye production, requiring no dyeing and reducing filtration costs [29].

Higher glucose syrup with dextrose appears to be more suitable for use in food products. As dextrose increases, the moisture content and tendency of dryness in the product decrease [6]. Basically, glucose syrup creates a thin layer on the surface of the product, resulting in softness and freshness of the product [31].

3-2-2- Glucose Syrups pH Values

Regarding the obtained results (Table 6), corn replacement effect on pH was influenced by different amounts of corn flour (P<0.05) and adding flint corn flour within its final pH value reduced the final pH of glucose syrups too. The correlation results of statistical analysis showed that there was no significant difference between glucose syrup pH in the control and the treatment containing 30% hard corn flour (P>0.05). But in control was significantly higher than those of the treatments containing 50 and 70% hard corn flour. The highest (5.07) and lowest (4.90) glucose syrup pH values were observed in control and 70% hard corn flour, respectively. The results of this analysis corresponded to the standard values provided and were within acceptable limits. Organic acids cause an acidic state in the syrup and play a major role in the characterization of the syrup flavor. The main acid found in syrup is gluconic acid [32]. The pH range of glucose syrups are between 5.5 - 4.5 [33], which in the present study all treatments were within the permissible range.

Table 7 Analysis of variance. Effect of corn flour replacement on mean physicochemical properties of glucose syrup treatments.

Variable	Source	Degree of freedom	sum of squares	average of squares	Test statistics	Significant levels observed
pH	Between groups	3	0.056	0.019	4.32	0.041*
	Within groups	8	0.033	0.004		
	Total	11	0.89			
Dextrose Equivalent	Between groups	3	5.78	1.92	25.22	0.000*
	Within groups	8	0.611	0.076		
	Total	11	6.39			
Soluble Solids	Between groups	3	7.8	2.6	31.21	0.000*
	Within groups	8	0.667	0.083		
	Total	11	8.47			
Sulfated Ash	Between groups	3	0.015	0.005	11.22	0.003*
	Within groups	8	0.004	0.000		
	Total	11	0.018			
Color Parameter	Between groups	3	7610.25	2536.75	140.93	0.000*
	Within groups	8	144.00	18.000		
	Total	11	7754.25			

* Significance at the 5% level.

3-2-3- Glucose Syrups Soluble Solids (°Brix)

The results of comparing the mean initial soluble solids (°Brix) in different amounts of hard corn flour replacement (Fig. 2) were correlated with those of for dextrose equivalents and pH and showed that the addition of hard corn flour significantly reduced the soluble solids content in glucose syrups (P<0.05). The highest soluble solids content (27.47) was observed in control (100% soft corn flour) and the lowest one (25.57) was observed in 70% hard corn flour (30% soft corn flour) (P<0.05). According to the main procedure of glucose syrup preparation (depicted in Fig. 1 as a schematic diagram), the final stage includes the evaporation step in order to concentrate the obtained syrups and level up the initial brix up to the final brix by more than 80 (~82.5-85) which is corresponded to the standard values provided and were within acceptable limits.

When the inversion process occurs, due to the water involved in the process, an increase in solids (Brix) and soluble solids occurs, this increase in solids is known as hydrolysis gain [19]. Solids for an acid conversion process can be about 40%, but for an enzymatic conversion process, solids are usually less than 35% [1]. In soft corn flour, starch is readily available to the enzyme, which increases the solubility of glucose syrup solids [34]. By increasing the fructose content and DE, it is possible to increase the syrup solids content [19]. The amount of final solids depends specifically on the DE syrup, its viscosity and osmotic pressure. As a general rule, the lower the amount of DE, the less dry syrup solids will be. This is because syrups with less DE are viscous and once their solids content is increased, it will no longer be possible to be processed [30].

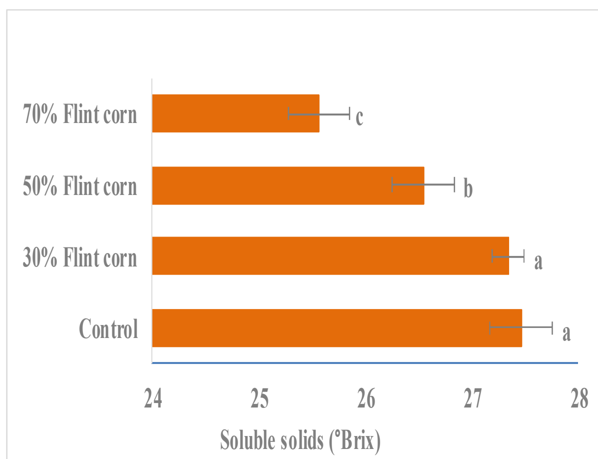

Fig 2 Soluble Solids (°Brix) of glucose syrups obtained from different individual treatments. Reported values are the means ± standard deviation of three replicates. Error bars indicate the maximum deviation from the mean values. Different letters represent significant differences in mean (P<0.05).

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3-2-4- Glucose Syrups Sulfated Ash Content

The results of comparison of the mean effect of different amounts of hard corn flour replacement (Fig.3) showed that glucose syrup sulfate ash was affected by hard corn flour and the change in hard corn flour content caused a change in glucose syrup ash. The highest amount of sulfated ash was observed in 50 and 70% treatments of hard corn flour (0.51 and 0.50%, respectively) ($P>0.05$) and the lowest was in control (100% soft corn flour) (0.41%) ($P<0.05$). The results of this analysis corresponded to the standard values provided and were within acceptable limits.

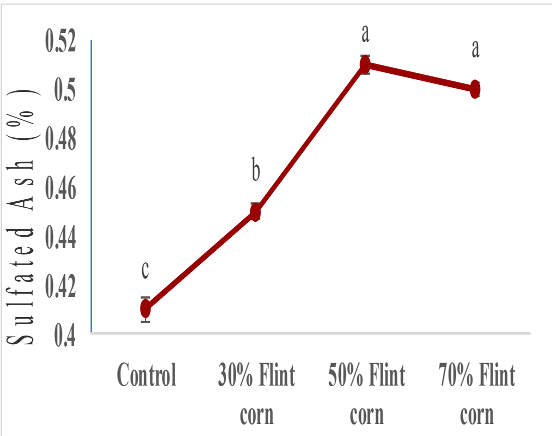

Fig 3 Sulfated ash (%) of glucose syrups obtained from different individual treatments.
Reported values are the means ± standard deviation of three replicates. Error bars indicate the maximum deviation from the mean values. Different letters represent significant differences in mean ($P<0.05$).

Ash is produced during the hydrolysis process. Ion exchange resins are typically used to separate these ions from the syrup [17]. Although the presence of high ash reduces the efficiency of the activated carbon process, but suitable ion exchange systems in the production of glucose and fructose syrups would significantly reduce syrup ash and contributes to the color and taste stability due to the removal of color precursors and catalysts [14]. Glucose and fructose syrups are highly fermentable, due to the high content of mono and disaccharides (1–2%) and the small amount of oligosaccharides. The higher the ash, the more the color intensifies in the syrup [10]. In flint (hard) corn flour, minerals would hardly be separated, which increases the amount of sulfated ash in corn flour [16]. Therefore, the use of hard corn flour instead of soft corn flour increased the amount of sulfated ash in glucose syrup. The maximum limit for sulfate ash in glucose syrup is equal to 0.7% [8], which was within the permissible range for all treatments in the present study.

3-2-5- Glucose Syrups Colorimetric Parameter

Fig. 4 depicts the effect of hard corn flour substitution (at 30, 50 and 70%) on glucose syrup color. It was shown that substitution of hard corn flour in different percentages caused significant changes in syrups color ($P<0.05$). The color of glucose syrup increased with the addition of hard corn flour. The highest (140.33) and the lowest (75.33) amounts of syrup color were observed in 70% treatment (30% soft corn flour) and control (100% soft corn flour), respectively. The results of this analysis corresponded to the standard values provided and were within acceptable limits.

Ion exchange resins as well as activated carbon are used to bleach the syrup. In the case of powdered activated carbon, 50 grams of activated carbon is typically used for every 5 kg of corn flour (approximately 1% by weight). In addition, the values obtained regarding the measurement of colorimetric parameters showed an acceptable range of optimal treatment, which is usually applicable to the final syrup in the baking industry and bakery and confectionery products (cakes, cookies, etc.) [8,15,27].

Turbidity in glucose syrups is an important factor and the lower the turbidity, the more customer-friendly and marketable it will be [8]. Unlike sucrose, fructose and glucose are inextricably linked to fructose syrup, which is why they are more involved in browning reactions and intensify coloration [7]. Heating of glucose and fructose sugars intensifies the production of hydroxyl-methyl furfural, thereby increasing colorimetric indices [34]. The increase in browning and the color of the syrups is more pronounced with the increase in the ratio of hard corn flour, due to the increased acidity and lower pH in the hard flour samples. Occasionally an increase in the amount of ash and minerals can also increase the amount of dye in glucose syrups [1,36].

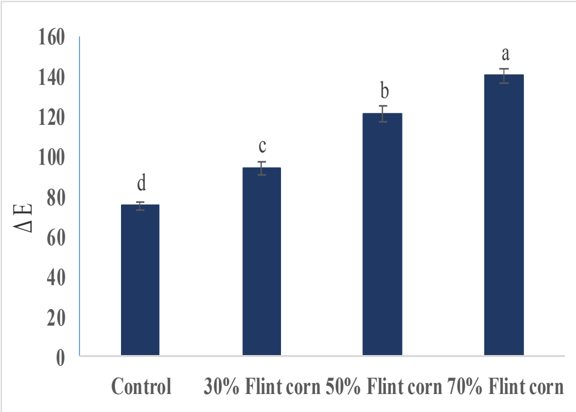

Fig 4 Color Parameter (ΔE) of glucose syrups obtained from different individual treatments.

Reported values are the means ± standard deviation of three replicates. Error bars indicate the maximum deviation from the mean values. Different letters represent significant differences in mean (P<0.05).

On the other hand, the pigment content of hard corn flour is higher than that of soft corn flour because in hard corn flour, pigments stick to the starch and increase the color of the syrup, while in the corn flour the pigments are easily separated from the starch [6]. So that, depending on the type of glucose syrup used and the total starch hydrolysis, bleaching is essential. In addition to the separation of dye compounds, the separation of dye-producing materials is also important for increasing the shelf life of the product during storage without creating opacity and color [14,15].

3-2-6- Glucose Syrups Sensory Evaluations

The results of analysis of variance and post-hoc test (LSD) (Table 8) showed that the effect of hard corn flour substitution (30% and 50%) on glucose syrup organoleptic parameters including flavor, aroma, appearance and overall acceptability was not significantly different in comparison with the control (P>0.05).

But regarding to the treatment consisted of 70% hard corn flour, all the sensory parameters were significantly different from those of other samples (P<0.05). Increasing higher amounts of flint corn flour had affected these parameters in the syrup and reduced them in the case of being probable. In this way, organoleptic parameters of the treatment with 70% flint corn flour were not approved by the evaluators.

Table 8 Analysis of variance. Effect of corn flour replacement on mean organoleptic parameters of glucose syrup treatments.

Variable	Source	Degree of freedom	sum of squares	average of squares	Test statistics	Significant levels observed
Flavor	Between groups	3	2.91	0.972	3.88	0.055
	Within groups	8	2.000	0.250		
	Total	11	4.91			
Aroma	Between groups	3	1.000	0.333	0.667	0.596
	Within groups	8	4.000	0.500		
	Total	11	5.000			
Appearance	Between groups	3	2.91	0.972	3.88	0.055
	Within groups	8	2.000	0.250		
	Total	11	4.91			
Overall Acceptability	Between groups	3	2.91	0.972	3.88	0.055
	Within groups	8	2.000	0.250		
	Total	11	4.91			

* Significance at the 5% level.

Also according to Fig. 5, 70% replacement of flint (hard) corn flour reduced the overall acceptance (acceptability) of the obtained glucose syrup. Therefore, this treatment did not seem appropriate for producing glucose syrup. Generally, glucose syrups are clear colorless and tasteless syrups with no smell and have a viscosity similar to liquid sugar [3].

Using flint corn leads to an increase in the fructose content of the syrups and is evaluated as less favorable due to their darker appearance, due to increased brown reactions which is due to reducing sugars [25]. Treatment with ion-exchange and activated carbon resins destroys

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any minerals, undesirable color and taste. Therefore, it may be possible to control and inhibit the relatively altered color and taste in hard corn flour samples using auxiliary treatments [19].

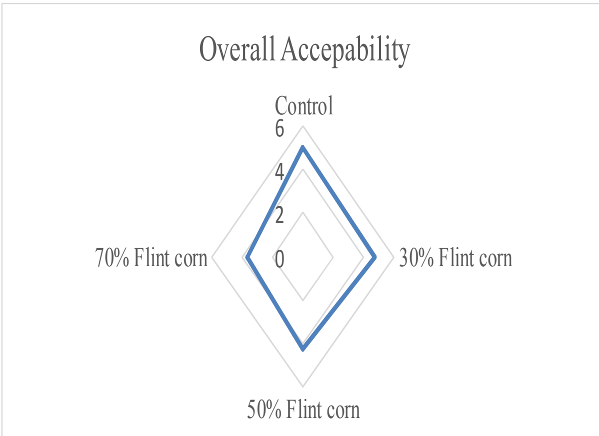

Fig 5 Overall acceptance of glucose syrups obtained from different individual treatments.
Reported values are the means of three replicates.

3-2-7- Cost-Effective Evaluation of Production

Table 9 represents an evaluation on cost effective production of glucose syrups. Calculations carried out according to the different replacements of flint (hard) corn flour instead of floury (soft) corn flour which led to different proportional formulations of glucose syrups.

Any type of starch can be used to make glucose syrup, but in practice, a combination of factors such as starch availability, price, trade policy, cost estimation and acceptable technology in converting starch to glucose syrup is considered. Regarding the main sources of starch production, including corn, wheat, potatoes and rice, these three products are not used to produce syrup, due to the high cost of potatoes and rice and also due to the common use of wheat in the baking industry. But corn is used as the main and available source for syrup production [14,17,18]. Soft corn is generally used to produce glucose syrup, but since most corn products are imported, it is not always possible to access the desired varieties, and in practice, different types of corn varieties are used [11,14]. Although in the starch industry, mainly soft (floury) corn is consumed and in our country, sometimes imported corn is hard corn, so combining these two types of corn is inevitable.

On the other hand, regardless of the price debate, at certain times of the year, only soft corn is grown (in the southern hemisphere), so at times of the year when it is not cultivated, it is sold at a higher price (at full price) and storage costs will also increase. But hard corn is grown at all times of the year in both the northern and southern hemispheres and usually has a fairly fixed price. It is also preferable to use a dry mill instead of a wet mill to grind corn [11,17]. Since rainfall is low in Iran, saving water consumption is naturally very important, especially in industry and the use of dry mills consumes less water compared to wet mills.

By calculating the cost of raw materials related to a batch of treatment formulations, the results indicated a reduction in the cost of the final product as a result of increasing the percentage of hard corn flour. In this way, the price of the finished product has decreased as the amount of hard corn flour has increased. In the wet corn milling method for producing glucose syrups, the water consumption is 2.5 to 3 times the product weight, depending on the amount of wastewater turn back to the production line. But in dry corn milling method without ion exchange system, the amount of water consumption is 0 - 1 times the final product [9, 37].

The flour used in this study was a by-product of dry mill which reduced water and wastewater consumption. Products derived from natural materials are considered to be more acceptable. Glucose syrup is used as a natural ingredient in many food processes. Glucose syrup is one of the best sugar substitutes and its sweetness is 0.7% sugar which can be used in food, pharmaceutical and chemical industries for various applications. So that, manufacturing of corn syrup on an industrial scale and taking into account the production process during the year, replacing glucose syrups in different scale of corn flour and according to the technological need of each industrial unit, would be effective in cost calculation and economically cost effective of the production.

Table 9 Comparison of glucose syrups final costs, according to the raw material for individual formulations in a batch.

Samples	**Mixtures (flint corn + floury corn)**	**Variable formulation components (W / W)**		**Fixed formulation components (W / W)**			**Total Price* (USD)**
		Floury (soft) corn flour (kg)	Flint (hard) corn flour (kg)	α-amylase (mL)	Glucoamylase (mL)	Water (L)	
Control	0 + 100	5	0	2	0.2	15	
1	30 + 70	3.5	1.5	2	0.2	15	
2	50 + 50	2.5	2.5	2	0.2	15	
3	70 + 30	2.5	3.5	2	0.2	15	
Price* (USD)	0 + 100	1.375	0	0.01	0.0012	1.35	2.768[a]
	30 + 70	0.962	0.375	0.01	0.0012	1.35	2.699[b]
	50 + 50	0.687	0.625	0.01	0.0012	1.35	2.657[c]
	70 + 30	0.412	0.875	0.01	0.0012	1.35	2.631[d]

* Based on US dollar day price, 2018.

4- Conclusion

Glucose syrup is one of the many industrial uses. Therefore, the purpose of this study was to design an experiment to investigate different treatments of corn flour for the production of this high consumption material.

Different amounts of hard corn flour were used to make glucose syrup. The results showed that the use of flint (hard) corn flour in combination with floury (soft) corn flour up to 30% did not make a significant difference in the physicochemical properties of glucose syrups. Although at higher concentrations the physicochemical properties of glucose syrup changed, but the characteristics of treatments containing 50% and 70% hard corn flour were also within the standard range of glucose syrups. Only the treatments with 70% flint flour were not approved by the evaluators in case of organoleptic properties. However cost estimates indicated a reduction in cost of raw materials and consequently a general reduction in production costs by replacing hard corn flour. According to the obtained results, it can be concluded that hard corn flour can be used on all surfaces, but the best treatment was 50% replacement level or a bit more which had a good effect on the properties of the produced glucose syrups and showed more similarity with the control sample. All of these, along with being cost effective, appeared this treatment to have the potential of supposing as a sugar substitute in food industry.

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مقاله علمی_پژوهشی

تولید شربت گلوکز از طریق هیدرولیز آنزیمی مخلوط‌های آرد ذرت سخت و نرم و ارزیابی ویژگی‌های آن به‌عنوان شربت مقرون به صرفه اقتصادی

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چکیده

مشتقات نشاسته ذرت، مشتمل بر شربت‌های گلوکز، امروزه به‌طور گسترده‌ای در صنایع غذایی موردمصرف قرارمی‌گیرند. شربت گلوکز نه‌تنها صرفاً به‌دلیل قدرت شیرین‌کنندگی و ارزش غذایی‌اش، بلکه به‌دلیل خواص عملکردی شایان توجهش (قابلیت تثبیت رطوبت، نرم‌کنندگی، بهبود بافت و قابلیت جلوگیری‌از کریستالیزه‌شدن ساکارز) در صنایع غذایی مورد استفاده قرار می‌گیرد. برای تولید شربت گلوکز معمولاً ذرت آردی (نرم) (*Zea mays var. amyalcea*) مصرف می‌شود. اما بیشترین ذرت وارداتی به ایران ذرت سخت (*Zea mays var. indurate*) است که در تمام طول سال قابل دسترس است.نتیجتاً استفاده از هر دو نوع آرد ذرت به‌منظور تولید شربت گلوکز در ایران اجتناب‌ناپذیر است. بنابراین، هدف از این مطالعه، بررسی تأثیر استفاده از آرد حاصل‌از ذرت سخت و ذرت آردی (نرم) برای تولید شربت گلوکز بود. چهار تیمار شامل مخلوط آرد ذرت سخت + آرد ذرت نرم در چهار نسبت ۷۰٪+۳۰٪، ۵۰٪+۵۰٪، ۳۰٪+۷۰٪ و نیز ۱۰۰٪ آرد نرم به عنوان نمونه کنترل تهیه شدند و ویژگی‌های فیزیکوشیمیایی و ارگانولپتیکی شربت‌های تولیدشده مورد ارزیابی قرار گرفتند. با توجه به نتایج به‌دست‌آمده، استفاده از آرد ذرت سخت بر ویژگی‌های فیزیکوشیمیایی و ارگانولپتیکی نمونه‌ها تأثیرگذار بود ($P<0.05$). افزایش نسبت به‌کارگیری آرد ذرت سخت، به‌طور معناداری میزان DE (معادل دکستروز)، مواد جامد محلول و pH شربت‌های گلوکز تولیدشده را کاهش‌داد ($P<0.05$). همچنین به افزایش پارامترهای رنگ و مقادیر خاکستر سولفاته شربت‌های تولیدشده منجر شد. با این‌حال با افزایش نسبت به‌کارگیری آرد ذرت سخت، برآوردهای هزینه حاکی از کاهش قیمت تمام‌شده مواد اولیه و در نتیجه کاهش کلی هزینه‌های تولید، دراثر جایگزینی آرد ذرت سخت بود. از نتایج به‌دست‌آمده چنین استنباط شد که آرد ذرت سخت می‌تواند در تمام مقادیر پیشنهادشده قابل‌استفاده باشد، اما بهترین تیمار، سطح جایگزینی ۵۰٪ یا کمی بیشتر در تولید بود که بر ویژگی‌های شربت گلوکز تأثیرات خوبی به‌همراه داشت و با ویژگی‌های نمونه کنترل مشابهت بیشتری داشت. تمام موارد بررسی و ذکر شده، به‌موازات مقرون‌به‌صرفه بودن از نظر اقتصادی، پتانسیل به‌کارگیری این تیمار را به‌عنوان جایگزین شکر در صنایع غذایی مطرح و مفروض می‌نماید.