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Utilization of Labneh Whey Lactose Hydrolyzed Syrup in Baking and Confectionery

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Abstract: Hydrolyzed labneh whey lactose syrup was obtained by the action of immobilized Beta-galactosidase enzyme using fluidized bed reactor system. The obtained sweet syrup was used as sugar replacement in three products (French - type bread, Cherry cake and Kunafah syrup). The sensory evaluation results showed that bread made with 25% sugar replacement level was better than the control one. For (Cherry cake and Kunafah) there were no significant differences between the products made with 25% sugar replacement level and the control samples. The bread dough mad with 25% sugar replacement had a better rheological properties with regards to stability, rate of absorption and mechanical tolerance indication. The nutritional value of the newly developed product was improved with regards to high amount of protein content compared to control products. No microbial growth was detected in both control and new developed bread products during storage.

Key words: Acid whey, whey lactose hydrolysis, whey lactose hydrolysate concentration, French type bread, bread dough, sugar replacement

INTRODUCTION

Whey is a by-product resulting from dairy industry especially cheese production. Both traditional and modern methods of cheese manufacture produce a huge amount of cheese whey (about 83% of the entire volume of milk used). Whey is classified to two types, sweet whey with a pH of about 6.02-6.58 and acid whey with a pH of 3.57-4.34 (Alsaed *et al*., 2011). The majority of the globally produced whey is sweet whey produced as a by-product of cheese industry (Boutin, 2010). On the other hand, acid whey is only produced in some Mediterranean countries including Jordan.

Whey had been a major world-wide disposal and pollution problem for the dairy industry. Thus, nutritionally valuable food resource was wasted and added to the environmental pollution. Biological Oxygen Demand (BOD) for whey is considered to be high varying from 30.000-60.000 mg/L (Ponsano and Castro, 1995). Boutin (2010) reported in the workshop held in Amman on 13 June 2010 by the U.S. Dairy Export Council, that nill of the American whey is wasted and there is a highly sophisticated whey industry in the country. He concluded the workshop by the following statement: "I hope I have opened your eyes to new possibilities associated with whey where new products can be made and old products can be made better and lower in cost".

In Jordan where the majority of the produced whey is of the acid type (a by-product of labneh production), there is still what can be called "the whey problem" i.e. disposing of acid whey in the sewerage system and increasing the pollution level.

The annual estimated amount of whey produced by the Jordanian dairy industry is about 40000 tons. More than three quarters of this quantity is acid whey and is produced from labneh, whereas the other is sweet whey that is produced from white cheese (Alsaed *et al*., 2011) It was noted that small amount of this whey (less than 1%) is used in the Jordanian food industry especially in beverages, whereas, the rest amount (99%) is disposed in the sewage causing an environmental contamination problem due to its high BOD (Yousif *et al*., 1997).

Yousif *et al*. (1998) studied the concentration of acid whey and its functionality in French type bread, they reported that the stability and the mechanical tolerance index of French bread dough was improved by the addition of whey solids, as were the internal and external characteristics of the bread. In addition, they found that the addition of whey solids had a retardation effect on bread staling and extended its storability by two days.

Additionally, Yousif *et al*. (1997) used the hot plate technique in drying the normal whey which was used in Arabic bread production, they found that the addition of whey solids (0.85-3.5%) improved the rheological properties of Arabic bread dough, i.e. increased absorption, dough devolvement time, improved machinability, as well as the specific loaf volume and sensory properties. It was found that whey contributed tenderness or shortness to cookies and crackers, pie dough and other baked products which otherwise require high levels of shortening to achieve the effect (Weisberg and Goldsmith, 1969).

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Many studies concentrated on the application of whey and whey powders in food industry.

The objectives of this study were to alleviate the environmental contamination problem caused by whey and the utilization of the whey lactose hydrolysate as a sugar replacer in the local food industry like bakery and confectionery.

MATERIALS AND METHODS

Sample collection and preparation: Acid labneh whey samples were collected from the dairy pilot plant at the University of Jordan. Potassium hydroxide (Sigma-Aldrich- St. Louis. Mo USA) was used to neutralize the acidity to the desired pH value (Rajakala and Karthigai, 2006). pH meter (Hanna instruments Hi - 8519-Italy) was used to monitor the pH of the treated whey. Whey sample was sterilized by membrane filtration (cellulose nitrate with 0.2 mm pore size and 47 mm diameter, Microfiltration system, U.S.A); the sample was introduced into the fluidized bed reactor for lactose hydrolysis. All conditions with regards to lactose hydrolysis were optimized (pH, temperature, flow rate, enzyme concentration) to attain maximum lactose hydrolysis (Alomari *et al.*, 2011).

Hydrolyzed lactose (sugar syrup) concentration: The hydrolyzed lactose in acid whey samples were concentrated using vacuum scraper concentrator (manufactured by Zhejiang Wenxiong Machine Valve Co., China; model ZN50). The concentration process was carried out at temperature 70°C, vacuum and pressure at 0.03, mpa, time or duration needed for concentration was 45 min), the total solids achieved (20 and 40%). Many samples of the concentrated sugar syrup obtained were evaluated organoleptically with regard to color, flavor and consistency.

Rheological properties of the bread dough: The rheological properties of the bread dough were evaluated using Farinograph according to the AACC method (2000).

Test baking: The experiments regarding the replacing of sugars by the concentrated sugar syrup in one bakery item (French-type bread) and two confectionery products (Kunafah and Cherry cake) were carried out in the University of Jordan (JU) restaurant. The methods and recipes for preparation of the 3 mentioned items by the (JU) restaurant were followed.

French-type bread: The control dough was prepared using the straight dough bulk fermentation process. All ingredients i.e flour (2 kg), water (about 1000 ml), salt (30 g), yeast (50 g), improver (30 g) and sugar (200 g). Three experimental bread doughs were prepared having 25, 50 and 75% actual sugar replacement. The doughs were mixed until they had achieved proper development. The doughs were first proofed for 5-10 min at 35°C, then divided into 80 g pieces, moulded, panned and finally proofed at 45°C and 85% relative humidity until a suitable height had been attained. The loaves were baked at 230°C for 15 min using a rotating oven and then allowed to cool in the pans for 10 min before deepening.

Cherry cake: The control dough was prepared using the straight dough preparation. All ingredients i.e water (1 L), Sugar (150 g), powdered cream (350g), gelatin (50 g), canned cherry (10 g) as flavoring agent. Three experimental cake doughs were prepared having 25, 50 and 75% sugar replacement by sugar syrup. The doughs were mixed until they had achieved proper development. Then the product was kept in the refrigerator to achieve the desired firmness and consistency.

Kunafah syrup: The control syrup was prepared using (600 ml) water and (300 g) sugar. The syrup was heated until boiled and becomes semi viscous, three experimental sugar syrup were prepared having 25, 50 and 75% sugar replacement by hydrolyzed lactose syrup. Then the prepared syrups were added to the previously prepared Kunafah; consisting of (500 g) ready Kunafah dough, (100 g) ghee and (1 kg) cheese. The doughs were mixed until they had achieved proper development. Then the previous ingredients were added and heated for proper period. The prepared sugar syrups (control, 25, 50 and 75%) were added to end product and served hot.

Chemical analysis of product samples: Protein and ash content were determined using AOAC method (1995). Water activity was measured using Novasina instrument (Axier Ltd); the method given in the manual of the instrument was followed; the temperature was calibrated to reach 25°C, then switching on the instrument for 10-15 min. The sample was put in plastic plate then placed in the specified chamber. The instrument was left until the reading of the water activity appears on the screen of the instrument.

Sugar extraction and determination: The sugar contents of the developed products were extracted using a method described by (Langemeier and Rogers, 1995), with slight modifications. Five g of each sample (bread, Cherry cake and Kunafah syrup) was placed in 100 ml volumetric flask with 70% ethanol. The sample was homogenized in a homogenizer (Ultra turex mixture, Type 25, Germany) for 5 min at 15000 rpm and then sonicated for 15 min at 45°C. The sample was then filtered using (Whatmon filter no.1). The filtrate was micro filtered using 0.45 μm nylon-type membrane. Then the sample was ready for HPLC analysis. Using (NH2-R-P-Macherey-Angel-Germany) with 79:21 aceotonitril: water at flow rate of 1 ml/min.

Sensory evaluation: The produced products (French-type bread, Cherry cake and Kunafah) were evaluated by a panel of assessors (12 semi-trained subjects) from the Department of Nutrition and Food Technology at the University of Jordan. Bread samples were evaluated for the sensory attributes on a 9 point hedonic scale, with 9 indicating "dislike extremely" and 1 "like extremely". Testing was conducted in one session in a well- lighted and odor-free environment with specific instructions for sample evaluators. Kunafah and Cherry cake were evaluated for taste and aroma. The sensory evaluation for the three products were done twice in duration of 1 week (Ogunrinola *et al*., 1988; Yousif *et al*., 1991).

Bread storage study: The bread samples was wrapped in polyethylene bags, sealed and stored for 5 days at 20°C. Bread samples were checked every day (Yousif *et al*., 1998).

Statistical analysis: The Analysis of Variance (ANOVA) was done using mixed procedure (Proc Mixed). Differences between treatment means were tested using protected Least Significant Difference (LSD) test at $p<0.05$. All statistical analyses were performed using the SAS software, version 9 (SAS Institute, 2002).

RESULTS AND DISCUSSION

Farinograph results: Figure 1 a, b, c and d show the results of farinograph analysis. The results indicated that, the addition of Concentrated Lactose Hydrolyzed Acid Whey Syrup (CLHAWS) increased significantly the rate of water absorption ($p\leq0.05$), although this increase was relatively slight. It ranged from 60% for control sample to 66% for the 75% sugar replacement level. These changes may not be due to sugar only because whey contains proteins, salts and other soluble components (Yousif *et al*., 1998). However, these results agree with those reported by Yousif *et al*. (1997) and Thompson and Baker (1983). They reported that the addition of total solids from whey increased the rate of water absorption.

Addition of low percentage of sugar syrup (25% replacement level) positively improved dough stability. The obtained results are in agreement with those reported by Bilgin *et al.* (2006) who found that, the addition of whey and buttermilk significantly increased dough stability, due to the role of physicochemical properties of whey and buttermilk which contain different protein sources, milk fat and lactose. On the other hand, negative effect was noticed by increasing the added percentage of sugar syrup. Such results agree with those obtained by Abdalla *et al.* (2011) who reported negative effect for the addition of whey on wheat flour dough stability. These results may be due to the different reactions under the conditions of dough preparation. Sulphydryl/disulfide group may play an important factor in controlling the properties of dough.

It is clear from (Fig. 1d) that the Mechanical Tolerance Index (MTI) significantly increased ($p\leq0.05$) in the three levels of sugar replacement. MTI measure the degree of softening during dough mixing, the higher the (MTI) the weaker the flour. MTI reached its maximum value (60 BU) at 50% replacement level, whereas it was (50 BU) at 25% and (21 BU) at 75% replacement level. These results indicated that, the addition of hydrolyzed syrup did not affect significantly ($p>0.05$) the dough development time. These results agree with those reported by Yousif *et al.* (1998).

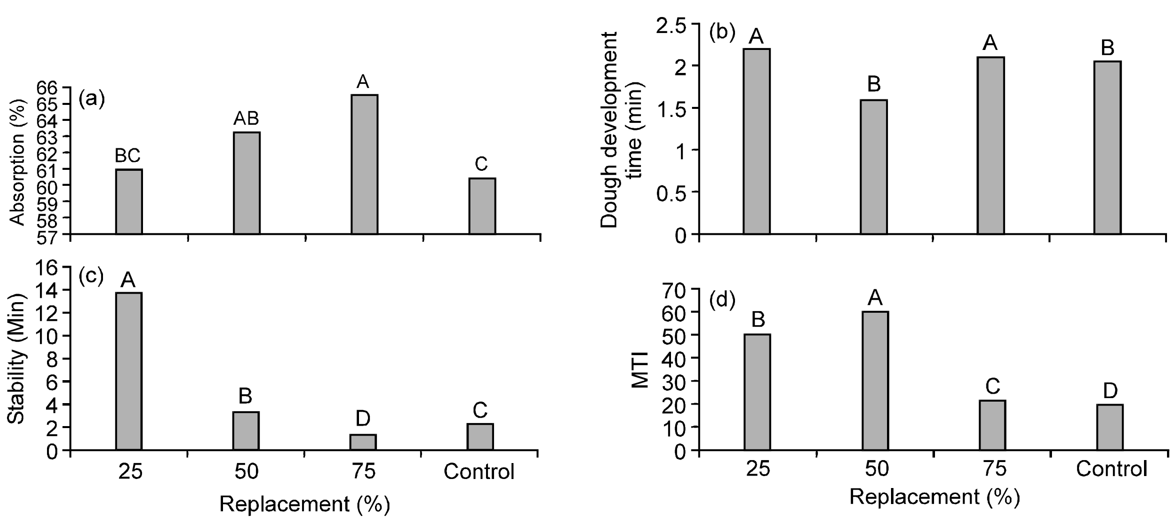

Fig. 1: (a) Farinograph analysis (Absorption); (b) Farinograph analysis (Dough development time); (c) Farinograph analysis (Stability); (d) Farinograph analysis (MTI: Mechanical tolerance index)

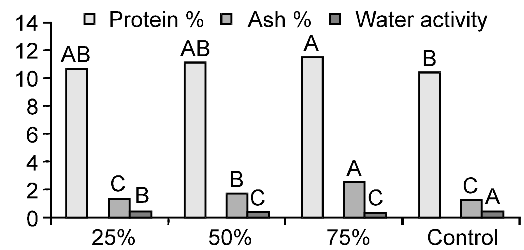

Fig. 2: Chemical analysis of French bread sample

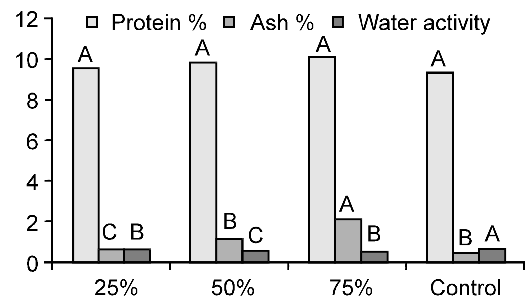

Fig. 3: Chemical analysis of Cherry cake sample

Chemical analysis: Protein and ash percentages as well as water activity content were presented in (Fig. 2 and 3). The results showed that the addition of (CLHAWS) a slight although insignificant increase in the protein content of both bread and Cherry cake. It was clear (Fig. 2) that the protein content was increased as a function of increased level of added whey syrup. Protein content of the bread ranged from (9.37%) in the control to (10.13%) in the bread with 75% sugar replacement level. On the other hand, in cake products they ranged from (10.5%) for the control to (11.50%) for the 75% sugar replacement level. Jooyandeh *et al.* (2009) found that, supplementation of flour by fermented whey protein concentrate and whey permeate at 25, 50, 75 and 100% levels, increased the bread contents of protein, fat and ash. The values of these components increased with increasing levels of substitution.

Regarding the ash content, it was significantly ($p \leq 0.05$) increased as the level of sugar replacement level increased from 25-75%. It ranged from (1.32%) for the control sample to (2.35%) for the 75% replacement level for bread. For Cherry cake, the values ranged from (0.50%) for the control to (2.12%) for the 75% replacement level. These results are in agreement with those reported by Bilgin *et al.* (2006) who found that, the replacement of water with pasteurized whey and butter milk increased the macro minerals concentration, particularly the calcium and potassium levels ($p \leq 0.05$) of the bread samples compared to the control. The ash content of the bread samples reflects its contents of minerals. Such results were expected since whey contains different amounts of minerals like Ca, P, Mg, K and Fe.

Due to the low water activity level of bread (Fig. 2) the 75% replacement level had a better stability or shelf life than other treatment, water activity was (0.39) compared to (0.49) for the control. However, the water activity of samples with 25%, 50% replacement and the control sample was less than (0.6) indicating a good stability and shelf life for these products (Yousif *et al.*, 1990). This may indicate the possibility of longer storage of bread at ambient temperature (20±5°C) without deterioration. However Cherry cake water activity was significantly ($p \leq 0.05$) decreased as the sugar replacement level increased.

Water activity can be defined as the amount of water available for chemical reactions and microbial growth. The reported decrease in the water content as a function of increased level of sugar replacement might be due to the available total solids like protein, sugars, salts and minerals. Proteins become denaturated during baking process, as a result, the functional groups of denaturated whey protein becomes active and hydrated by free water. The same trends can be also followed to explain the effect of sugars on water activity. Sugars have a hydrophilic nature so, it can bind the free water and reduce its quantity and thus, the water activity. These results, however, agree with other earlier findings on water activity in baking products. It was found that, sugars are used in the food industry to lower water activity. They decrease the water activity as the concentration increase; however, the rate of changed of water activity per unit of concentration is different for different sugars (Sloan and Labuza, 1968; 1975). In addition to that, it was reported that, small solutes such as sugars and salts lower water activity in baking products by increasing starch gelatinization temperature (Sloan and Labuza, 1975; Ghiasi *et al.*, 1982). Dramatically physical, chemical and biological changes took place during bread making, such as evaporation of water, formation of porous structure, volume expansion, protein denaturation, starch gelatinization, crust formation ...etc. All of these changes might affect the chemical and physical analysis of the bread samples (Arapita and Datta, 2008).

Sugar analysis: The results of sugar analysis of the developed three products (French - type bread, Cherry cake and Kunafah syrup in which 50% sugar syrup was chosen as an intermediate added percentage) were presented in Fig. 4, Significant differences ($p \leq 0.05$) were observed between (sucrose, glucose, galactose, fructose and lactose) contents. Control bread contained (2.15%) sucrose, whereas in the 50% replacement level it was (1.95%). Since the French-type bread dough contains about 8% sucrose, so a marked decreased is clear in sucrose content of the bread. These results were in agreement with other findings (Yousif and Alghzawi, 1990) who reported that roasting of carob powder caused some reduction in sugar content due to

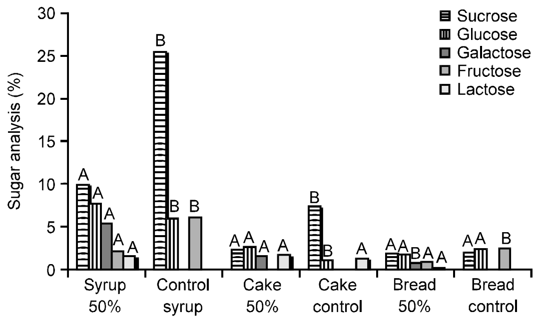

Fig. 4: Sugar analysis of developed products

the Millard reaction and carmalization. Nearly all sugars in the hydrolyzed syrup are invert sugars and therefore it is expected to play a major role in the browning process. During baking above (160°C) sugar undergoes a series of complex browning reactions (Czernohorsky and Hooker, 2010). The products of such reactions form the brown crust of many baked goods, these reactions are known as Millard reactions and are essentially amino acid-catalyzed caramelisation reactions in which a sugar aldehyde or ketone is converted to an unsaturated aldehyde or ketone (Czernohorsky and Hooker, 2010). Furthermore, it was found that, lactose is responsible for the improved crust color and flavor development in bakery goods when a dairy ingredient is used, as it combines with protein in Millard reaction (Singleton and Robertson, 1966). The formation of crust and browning during baking appears to be the primary contribution to the formation of bread flavor. The browning is mainly the result of Millard type browning reaction rather than of carmalization. Crust browning occurs when the temperature is greater than 110°C and it showed an experimental correlation with weight loss during baking and with oven temperature (Mondal and Datta, 2008).

Another chemical reaction that might affect the sugar content was fermentation process, where sucrose is considered the main ingredient to produce carbon dioxide and moisture. Fermentation is the process by which yeasts act on the sugars and starches to produce carbon dioxide and alcohol. This process is the fundamental step to making bread dough (Mondal and Datta, 2008). It was demonstrated that lactose was not hydrolyzed by Bakers yeast during normal fermentation steps in bread making and was thus available to undergo the Millard reaction which contributes to color development in bread crust (Guy, 1978).

The same trends can also be noted for sucrose level in the 50% replacement level treatment. It was clear from Fig. 4 that, galactose was not detected in the control sample compared to (0.95%) in the 50% replacement level. Presence of galactose in the 50% replacement level treatment was due to the hydrolysis process conducted on lactose which produce glucose and galactose that comes from added syrup. Regarding lactose, small amount of this sugar (0.3%) was detected in the 50% replacement level treatment compared to (0.0%) in control sample. This residual amount of lactose originated from the added syrup, because the hydrolysis rate of lactose was optimized at 85% after 6 hrs. These results were in agreement with the previous results reported about lactose hydrolysis (Alomari *et al.*, 2011); 87% of lactose hydrolysis was reported in milk using packed bed reactor with an immobilized yeast cell in Na-alginate beads after 7 hrs of incubation (Reeba *et al.*, 2010). In addition to that, Shah and Nickerson (1978) found that, 100% lactose hydrolysis was not necessary, since maximum sweetness was achieved with 70-90% hydrolysis rate, due to the synergistic sweetening effect of lactose, glucose and galactose.

With regards to Cherry cake product, it is clearly evident from (Fig. 4) that the glucose and galactose content were significantly ($p \leq 0.05$) different between the control sample and other treatments. These differences might be due to different quantity of the added syrup. Data in Fig. 4 also showed that, sucrose level was (7.55%) in the control sample, whereas it was (2.45%) in the 50% replacement level. The relatively high level of lactose (1.7%) in both control sample and 50% sugar replacement level might be due to the added cream (a major component of Cherry cake) that contains skim milk powder, as well as the residual amount of non hydrolyzed lactose that is already found in the added syrup.

Regarding Kunafah syrup product, the same trend for sugar composition can also be noticed. The sucrose level was (25.5%) in control sample compared to (10%) in the 50% sugar replacement level. The glucose level reached (7. 8%) in the 50% sugar replacement level compared to (6.1%) in the control syrup. Additionally, the 50% sugar replacement level contained (2.5%) fructose compared to (6.25%) in the control syrup. These fructose and glucose values were produced as a result of sucrose conversion under the effect of heat treatment during syrup preparations. It was found that sucrose level showed pronounced decrease during storage of date jelly (Yousif *et el* ., 1990) . Whereas the glucose and fructose levels were significantly increased. These changes might be due to the sucrose inversion due to the acid environment (Yousif *et al.*, 1990). Finally, galactose (5.5%) and lactose (1.65%) in the 50% sugar replacement is due to the addition of the lactose hydrolyzed syrup that contained galactose and residual amount of non hydrolyzed lactose. On the other hand (0.0%) of galactose and glucose were found in the control sample.

Sensory evaluation results: Table 1 showed that there were significant differences ($p \leq 0.05$) between the new developed three products and the control samples. The

Table 1: Sensory analysis of developed products

Product	25% replacement	50% replacement	75% replacement	Control	LSD values
French bread	2.4667C*	2.7000BC	3.4333A	3.0000AB	0.50093
Cherry cake	2.3667BC	2.9333B	4.5667A	2.1000C	0.57936
Kunafah	1.9333B	4.7333A	4.7333A	1.9618B	0.43285

*Means of duplicate. **Means in each row followed by the same letter are not significantly different at 95% confidence

sensory evaluation results of French-type bread showed that, bread prepared at 25% sugar replacement level was better than the control bread which achieved the best scores (2.46) compared to (3.00) of the control sample. But no significant differences were detected between the replacement level of 50 and 75% compared to the control sample since the 50% replacement sample achieved (2.70) scores and the 75% replacement achieved (3.43) scores. These results were comparable to those obtained by Ogunrinola *et al.* (1988), who reported that, 22% of the panelist scored 'like very much" for the mineralized 75% Hydrolyzed Whey Permeate Syrups (HWPS) breads, 17% for the demineralized 50% HWPS bread and 19% for control breads.

With regards to the Cherry cake product, the sensory results revealed that there were no significant differences between the 25% replacement level treatment compared to the control sample. Whereas the control sample was significantly better than 50% and 75% replacement level. It can be also noted that, there were no significant differences between the two treatments 25 and 50% replacement level.

For Kunafah product, it was noticed that there was no significant differences between the 25% replacement level and the control sample; the 25% treatment achieved (1.93) scores whereas the control sample a achieved (1.96) scores. On the other hand, it was noticed from the sensory evaluation results that, there were significant differences between the 50 and 75% replacement level compared with the control sample; the control sample achieved the best results (1.96) scores compared to (4.73) scores of both 50 and 75% replacement level. This result might be explained on the relative sweetness of added sugar. It is known that sucrose sugar has a high level of sweetness (100) compared to only (35) and (70-80) for the galactose and glucose respectively. However, most consumers favor the sweet taste especially for the confectionary products like (Kunafah and Cherry cake).

Studies on the relative sweetness of hydrolyzed lactose syrups have been reported by different researchers. Shah and Nickerson (1978) used a model system based on unflavored ice cream mix. The simulated hydrolyzed syrups were evaluated at two percent of substitutions for sucrose (25 and 50%) in the control mix, which contained 15% sucrose. The results showed a synergistic effect i.e., for example less sucrose was needed for equal sweetness when 25% of the sucrose was replaced with 70% hydrolyzed lactose syrup than when was replaced with 100% hydrolyzed syrup. Poutanen *et al.* (1978) examined enzymatic conversion of glucose to fructose in glucose/galactose syrups and found that the relative sweetness of the product was almost equal to that of sucrose. This approach may offer considerable potential to the dairy industry. There is also an application for a system that could be used for conversion of the comparatively low-sweetness galactose to either glucose or fructose. The specific enzyme should be found and its source should be developed commercially. It would have a substantial effect on potential application of hydrolyzed products from lactose and on process economics (Zadow, 1984).

Bread storage study: The bread samples were evaluated organoleptically through 5 days of storage. Crakes were noted in the crust of the control sample compared with the treated samples (25, 50 and 75% sugar replacement level). This might be attributed to the evaporation of free water with storage and that leads to shrinkage of the crust leading to formation of broken surface crust. This result might be ascribed to that the free water availability in the control sample was more than that of the treated sample. Additionally, such results might be due to the availability of more functional groups in the treated samples compared to the control. These functional groups like aldehyde, amines and carboxyl group will be bound with free water reducing the amount of free water in the treated sample compared to the control. Both moisture and weight loss (approximately 1%) during storage of bread samples at 30°C were observed (Pisesookbunterng *et al.*, 1983). The Inspection for visible microbial growth on bread samples was done daily through the unopened packages. No microbial growth was detected in all stored samples. These results might be explained based on the water activity, in which the level of water activity in both control and treated samples were extremely less than the minimal water activity required for any microbial growth which is (0.6) (Yousif *et al.*, 1990). All stored samples were evaluated each day through the mentioned storage period. It was noted that all evaluated samples were accepted with regard to the flavor. The bread was considered unacceptable if the bread showed visible signs of mould. Obvious changes in the bread color of the stored samples was not also recorded.

Conclusion: This study showed that 25% sugar replacement level of the three products (French-type bread, Cherry cake and Kunafah syrup) was almost equal or better than the control with regards to all tested quality parameters carried out in these developed products (rheological properties, chemical analysis and bread storage study). Since no harmful effect on the quality was detected during this study, these results will enhance the idea of using whey lactose hydrolyzate in the formulating of food products.

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