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POSSIBLE CONSEQUENCES OF THE SUCROSE REPLACEMENT BY A FRUCTOSE-GLUCOSE SYRUP

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ABSTRACT

The fructose-glucose syrup is currently used instead of sucrose in bakery products for economic and technological reasons. The authors investigated the extent to which this change affects the formation of non-enzymatic browning products (Advanced Glycation End – AGE-Products and melanoidins). Formation of these products in model systems – mixtures of various sugars (sucrose, fructose, glucose – concentration 6%) with glycine (concentration 0.7%) or/and lysine (concentration 0.3%), heat-treated 60 – 100 °C for 15, 30, 45 and 60 min, was studied. The formation of AGE products and melanoidins was determined on the basis of absorption at 294 nm (AGE-products) and 420 nm (melanoidins), respectively. The results pointed out notable difference in the AGE-products and also melanoidins formation for a variety of sugars. The reactivity of sucrose was low even at 100 °C/60 min. Fructose and glucose originated a significantly increasing of the non-enzymatic browning products formation. The reactivity of fructose was in the caramelisation and also in Maillard reactions the highest in any combination of composition. Lysine is the most reactive amino acid which takes part in Maillard reactions even if it is bound to protein. The non-enzymatic browning reactions result in the formation of non-digestible cross-linked proteins. Lysine is also the limiting essential amino acid of most cereals. Due to the lysine properties, reduction in protein quality is the most important nutritional effect of Maillard reactions in food. The sucrose replacement by fructose-glucose syrup in bakery products leads to more extensive non-enzymatic browning reactions, i.e. caramelisation and also Maillard reactions, while changes are in the Maillard reaction more pronounced.

Keywords: non-enzymatic browning reaction; sucrose; high fructose corn syrup; caramelisation; Maillard reaction

INTRODUCTION

Sucrose is one of the oldest sweeteners and flavour ingredients **(Rippe and Angelopoulos, 2013)**. Global trends in the sucrose consumption slightly decrease **(Sitárová, 2011)**. On the other hand, the consumption of soft drinks, sweet stuff, and other goodies significantly increases. Food producers look for sucrose alternatives for economic and technological reasons. Currently, mainly high fructose-glucose syrup from corn (high fructose corn syrup – HFCS) competes to traditional sucrose (Figure 1).

HFCS is used not only in the production of soft drinks but also in the production of confectionery, bakery, and dairy products. HFCS can be produced with various proportions of glucose and fructose **(Lo et al., 2008)**. The most commonly used types of these syrups are HFCS-42 (42% fructose), and HFCS-55 (55% fructose), a composition similar to the composition of sucrose (50% fructose and 50% glucose), or HFCS-90 containing 90% fructose. The advantage for food manufacturers is that the monosaccharides in HFCS provide improved taste, stability, freshness, texture, colour, and consistency of food, as compared to sucrose **(Moeller et al., 2009)**. Handling the HFCS is much easier because of lower glucose and fructose solution viscosity compared to viscosity of the sucrose solution at the same concentration **(Johnson et al., 2009; Nordic Sugar, 2015)**.

Fructose affects human health directly and indirectly. The *direct health effects* of fructose represent the subject of many scientific studies. Fructose promotes the accumulation of fat in the abdomen and abdominal organs (abdominal obesity) and increases production of triglycerides, phospholipids, and cholesterol in the liver from non-fat sources (hepatic lipogenesis) **(Hu and Malik, 2010)**. These processes may lead to hepatic steatosis **(Akram and Hamid, 2013)**. Fructose contributes to the production of uric acid which may adversely affect the development of gout **(Bray, 2013)**. Compared with other caloric sweeteners, high intake of fructose (e.g., in the form of HFCS) increases the risk of obesity **(Gaby, 2005; Moeller et al., 2009)** and is related to the metabolic syndrome (abdominal obesity, impaired glucose tolerance associated with insulin resistance and hyperinsulinaemia, hyperlipoproteinaemia characterized by low HDL,

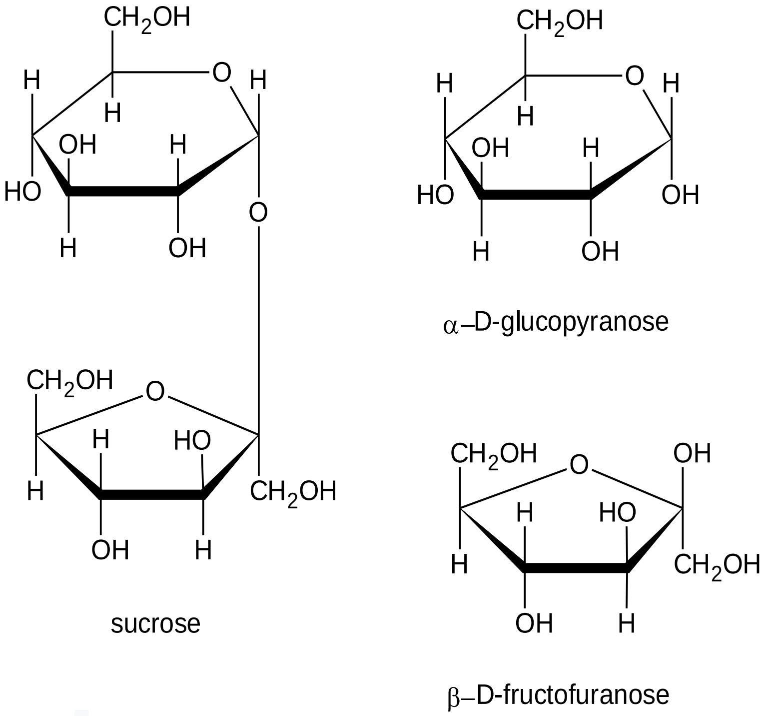

Figure 1 Structure of sucrose (β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside) and components of HFCS (β-D-fructofuranose and α-D-glucopyranose).

elevated triglycerides levels, hypertension) **(Akram and Hamid, 2013)**.

The *indirect effect* of free monosaccharides is connected with thereof higher reactivity during food processing when compared with the sucrose. Reducing sugars undergo non-enzymatic browning reactions with proteins and amino acids at higher temperature directly, while the non-reducing sucrose must be at first hydrolysed to monosaccharides **(Dills, 1993)**. The intermediates and products of these reactions are responsible for essential sensory attributes of thermally processed food products, contributing to their flavour, aroma, texture, and colour. Their antioxidant properties also contribute to the increased food stability **(Velíšek, 2014)**. On the other hand, a lot of carcinogenic, toxic and mutagenic compounds are formed there **(Brands et al., 2000; Sumaya-Martinez et al., 2005)**. Acrylamide is one of the most important toxic substance predominantly formed in food characterized by high content of starch (**Vlčáková and Vieriková, 2010)**.

Non-enzymatic browning reactions depend on many parameters, such as temperature, time of heating, water activity, pH, source of reactants, and concentration of the reactants **(Rystov et al., 2011)**. The sugar degradation reactions in the absence of amino groups (caramelisation) lead to formation of brown pigments. In the presence of nitrogen-containing compounds, Maillard reactions take place to form Maillard products (MRPs). They are colourless (Advanced Glycation End Products – AGE-products) in the first phase of the reactions, and subsequently brown pigments (melanoidins) are formed.

Scientific hypothesis

In the present study, the effect of temperature, time of heating, and type of reactants on MRPs (AGE-products and melanoidins) formation was investigated in the sugar solutions (sucrose, fructose and glucose syrup) – caramelisation reactions, and in blend of these sugars with glycine and lysine – Maillard reactions.

MATERIAL AND METHODOLOGY

Chemicals

- *Fructose syrup* (VUC Services Ltd., Czech Republic); content of fructose 99.8%; content of glucose <0.1%; (*Fru*)
- *Glucose syrup C*Sweet D 02767* (Cargill Inc., USA); content of glucose 75%; content of water 25%; (*Glc*)
- *Sucrose* (*Suc*), *Glycine* (*Gly*), *Lysine* (*Lys*), *$NaHCO_3$* (Merck Germany); all reagents were of analytical grade
- *Deionized water* adjusted to pH 7.4 with $NaHCO_3$.

Samples preparation

During caramelisation and Maillard reactions, the formation of AGE-products and terminal melanoidins was observed in model systems. Tested samples were aqueous mixtures of sugar (Suc, Fru and Glc) with glycine and/or lysine. The final content of components in the reaction mixture was 6% (w/w) for the sugar, 0.7% (w/w) for glycine, and 0.3% (w/w) for lysine. Composition of reaction mixture is imitating the content of the components in sweet bakery products.

The following combinations were studied:
Sugar (***Suc; Fru; Glc***);
Sugar + Glycine (*Sugar + G*);
Sugar + Lysine (*Sugar + L*);
Sugar + Glycine + Lysine (*Sugar + G + L*).

All samples were heated at 60 °C, 70 °C, 80 °C, 90 °C and 100 °C for 15, 30, 45 and 60 min in boiling glass tubes and then immediately cooled in ice.

Determination of AGE-products and melanoidins

Samples were measured spectrophotometrically at 294 nm (AGE-products) and 420 nm (melanoidins), respectively, according to **Yu et al. (2012)**, using an UV spectrophotometer (λ-Helios, G. B.). When necessary, appropriate dilutions were made in order to obtain required optical density.
Results are expressed as:

$\Delta A = A_{sample} - A_0$.

A_{sample} – the absorbance of the heated solution
A_0 – the absorbance of the control (25 °C/0 min)

Statistical analysis

All model systems were prepared in triplicate. Data were expressed as the mean ± standard deviation (SD) and represent three independent analyses. Statistical significance of sucrose replacement was examined using the Student´s paired t-test. A $p < 0.05$ (*) and $p < 0.01$ (**) was considered statistically significant.

RESULTS AND DISCUSSION

The effect of temperature, time of heating, and composition of reaction mixture was investigated in this study. The content of sugars in model system was calculated according to usual recipes for cakes. The content of amino acids has gone out from the average content of glycine and lysine in proteins of common cereals. Our results showed that the temperature and time of heating significantly affect the formation of MRPs in the caramelisation and in the Maillard reactions. Formation of these products was proportional to the temperature and time of heating. In the samples containing reducing sugars (Fru, Glc) in the *caramelisation reactions* already a 15 min exposure to 100 °C resulted in the formation of AGE-products (Figure 2). AGE-products start to form already at 80 °C in a fructose sample and at 90 °C in glucose during the caramelisation reactions in the samples heated for 60 min. Formation of melanoidins was very low under these conditions (maximum value $\Delta A = 0.18$). Fructose seems to be the most reactive sugar.

Heat treatment of samples, mixtures of sugar and amino acids results in *Maillard reactions*. Levels of AGE-products (294 nm) and melanoidins (420 nm) have not significantly changed after glycine addition when compared to caramelisation reactions (Figure 3 and Figure 4; Sugar versus Sugar + Gly). However, ΔA values rose enormously in the presence of lysine for fructose and glucose. Larger differences among the samples were observed in the formation of melanoidins. The most of AGE products and melanoidins were formed in samples with fructose or glucose syrup (Figure 3 and Figure 4; Fru versus Suc, Glc versus Suc).

The non-enzymatic browning reactions and the oxidation reactions are the most important and the most extensive reactions during food storage and processing mostly at high temperatures **(Delgado-Andrade et al., 2010)**. The main components participating in these changes are the carbohydrates, particularly reducing mono- and oligosaccharides or polysaccharides undergoing hydrolysis upon food processing (e.g. starch). The saccharides (especially reducing sugars) are susceptible to caramelisation reactions. Their products are brown pigments – melanoidins. The reactions of carbonyl compounds (carbohydrates or their derivatives) with compounds containing free amino group (amino acids, peptides, proteins) is a special type of non-enzymatic browning reactions known as the Maillard reactions (MR). The chemistry of the Maillard reactions is very complex and their mechanism is still not fully understood. Products of these reactions (Maillard reaction products – MRPs) are important for their organoleptic characteristics and modify the nutritional value of food. Their antioxidant properties support the food stability **(Markowicz-Bastos et al., 2012)**. However, the MR have also been associated with the formation of potentially harmful compounds and

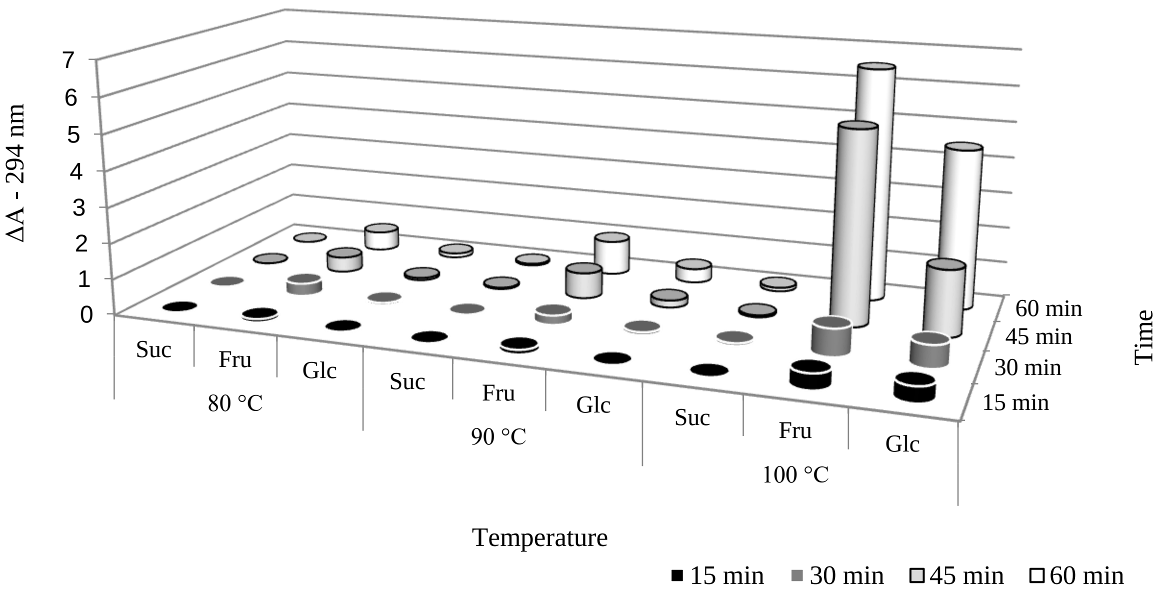

Figure 2 Formation of AGE-products in the caramelisation reactions of various sugars heated at 80, 90, and 100 °C for 15, 30, 45, and 60 min.

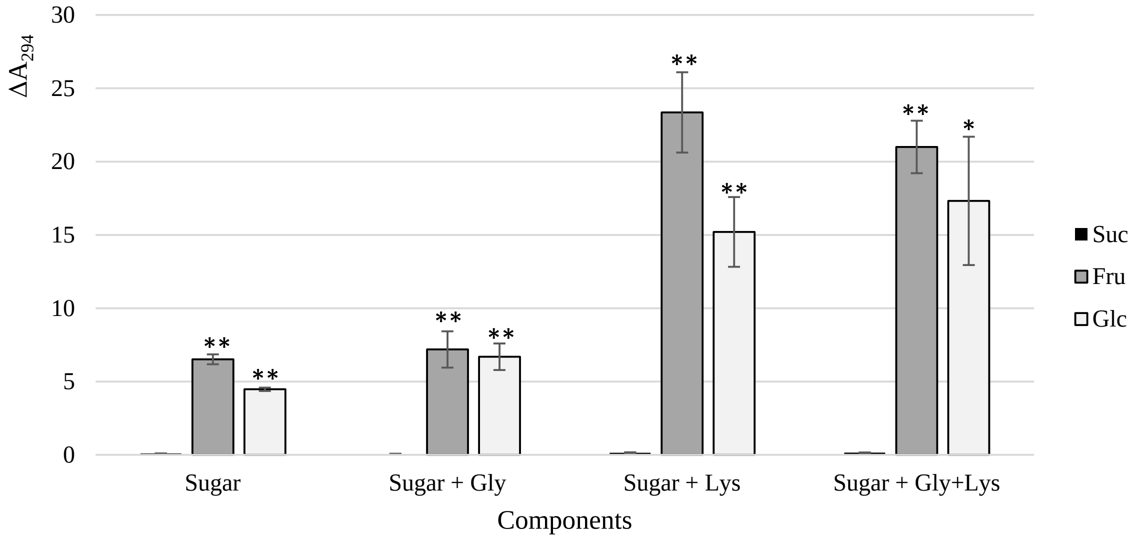

Figure 3 Formation of AGE-products (294 nm) in the reaction mixtures of different composition heated at 100 °C for 60 min.
Note: Results are expressed as mean ±SD (n = 3). Statistical significance of sucrose replacement was tested by Student´s t-test *p <0.05; **p <0.01.

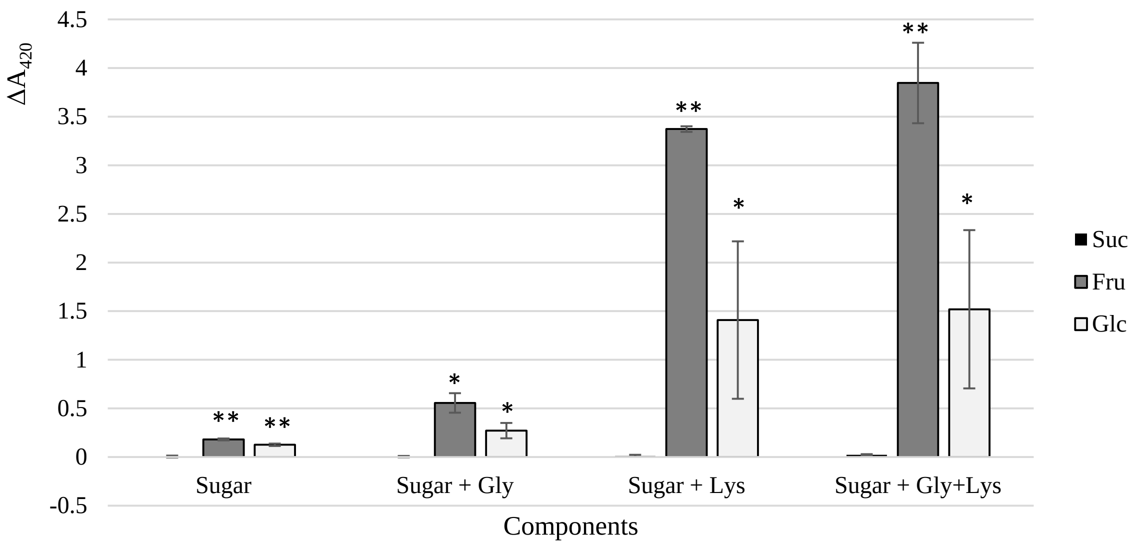

Figure 4 Formation of melanoidins (420 nm) in the reaction mixtures of different composition heated at 100 °C for 60 min.
Note: Results are expressed as mean ±SD (n = 3). Statistical significance of sucrose replacement was tested by Student´s t-test *p <0.05; **p <0.01.

reduction of the bioavailability of proteins and amino acids **(Purlis, 2010; Jiang et al., 2013)**. The most reactive amino acid in MR is lysine in both forms, the ε-amino group of free amino acid and the lysyl residues in proteins **(Velíšek, 2014)**. Protein nutritional impairment, the consequence of the destruction of essential amino acids or decrease of their bioavailability, is one of the oldest known nutritional implications of these reactions **(Markowicz-Bastos et al., 2012)**. The Maillard reactions result in a decrease in protein quality and nutritional value of food due to the loss of amino acids or their binding to unavailable complexes and the decreased protein digestibility **(Nagai et al., 2014)**. This is particularly important for bakery products, because the lysine is limiting amino acid of cereals and cereal products **(Sarwar, 1985)**. Deficient lysine can represent a considerable problem for vegetarians consuming relatively high amount of cereals. Limitation of the Maillard reactions during the food processing or cooking is therefore required.

High-fructose corn syrup (HFCS) has been used in the food industry since the 1970´s of last century as its production is cheap, easy to handle, and have a slightly sweeter-perceived than similar dose of sucrose. Sucrose is a disaccharide consisting of the glucose molecule linked with the fructose molecule. HFCS is a liquid blend of these two monosaccharides. Glucose and fructose molecules float in a solution rather than being bound to each other. Sucrose, as a non-reducing sugar, is considerably less reactive than free glucose or fructose. Reducing sugars are capable of forming an open chain structure and expose the carbonyl group. Sucrose has to be at first hydrolysed to glucose and fructose which can subsequently enter these reactions **(Rippe and Angelopoulos, 2013)**. Percentages of acyclic forms and electrophilicity of the carbonyl group are important factors affecting the reaction rates of individual sugars. Open chain structure of fructose occurs more frequently than that of glucose and, as a consequence, the higher reactivity of fructose can be expected in the Maillard reactions **(Velíšek, 2014)**. The suggestions of scientists regarding the higher reactivity of fructose are not uniform because of lower electrophilicity of ketoses **(Yamada et al., 1994; Naranjo et al., 1998; Yeboah et al., 1999)**.

Many studies and statistics show that the use of HFCS in the food industry is related to increased incidence of obesity **(Bray et al., 2004)**. Increased intake of fructose in a food is connected with obesity, dyslipidaemia, insulin resistance, hypertension, atherosclerosis, vascular diseases, Alzheimer's disease, and renal diseases **(Tappy and Lê, 2010; Nagai et al., 2012; Rippe and Angelopoulos, 2013)**.

Our results showed that the temperature and time of heating significantly affect the formation of the caramelisation and also the Maillard reactions products. Formation of the products was proportional to the temperature and time of heating. The formation of AGE-products was already observed at 70 °C (Fru, Glc). Melanoidins were formed only at higher temperatures (90 – 100 °C) for prolonged exposure. MRPs were hardly formed when sucrose was used. The most significant changes occur at 100 °C/60 min for all studied samples. The changes in the samples, as the consequence of Maillard reactions, were markedly more extensive than those of the caramelisation reactions. Formation of MRPs in Maillard reactions requires lower temperature and shorter time of heating, as compared with the caramelisation reactions. Intensive caramelisation takes place at 120 °C or more **(Velíšek, 2014)**. To observe the beginning of the MRPs formation, the temperatures of up to 100 °C were tested in our model experiments. In food processing, higher temperatures are used. Considering the fact that such significant differences between the reducing sugars and non-reducing sucrose were observed at 100 °C, what changes can be expected at higher temperatures?

Our results confirmed high reactivity of lysine. The addition of glycine alone (without lysine) induced only minor MRPs formation, as compared with caramelisation reactions. Reactivity of sucrose in non-enzymatic browning reactions was very low. Bakery products prepared with sucrose will contain significantly less toxic Maillard products than products prepared with fructose and glucose syrup. Particularly high level of MRPs was observed using fructose syrup. HFCS in human diet is the main source of reactive dicarbonyl compounds **(Lo et al., 2008)**. Increased intake of AGE-products and melanoidins through bakery products could represent another negative effect of HFCS **(Bray et al., 2004; Tappy and Lê, 2010; Bray, 2013)**.

Healthy human organism can generally compensate the increased intake of harmful substances. But how long can a human stay healthy? What may accelerate pathological processes in the body? The generation receiving an increased amount of MRPs through the bakery products prepared with fructose syrup all their lives has not yet reached the age when health problems usually start to develop.

Type 2 diabetes is a disease typical for elderly persons. Intake of higher amount of Maillard products may by potentially dangerous for their organisms. May it affect the rate of their aging? And may it affect aging of healthy people? Answers to these questions are still unknown. In the meantime, we must confront unethical advertisement of "quickly available energy" and the prevalence of producers´ interests over interests of consumers.

CONCLUSION

Sucrose is a low reactive sugar in terms of non-enzymatic browning reactions. Bakery products with sucrose contain significantly less toxic Maillard products than those prepared with fructose-glucose syrup. Extremely increased levels of Maillard products have been observed when fructose syrup was used. Increased intake of AGE products and melanoidins via bakery products may be considered as another undesirable effect of fructose syrup usage. However, producers prefer low costs and technological advantages of fructose-glucose syrup to potential dangerous effect on human health.

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