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Effect of pH, temperature and heating time on the formation of furan from typical carbohydrates and ascorbic acid

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Abstract

The presence of furan levels in foods has attracted considerable attention worldwide because furan is considered to be a possible carcinogenic substance to both humans and animals alike. Furan formation in the presence of carbohydrates and ascorbic acid in food during thermal processing is influenced by several factors including pH, temperature and time. The aim of this study was to investigate the effects of pH, heating temperature, and heating time on furan formation in glucose, fructose, sucrose and ascorbic acid solutions model. Headspace gas chromatography mass spectrometry (HS-GC-MS) was used to monitor the furan level in each of the four model systems. The results revealed that the most level ($P < 0.05$) of furan was found in glucose, fructose and ascorbic acid at pH 9.40, pH 7.00 and pH 4.18, respectively, when heating temperature was more than 90ºC, but sucrose solution produced significantly ($P < 0.05$) more furan at pH 4.18 at heating temperature > 130ºC. Furthermore, the ascorbic acid solution produced significantly ($P < 0.05$) more furan than glucose, fructose and sucrose solution during heat processing. From these results, it was indicated that heat-induced furan formation from four experimental models using glucose, fructose, sucrose and ascorbic acid was significantly influenced by different heat processing parameters such as pH, heating temperatures, and heating time. In addition, it was also suggested that higher heating temperature was needed to produce furan for sucrose. Further studies will use various carbohydrate mixture models to stimulate the carbohydrate and ascorbic acid complexes found within various food matrices.

Key words: Furan formation, typical carbohydrates, ascorbic acid, model systems, HS-GC-MS.

Introduction

Furan (C_4H_4O) is present in a wide range of thermal processed foods such as canned and jarred foods with an average level of 170 ng/g [1, 2]. The presence of such furan levels in foods have attracted considerable attention worldwide because furan is considered to be a possible carcinogenic substance to both humans and animals alike [3-5]. Subsequently, several international food organizations such as the United States Department of Food, Drug and Agriculture (US FDA) and the European Food Safety Authority [6] have launched investigation programs to survey the occurrence of furan in commonly consumed foods. Three furan research areas have become major topics of investigation including rapid and precise quantitative furan assessments, furan origins including its formation mechanisms as well as the determination of the impact of furan on human health.

Understanding of the possible sources of furan in foods may provide valuable information with regard to thermal processes control and management procedure for the control and elimination of furan in foods. Several experimental models have been used to identify potential sources of furan including simulated commercial pasteurization and sterilization models in food processing [7-10]. Several experimental models have also been reported to find furan formation including high temperatures induced pyrolysis models[11], roasting model for certain foods (coffee) [9, 10], ionizing radiation [7, 12] and ultraviolet treatment model[13].

Furan formation (Fig. 1) is induced by five possible chemical reactions including: 1) thermal degradation of carbohydrates, 2) the Maillard reaction involving a reaction between an amino acid and a reducing sugar at a high temperature, 3) the oxidation of polyunsaturated fatty acids, 4) the decomposition of ascorbic acid or its derivatives, and 5) the thermal oxidation of carotenoids[14].

Carbohydrates, lipids, proteins and amino acids (AAs) play important role in the formation of furan in food thermal processing. Maga [15] believed that carbohydrates, such as glucose, lactose, and fructose, may be the major sources of furan in food. Perez and Yaylaya [11] suggested that ascorbic acid had the highest potential to produce furan during thermal treatment followed by some sugar/amino acid mixtures. Becalski and Seaman [8] proposed that furan could be formed through the oxidation of polyunsaturated fatty acids at elevated temperatures as well as from the decomposition of ascorbic acid or its derivatives. In addition, Fan [7] suggested that the furan could be formed from sugars, ascorbic acid, and organic acids during irradiation or thermal treatment. Previous

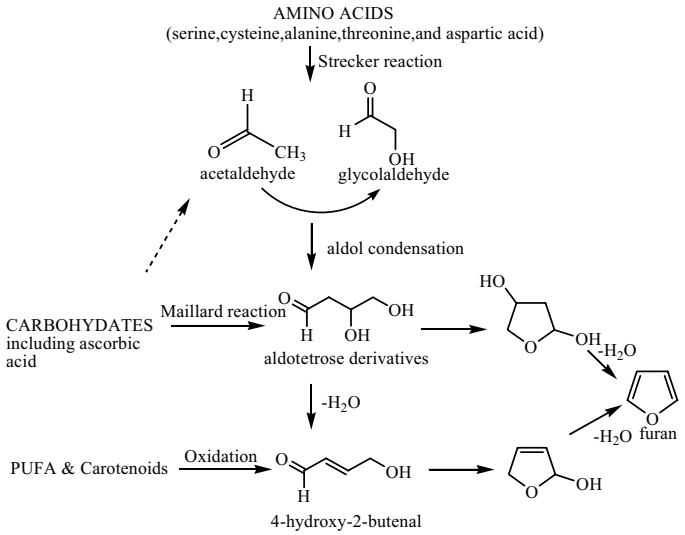

Figure 1. Proposed pathways and precursors of furan according to previous studies [8, 11, 14, 15] with some modifications.

studies using ^{13}C-labeled glucose and/or serine have shown that acetaldehyde and glycolaldehyde are key fragments which, upon aldol condensation, cyclization, and dehydration, result in furan (Fig. 1) [8, 11, 14, 15]. The key intermediates (acetaldehyde and glycolaldehyde) may originate from sugars and/or defined amino acids. Alternatively, ascorbic acid may be transformed under non-oxidative pyrolytic conditions to 2-deoxyaldotetrose as a key intermediate that can either lead directly to furan or, alternatively, via 4-hydroxy-2-butenal, a well-known lipid degradation product (Fig. 1).

The presence of certain carbohydrates and ascorbic acid in foods facilitate furan generation during thermal processing as well as the impact of heat processing on various parameters (i.e. pH, temperature, and time). As a result, the main objective of this study was to investigate furan formation in glucose, fructose, sucrose and ascorbic acid as reaction samples in aqueous model systems during heat processing with the change of important factors such as initial pH, heating temperature, and heating time. This will be helpful to find the better process conditions when these ingredients were used in food heat processing.

Materials and Methods

Chemicals and reagents: D-(+)-glucose (≥ 99%), D-(-)-fructose (≥ 99%), sucrose (≥ 99.5%), L-ascorbic acid (≥ 99%), furan (≥ 99%) d_4-furan (≥ 99%), and methanol (HPLC-grade) Sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$) and disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Effect of pH, temperature and heating time on the furan formation: Nine g of each precursor (glucose, fructose, sucrose and L-ascorbic acid) was dissolved in phosphate buffers mixed with $NaH_2PO_4 \cdot 2H_2O$ and Na_2HPO_4 and their concentrations were at 36 mg/ml. The pH value of buffers were adjusted to three levels at 4.18, 7.00, and 9.40 using $NaH_2PO_4 \cdot 2H_2O$ and Na_2HPO_4. Each precursor had three pH aqueous model systems (acidic, neutral and alkaline).

An aliquot of the homogenous solution (5 ml) was transferred into a reaction vessel (a 20-ml headspace vial) and then sealed with a crimp cap. The vials were heated for 30 min at 80, 90, 100, 110, 120, 130, 140 and 150ºC in an oil bath. Immediately afterwards, the samples were cooled down in an ice bath (about 0ºC) for 15 min to stop the reaction. Subsequently, 40 µl working solution of d_4-furan (2.5 µg/ml) was added through the septum with a gas tight syringe and the vial was vortexed. The samples were kept at room temperature for at least 30 min before analysis.

To determine the effect of heating time on the furan formation, each precursor (9 g) was dissolved in the sodium phosphate solution (pH 9.40). The vials were heated at 120ºC for 10, 20, 30, 40, 50 and 60 min in the oil bath.

Furan analysis: Furan was analyzed by a rapid, sensitive and automated HS-GC-MS. The addition of d_4-furan as the internal standard was used to quantitate furan. Furan and d_4-furan were extracted from the samples by Agilent Model G1888 Automated Headspace sampler. Separatation and identification were preformed using Agilent Model 7890A/7000 GC mass spectrometer equipped with a capillary column 19091P-Q04 HP-PLOT/Q, 30 m × 0.32 mm × 20µm.

Calibration curve: Stock, working and standard solutions of furan and d_4-furan were prepared according to previous report [16]. Standard samples at appropriate concentrations were injected with each analytical series in order to construct a 9-point calibration curve. Calibration standards (5, 50, 125, 250, 500, 800, 1000, 1200, and 2400 ng) were prepared by injecting 20-960 µl working solution of furan (0.25 or 2.5 µg/ml) and 40 µl working solution of d_4-furan (2.5 µg/ml) through the septum of 20 ml sealed headspace vials containing 5 mL water, respectively.

Headspace operating conditions: Oven temperature was set at 70ºC, loop at 110ºC, transfer line at 130ºC; 30 min thermal equilibration with low shake on 0.5 min pressurization, 1 min injection with vial pressurized to 10 psi.

GC-MS operating conditions: The gas chromatography (GC) oven temperature was initially set at 50ºC for 1 min and increased to 200ºC at rate of 10ºC/min for 10 min. The injector temperature was set at 200ºC with a 3:1 split ratio and a constant flow rate of 1.0 ml/min (UHP helium).

The high sensitivity EI ion source and transfer line temperatures were set at 230ºC and 225ºC, respectively, with a quadrupole temperature of 150ºC. The MS operating conditions were as follows: positive electron ionization mode (EI+) using automatic gain control with 70 eV of electron energy. Furan and d_4-furan were identified by comparison of spectra of sample compounds with those of standards and by comparing retention times of the samples with those of the standards. The mass spectrometer was operated in selected-ion monitoring mode (SIM) by recording the current of the following ions: m/z 68 $[M]^+$ and m/z 72 $[M]^+$ for furan and d_4-furan determination, respectively. For confirmation of furan and d_4-furan, the ions, m/z 39 $[M\text{-}CHO]^+$ and m/z 42 $[M\text{-}C_2HO]^+$ were monitored.

Statistical analysis: The experimental design was a completely randomized design with at least 3 replicates in each treatment. Data presented are the mean of triplicate determinations. The statistical analysis was carried out using Statistical Analysis

Software (SAS Version 8.2 (SAS Institute, Cary, NC, USA). The differences between treatments were analyzed by the least significant difference (LSD) test using the general linear model. Only significant differences ($P < 0.05$) are discussed unless stated otherwise.

Results

Effect of pH and temperature on furan formation from glucose model: The effect of pH and temperature on the formation of furan from glucose is illustrated in Fig. 2. With a heating temperature below 110°C, pH was not influencing furan generation until the oven temperature was greater than 110ºC. Once the oven temperature exceeded this temperature, the level of furan formation with glucose at pH 4.18 started to increase more rapidly compared to the glucose solutions at pH 9.40 and 7.00 after 30 min of heating. When the oven temperature reached 140ºC, 1212.39 ng/ml of furan was formed from the glucose solution at pH 9.40, a level significantly higher than the furan level in glucose solution at pH 7.00 (886.34 ng/ml) and pH 4.18 (82.06 ng/ml furan).

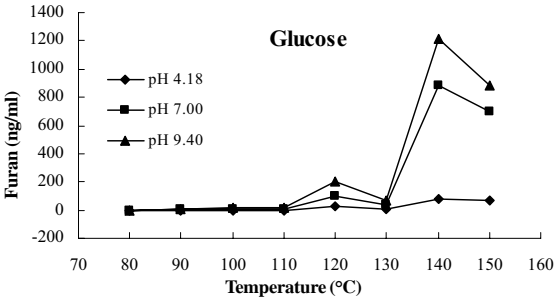

Figure 2. Effect of pH and temperature on thermally induced furan formation from glucose solution (36 mg/ml).

Beside pH, the increased temperatures also played an important role in furan formation from glucose. Generally speaking, five phases of furan formation appear as a function of heating temperatures from 80 to 150ºC: a slow increase between 80 and 110ºC and a rapid increase from 110 to 120ºC. A decrease between 120 and 130ºC followed by a remarkable increase from 130 to 140ºC, and finally a significant decrease between 140 and 150ºC in all solutions. At pH 4.18, the amount (22.57 ng/ml) of furan formed from glucose after heating at 120ºC for 30 min was significantly ($P < 0.05$) higher than that (0 ng/ml) heated at 80°C. As compared to heating at 120ºC, much higher levels of furan (82.06 ng/ml) were produced at 140ºC. At pH 7.00, the amount (99.36 ng/ml) of furan formed from glucose after heating at 120ºC for 30 min was significantly ($P < 0.05$) higher than that (0 ng/ml) heated at 80ºC. However, heat treatment of glucose at 140ºC produced approximately 9 times more furan (886.34 ng/ml) than that at 120ºC. Similarly, at pH 9.14, the amount (203.01 ng/ml) of furan formed after heating at 120ºC for 30 min was significantly ($P < 0.05$) higher than that (0 ng/ml) heated at 80ºC. Compared to heating at 120ºC, about 6 times amount (1212.39 ng/ml) of furan was formed at 140ºC. These results suggested that temperature is also a crucial factor affecting furan formation from glucose.

Effect of pH and temperature on furan formation from fructose: Fig. 3 shows the effects of pH and temperature on the formation of furan from fructose due to thermal processing. No significant influence was observed between pH and furan formation when oven temperature was below 120°C. The furan level was increased in fructose solution when the temperature was over 120°C. At pH 4.18, much less amounts of furan were formed from fructose in all solutions when compared to those observed at pH 7.00 and pH 9.40. For example, after 30 min of heating at 130ºC, 423.54 ng/ml furan was formed from fructose solution at pH 9.40, 1112.33 ng/ml furan was formed from fructose solution at pH 7.00, and 88.78 ng/ml furan was formed from fructose solution at pH 4.18, respectively.

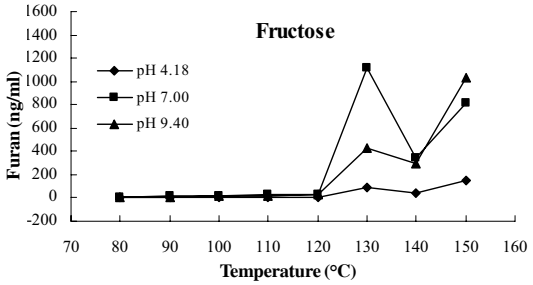

Figure 3. Effect of pH and temperature on thermal-induced furan formation from fructose solution (36 mg/ml).

In addition, changes in the four phases of furan formation heating temperature from 80 to 150ºC were observed with a slow increase between 80 and 120ºC and a rapid increase from 120 to 130ºC. A rapid decrease, however, between 130 and 140ºC followed by a remarkable increase from 140 to 150ºC in all solutions. At pH 4.18, the level (88.78 ng/ml) of furan formed from fructose after heating at 130ºC for 30 min was significantly ($P < 0.05$) higher than that (0 ng/ml) heated at 80ºC. As compared to heating at 130ºC, much higher level (147.53 ng/ml) of furan was produced at 150ºC. At pH 7.00, the amount (1112.33 ng/ml) of furan formed from fructose after heating at 130ºC for 30 min was significantly ($P < 0.05$) higher than that (8.18 ng/ml) heated at 80 ºC. However, at pH 9.14, the amount (423.54 ng/ml) of furan formed from fructose after heating at 130ºC for 30 min was significantly ($P < 0.05$) higher than heated at 80ºC (1.48 ng/ml). As opposed to heating at 130ºC, much higher amount (1026.20 ng/ml) of furan was formed at 150ºC. These results suggested that temperature is also a crucial factor influencing furan formation from fructose.

Effect of pH and temperature on furan formation from sucrose: The effect of pH and temperature on the formation of furan from sucrose as a function of thermal processing is shown in Fig. 4. Temperatures less than 130ºC did not lead to significant amounts of furan formation regardless of pH. At pH 4.18, no furan was produced from sucrose as heating temperature increased from 80 to 120ºC while furan levels increased from 1.29 to 84.26 ng/ml as

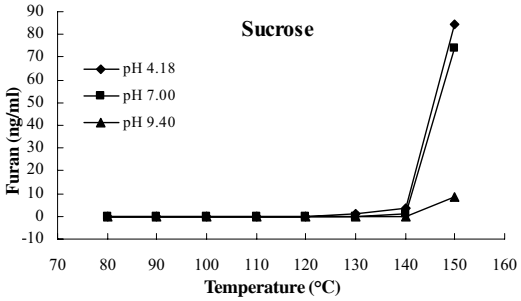

Figure 4. Effect of pH and temperature on thermally induced furan formation from sucrose solution (36 mg/ml).

temperature increased from 130 to 150ºC. Similarly, at pH 7.00 as heating temperature increased from 80 to 130ºC, no furan was produced from sucrose. As the heating temperature increased from 140 to 150ºC, however, the furan level increased from 1.12 to 74.20 ng/ml. At pH 9.40, as heating temperature increased from 80 to 140ºC, no furan was formed. However, only 8.74 ng/ml of furan was formed even after heating at 150ºC for 30 min. These results indicate that both pH and temperature have a combined effect on furan formation from sucrose.

Effect of pH and temperature on furan formation from ascorbic acid: The effect of pH and temperature on the formation of furan from ascorbic acid due to thermal processing is presented in Fig. 5. Heating temperature ≤ 90ºC did not lead to significant amounts of furan formation regardless of pH. Results show that pH had a significant effect on thermally induced furan formation at heating temperature > 90ºC. At pH 4.18, ascorbic acid solution produced significantly ($P < 0.05$) more furan than ascorbic acid prepared at pH 7.00. Higher amounts of furan were formed from ascorbic acid at pH 7.00, compared to that at pH 9.40 suggesting that pH is an important factor influencing furan formation from ascorbic acid. For instance, after 30 min of heating at 150ºC, 3791.15 ng/ml furan was formed from ascorbic acid solution at pH 4.18, 2651.27 ng/ml furan was formed from ascorbic acid solution at pH 7.00, and 2358.08 ng/ml furan was formed from ascorbic acid solution at pH 9.40, respectively. Our results suggested that the highest level of furan was formed from ascorbic acid at acidic pH.

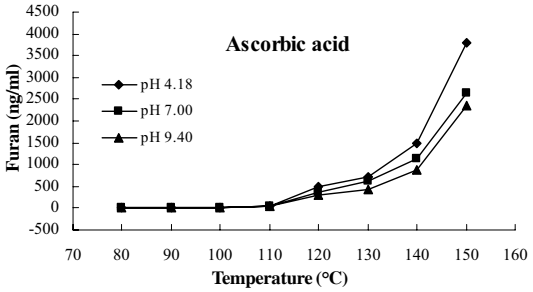

Figure 5. Effect of pH and temperature on thermal-induced furan formation from ascorbic acid solution (36 mg/ml).

Additionally, as heating temperature increased from 80 to 150ºC, furan formation from ascorbic acid in all solutions increased rapidly. At pH 4.18, the amount (500.60 ng/ml) of furan formed from glucose after heating at 120ºC for 30 min was about 167 times higher than that (3.07 ng/ml) heated at 80ºC. Compared to heating at 120ºC, much higher level (3791.15 ng/ml) of furan was produced at 150ºC. At pH 7.00, the amount (350.69 ng/ml) of furan formed from glucose after heating at 120ºC for 30 min was approximately 300 times higher than that (1.20 ng/ml) heated at 80ºC. However, heat treatment of glucose at 150ºC produced significantly ($P < 0.05$) more furan (2651.27 ng/ml) than at 120ºC. Similarly, at pH 9.14, the amount (315.98 ng/ml) of furan formed from glucose after heating at 120ºC for 30 min was about 300 times higher than that (1.07 ng/ml) heated at 80ºC. Compared to heating at 120ºC, about 8 times amount (2358.08 ng/ml) of furan was formed at 150ºC. These results suggested that temperature plays a crucial part in affecting furan formation from ascorbic acid.

Effect of heating time on furan formation from solutions of glucose, fructose, sucrose and ascorbic acid: To test whether heating time affects furan production from solutions of glucose, fructose, sucrose and ascorbic acid, we determined furan levels in all solutions of pH 9.40, which were heated at 120ºC for 10, 20, 30, 40, 50 and 60 min in the oil bath (Fig. 6). The results were as follows: as heating time increased from 10 to 60 min, furan formation from solutions of glucose, fructose, and ascorbic acid increased linearly except sucrose. For example, the amount (1228.52 ng/ml) of furan formed from glucose after 60 min of heating at 120ºC was about 500 times higher than that (2.46 ng/ml) heated for 10 min. As for fructose solution, the amount (445.75 ng/ml) of furan formed after 60 min of heating at 120ºC was significantly higher than that (2.44 ng/ml) heated for 10 min. In addition, the amount (1991.67 ng/ml) of furan formed from ascorbic acid after 60 min of heating at 120ºC was about 616 times higher than that (3.23 ng/ml) heated for 10 min. From these results, we could conclude that long time heating also promotes furan formation.

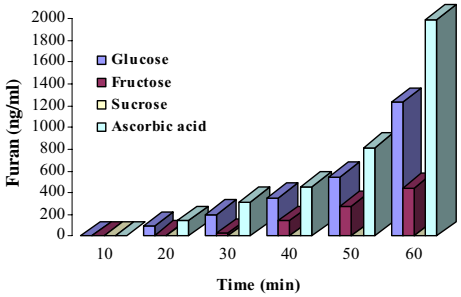

Figure 6. Effect of heating time on furan formation from solutions of glucose, fructose, sucrose and ascorbic acid (36 mg/ml each).

Discussion

In this study, glucose, fructose, sucrose and ascorbic acid were selected as model systems to investigate the factors (pH, heating temperature, and heating time) on the furan formation as earlier studies on furan formation from precursors tended to be contradictory [17]. Phosphate buffer may have effects on furan formation [9, 10]. The formation of furan would be significantly different if prepared in the absence of phosphate [18]. Previous researchers [8, 11, 14, 15] have proposed potential approaches to reduce furan formation through intermediates in foods. Furan may be reduced under the following situations: (i) Some key intermediates such as 2,3-diketogulonic acid (DKG), aldotetrose derivatives, and 4-hydroxy-2-butenal may be eliminated under the change of reaction conditions; (ii) Other compounds other than furan are formed via the control of processing conditions at final reaction stage; (iii) Some key pathways such as the formation of DKG, aldotetrose derivatives, and 4-hydroxy-2-butenal are blocked.

This paper presented the possibility of reducing or eliminating the formation of furan in heat processed foods by the modification of processing parameters to limit the formation from furan intermediates (i.e. glucose, fructose, sucrose, and ascorbic acid). These parameters include heating temperature, heating time, pH, etc. with pH and heating temperature are important factors influencing furan formation from solutions of carbohydrates and ascorbic acid, Lower temperature, shorter heating time, and acid conditions would be possible for reducing furan levels in the

food heating processing. The further study on reduction of furan level by modification of processing conditions and change of heat processing methods is under way in our laboratory.

Ascorbic acid and carbohydrates are also commonly used as additives in food products. The results of this study can be used for better formulation to minimize the formation of furan due to thermal processing. For example, if carbohydrates are required in formulation, sucrose would be a better choice than glucose and fructose to reduce the accumulation of furan due to heat treatment.

Conclusions

The results from this study indicated that heat-induced furan formation from solutions of glucose, fructose, sucrose and ascorbic acid in model systems was significantly influenced by heat processing parameters such as pH, heating temperature and heating time. For glucose solution, when heating temperature is more than 90ºC, the solution produced significantly ($P < 0.05$) more furan at pH 9.40 than that prepared in solution of pH 7.00 or pH 4.18. Fructose solution can significantly ($P < 0.05$) produce most furan at 7.00 when heating temperature is more 90ºC. Sucrose solution produced significantly ($P < 0.05$) more amounts of furan at pH 4.18 at heating temperature >130ºC. However, heat-induced ascorbic acid solution produced considerable levels of furan at pH 4.18 at heating temperature >90ºC. Furthermore, the ascorbic acid solution produced significantly ($P < 0.05$) more furan than glucose, fructose and sucrose solution during heat processing. From these results, it was suggested that higher heating temperature was needed to produce furan for sucrose.

The results from this study indicate that heat-induced furan formation from four experimental models using glucose, fructose, sucrose, and ascorbic acid was significantly influenced by different heat processing parameters such as pH, heating temperatures, and heating time. Optimized parameters could possibly limit the level of furan formation from foods containing these carbohydrates or AAs. Modification of processing conditions and changes of heat processing methods could possibly reduce or eliminate furan formation in heat processed foods containing glucose, fructose, sucrose or ascorbic acid. The limitation of previous studies is that only a single component in the food was investigated. Whether a mixture of these components (a mixture of sucrose and ascorbic acid) leads alterations of parameter is still unknown. Using carbohydrate mixtures stimulating those found in different food matrices therefore will be the focus of our future investigations.

Acknowledgements

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