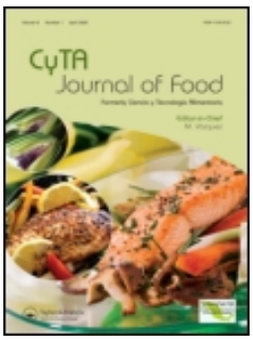

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Usefulness of determination of glucosylisomaltol and hydroxymethylfurfural to control browning reaction during storage of baby cereals

Utilidad de la determinación de glucosilisomaltol e hidroximetilfurfural para el control de la reacción de pardeamiento durante la conservación de cereales infantiles

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Glucosylisomaltol (GIM) and hydroxymethylfurfural (HMF) were determined in gluten and gluten-free baby cereals stored under industrial conditions for 1 year and under laboratory conditions for 1 month at Aw = 0.65. In eight mixed cereals, GIM was detected at all times and temperatures assayed, increasing during storage. In rice cereals, GIM was only detected under industrial conditions after 3 and 6 months of storage at 55 and 32 °C, respectively; at Aw = 0.65, GIM was detected after 1 week of storage at 25 and 55 °C. In storage of rice–corn cereals under industrial conditions, GIM was only detected at 55 °C; under laboratory conditions, GIM was detected after 1 week at 55 °C and after 3 weeks of storage at 25 °C. HMF decreased during storage of baby cereals except in rice cereals, the only sample without caramel as an ingredient. HMF only proved to be a useful indicator in rice cereals, whereas GIM was useful in all samples.

Keywords: glucosylisomaltol; HMF; baby cereals; non-enzymatic browning; storage

Glucosilisomaltol (GIM) e hidroximetilfurfural (HMF) fueron determinados en cereales infantiles con gluten y sin gluten almacenados bajo condiciones industriales durante un año y en laboratorio durante un mes a Aw de 0,65. En la muestra de ocho cereales, GIM fue detectado en todos los tiempos y temperaturas ensayadas, incrementando durante la conservación. En los cereales de arroz, sólo se detectó GIM bajo condiciones industriales tras tres y seis meses a 55 y 32 °C respectivamente; a Aw = 0,65, GIM fue detectado tras una semana a 25 y 55 °C. En los cereales de arroz–maíz, bajo condiciones industriales, GIM fue sólo detectado a 55 °C; durante la conservación en laboratorio, GIM fue detectado tras una semana a 55 °C y tras tres semanas a 25 °C. HMF disminuye durante la conservación de los cereales, excepto en las muestras de arroz, la única muestra sin caramelo como ingrediente. HMF sólo demostró ser un indicador útil en cereales de arroz, mientras que GIM fue útil en todas las muestras.

Palabras clave: glucosilisomaltol; HMF; cereales infantiles; pardeamiento no enzimático; conservación

Introduction

Baby cereals are an important energy source for the nutrition of infants and form the basis of their weaning-feeding from the age of 3–4 months. These products have a long shelf-life and can usually be consumed up to 2 years after manufacture. Storage conditions and the particular composition of these cereals affect the progress of the Maillard reaction initiated during their processing (Fernandez-Artigas, Guerra-Hernández, & García-Villanova, 1999; Guerra-Hernández, Corzo, & García-Villanova, 1999). This reaction can produce a reduction in biological value due to a decrease in the content of lysine, the limiting amino acid in cereals. Commercial baby cereals are mostly composed of cereal (either with gluten or gluten-free) or legume flours (e.g. soy) and sucrose, glucose or fructose syrup, honey, powdered fruit, biscuits, minerals, vitamins and flavors.

Hydroxymethylfurfural (HMF) is a breakdown product of Amadori compounds during the Maillard reaction (Van Boekel, 1998) but is also formed from the dehydration of fructose and to a lesser extent, glucose when heated at high temperatures in slightly acidic media (Berg & Van Boekel, 1994). HMF is a valuable marker to control toasting and drying of cereals during the manufacture of baby cereals in samples that do not contain caramel (Fernandez-Artigas et al., 1999).

Glucosylisomaltol (GIM) is an intermediate product of the Maillard reaction between maltose and glutamine (Resmini, Pellegrino, Pagani, & De Noni, 1993). The former has been shown to be useful to monitor non-enzymatic browning during pasta drying (Resmini et al., 1993), bread manufacture, and wheat-based baby cereal storage (Guerra-Hernández, Ramirez-Jiménez, & García-Villanova, 2002) and biscuits (Rufián-Henares, Delgado-Andrade, & Morales, 2008).

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There are few papers in the literature where heat formed Maillard products have been evaluated for stability or shelf life (Fernández-Artigas et al., 1999; Gökmen & Senyuva, 2006).

In this report, the levels of GIM and HMF were evaluated as browning indicators in baby cereals with or without caramel prepared with gluten (eight mixed cereals) or gluten-free (rice and rice–corn) flour.

Materials and methods

Samples and reagents

Samples of three types of baby cereals were obtained from a dietetic products company: ''eight-cereals'' (brand containing wheat as the main cereal with rice, barley, rye, oat, corn, millet, and sorghum) (104 g/kg proteins and 6.54 g/kg sugars), ''rice cereal'' (4.41 g/kg proteins and 6.16 g/kg sugars), and ''rice–corn cereal'' (5.77 g/kg proteins and 3.93 g/kg sugars). According to the label information, the samples contained 800 g/kg flour plus soy flour, sucrose, vitamins, minerals, and flavors. All samples except for ''rice cereal'' also contain caramel. The commercial samples (packed under nitrogen atmosphere) were stored by the company at 32 or 55 °C for 1, 3, 6, or 12 months. These temperatures are used by industry to control normal and accelerated storage conditions. Identical samples were kept in our laboratory at 25 or 55 °C in oven for 1, 2, 3, or 4 weeks in air atmosphere with controlled water activity (Aw = 0.65). To maintain this water activity, the moisture of cereals was controlled according to the procedure described by Salmarch and Labuza (1980), placing the samples in a Petri plate on the upper shelf of a dissector containing saturated sodium nitrite solution. Samples were analyzed before their storage and again after storage conditions. Solid samples were stored at –50 °C until their analysis. All samples analyzed corresponded to the same batch.

Potassium ferrocyanide and zinc acetate were obtained from Merck (Darmstadt, Germany). Acetonitrile was purchased from Panreac (Barcelona, Spain) and HMF from Aldrich (Germany).

Analysis of HMF and GIM

Sample extraction

Extraction and HPLC determination were performed following the method applied to infant cereals by Guerra-Hernandez et al. (2002). The powdered sample (0.6 g) was placed into a 10 ml centrifuge tube to which 7 ml of deionized water was then added. The centrifuge tube was shaken vigorously for 1 min and the sample was then centrifuged for 10 min at 5000 rpm. The supernatants were clarified with 0.5 ml each of Carrez I (potassium ferrocyanide 15 g/L w/v) and Carrez II (zinc acetate 30 g/L w/v) solutions. The resulting mixture was centrifuged for 10 min at 5000 rpm. The solution was diluted to a total volume of 25 ml with deionized water. A 2 ml aliquot of this solution was filtered through a 0.2 μm disk filter before injection. The extraction procedure was repeated three times.

Chromatographic conditions

The liquid chromatographic systems used in this study consisted of a Waters model 600 (Milford, MA, US) with 50 μl injection loop chromatograph, a UV Konic detector model 200 UVIS (Reno, NE, US) at 280 nm, and Millenium program Waters integrator.

Fifty microlitres of purified solutions were separated in a reverse-phase C_{18} (Nova-Pack 4 μm; 250 mm × 46 mm id., Cartidge, Waters, Milford, MA,US) column. The mobile phase was acetonitrile:-water (5:95) and the flow rate was 1 ml/min.

Calibration of chromatography systems for HMF and GIM (previously synthesized by Guerra-Hernández et al., 2002) determination was made by the external standard method within ranges 0.05–0.25 and 0.00025–0.09 mg/l, respectively.

Additional determinations

Proteins were determined by the Kjeldahl method (Helrich, 1990). Reducing sugars were determined by means of a titrimetric method (Helrich, 1990).

Statistical analysis

Statistical analysis of data was performed by analysis of variance (SPSS for windows 15.0.1 Granada University Licence). The Student's test was used to compare means, and the level of significance was set at 95%.

Results and discussion

GIM determination

The method applied in this study was developed by our research group for similar samples (Guerra-Hernández et al., 2002). The relative standard deviation was 1.56% for a mean value of GIM of 7.68 mg/kg (Guerra-Hernández et al., 2002). The detection limit (signal-to-noise ratio >3) was 0.006 mg/kg and the quantification limit (signal-to-noise ratio >10) was 0.020 mg/kg.

Baby rice cereals

GIM was not detected in non-stored samples. Under industrial conditions, GIM was not quantified until the third month of storage at 55 °C and the twelfth month of storage at 32 °C. GIM values after 1 year of storage at 32 and 55 °C were 0.02 and 0.67 mg/kg, respectively (Supplementary Table 1). Changes between storage

times at the same temperature (32 or 55 °C) were statistically significant ($p < 0.05$), except between the third and sixth at 55 °C. Changes between temperatures for the same storage times were also significant ($p < 0.05$). At higher water activity (Aw = 0.65), GIM was detected from the first week of storage and increased during the 4-week study period (Supplementary Table 1). At 25 °C, the increases were statistically significant ($p < 0.05$) between the first and second week ($p = 0.026$) and between the third and fourth ($p = 0.035$). At 55 °C, there were statistically significant ($p < 0.05$) increases between all time periods except between the second and third week. The increase in storage temperature from 25 to 55 °C produced significant ($p < 0.05$) changes, with double the GIM concentration at 4 weeks of storage (Supplementary Table 1). After 1 month of storage at 55 °C, GIM was only detected when the Aw was high (Supplementary Table 1). After 1 month of storage at Aw = 0.65 (25 or 55 °C), GIM values were higher than after 1 year of storage of the samples (32 or 55 °C) at the proper water activity (Supplementary Table 1). The water content of this product rose from 3 to 8% after storage at Aw = 0.65. In this sample GIM always increased.

Baby rice–corn cereals

GIM was not detected in non-stored samples or after 1 year of storage at 32 °C. A minimum of 3 weeks of storage at 25 °C and Aw = 0.65 was required to quantify this compound in samples (Supplementary Table 2). At 55 °C/Aw = 0.65, GIM was detected after the first week of storage and increased to 0.65 mg/kg at 4 weeks, with statistically significant ($p < 0.05$) changes only between the second and third week. At 25 °C/Aw = 0.65, changes between the third and fourth week were statistically significant ($p < 0.05$). Significant ($p < 0.05$) changes were observed between temperatures for the same storage times. Under industrial conditions, significant ($p < 0.05$) changes in GIM were recorded during storage in nitrogen at 55 °C.

The GIM content was four-fold higher with elevated water activity (Aw = 0.65) for the same period and temperature of storage (55 °C/1 month) at the proper water activity (Supplementary Table 2).

GIM content was slightly higher in rice than in rice–corn cereal samples, it is conceivable that this is due to the greater reducing sugar content (62 g/kg for rice vs. 39 g/kg for rice–corn). Rufian-Henares, Delgado-Andrade, and Morales (2006a,b) also found slightly higher values in rice-based breakfast cereals than in maize-based breakfast.

Baby eight-cereals

The content of GIM in samples stored at 32 °C under industrial conditions was very low (<0.02 mg/kg) (Supplementary Table 3). At 55 °C, GIM levels were higher than at 32 °C and increased from 0.1 mg/kg at 1 month to 1.47 mg/kg at 12 months, with significant changes between the first and third month and between the sixth and twelfth ($p < 0.05$). At high water activity (Aw = 0.65) and 25 °C, GIM increased approximately 10-fold from the first to fourth week of storage, and these changes were statistically significant from the first to the third week of storage ($p < 0.05$). At the same water activity and storage periods but at 55 °C, there was also a marked increase ($p < 0.05$), which reached 3.66 mg/kg after 4 weeks, six-fold the value obtained at 25 °C. At the same temperature (55 °C) and storage time (4 weeks = 1 month), the GIM values obtained at Aw = 0.65 were 36-fold the values obtained with unmodified water activity.

GIM values obtained in rice-based samples (rice and rice–corn) were lower than those in the wheat-based sample (eight-cereals). Guerra-Hernández et al. (2002) also reported high GIM values in wheat-based samples stored under similar conditions. Rufian-Henares et al. (2006a,b) found much higher values than the present findings in wheat-based breakfast cereals, ranging from 6.21 to 61.8 mg/kg. Rice-based samples had a lower protein content (44.1 g/kg for rice and 57.7 g/kg for rice–corn) compared with wheat-based sample (104 g/kg for eight-cereals). On the other hand, Weiser, Seilmeier, and Belitz (1980) reported a higher glutamine content, the main GIM precursor, in wheat flour (31.1 g/100 g of protein) than in rice (15.4 g /100 g of protein) and corn (17.7 g/100 g of protein) flours.

Resmini et al. (1993) studied model system solutions (pH = 6.5) of sugars (fructose, glucose, maltose, or maltotriose) in the presence or absence of one amino acid (glutamine, asparagine or arginine), which were heated at 80 °C for 12 h. They reported that GIM was only produced by maltose solutions containing amino acids, especially glutamine. These same authors propose this compound as an indicator of overheating during drying pasta, so that the presence of GIM in our samples indicates that under these storage conditions (55 °C, 25 and 55 °C/Aw 0.65) the baby cereals suffer considerable thermal damage, even greater than that suffered during the processing, and therefore decreases the nutritional quality.

HMF determination

The method applied in this study was developed by our investigation group in similar samples (Guerra-Hernández, Garcia-Villanova, & Montilla-Gomez, 1992). The relative standard deviation was 2.10% for a mean HMF value of 3.25 mg/kg (Guerra-Hernández et al., 2002). Detection limit (signal-to-noise ratio > 3) was 0.0075 mg/kg and quantification limit (signal-to-noise ratio > 10) was 0.025 mg/kg.

Baby rice cereals

HMF was detected in non-stored samples (0.62 mg/kg). Under industrial storage conditions at 32 °C for 12 months, HMF increased during the 6 initial months and then decreased. After 12 months of storage, the HMF level was higher at 55 °C than at 32 °C (Supplementary Table 1), with significant changes between the third and twelfth month ($p < 0.05$). At high water activity (Aw = 0.65) and 25 °C, HMF increased during the first week of storage and then decreased. At the same water activity and storage periods but at 55 °C, there was a small but significant increase ($p < 0.05$), reaching 1.17 mg/kg at 3 weeks (Supplementary Table 1). Ramírez-Jiménez, Guerra-Hernández, and García-Villanova (2003) also reported increased during storage in baby rice cereals (samples without caramel) and Fernandez-Artigas et al. (1999) found an increase in HMF content during the toasting of cereal flours but not when samples were heated after the addition of ingredients with HMF.

Baby rice–corn cereals

HMF was detected in non-stored samples (11.2 mg/kg). The high content found is due to the addition of caramel during manufacture. At 55 °C, the HMF content showed no significant changes after 12 months of storage (Supplementary Table 2). Therefore, HMF was not determined at 32 °C. At high water activity (Aw = 0.65) and 25 °C, HMF was approximately five-fold lower in samples stored for 4 weeks than in non-stored samples. A similar behavior was observed at 55 °C/Aw = 0.65 (Supplementary Table 2).

Baby eight-cereals

HMF was detected in non-stored samples (13.0 mg/kg). This high content is due to addition of caramel during manufacture. At 55 °C, HMF content showed no significant changes after 12 months of storage (Supplementary Table 3). Therefore, HMF was not determined at 32 °C. At high water activity (Aw 0.65), HMF was approximately two-fold lower versus non-stored samples after 4 weeks of storage at 25 °C, and approximately six-fold lower ($p < 0.05$) at 55 °C (Supplementary Table 3).

The HMF content of the cereals with wheat markedly decreased during storage, especially under laboratory conditions; this suggests that the Maillard reaction products are prevalent (major degradation then generation), which indicates a nutritional decrease in these samples. Hidalgo and Pompei (2000), studying furosine and HMF kinetics in tomato products, found decreased HMF levels when such products were stored at room temperature. On the other hand Fallico, Arena, and Zappala (2008) studied the degradation of HMF in honey stored at 25, 35, and 55 °C finding that at room temperature HMF degradation kinetics was significant in comparison to the kinetics of formation, and the reaction of HMF degradation was characterized by lower activation energy (Ea) values compared to Ea formation values, which means that an increase of temperature promotes formation routes much more than the HMF degradation pathways. HMF content obtained in samples with wheat (eight-cereals) and rice–corn cereals were higher than that obtained in baby rice samples (without caramel).

Formation of HMF in foods varies with processing and storage conditions and is especially dependent on temperature and pH. This has been confirmed in model experiments with fruit juices and concentrates as well as in heat-treated milk (Gomis, Alvarez, Naredo, & Alonso, 1991; Kern, 1964; Morales, Romero, & Jimenez-Pérez, 1992). The level of HMF in food depends on the equilibrium between destruction by oxidation and formation from precursors (Morales et al., 1997). Dehydrated fruits and caramel are commonly included in baby cereals. The dehydration process that these ingredients undergo in their processing produces nonenzymatic browning, mainly due to carbohydrate degradation, which produces HMF (Fernandez-Artigas et al., 1999; Fernandez-Artigas, Guerra-Hernández, & García-Villanova, 2001).

It is not clear whether food-borne exposure to HMF represents a potential health risk for humans. HMF at high concentrations is cytotoxic, causing irritation to eyes, upper respiratory tract, skin, and mucous membranes. An oral LD_{50} of 3.1 g/kg body weight has been determined in rats (Ulbricht, Northup, & Thomas, 1984). This LD_{50} is very high compared to HMF levels found in baby cereals and therefore it does not pose a serious health risk to babies. Janzowski, Glaab, Samimi, Schlatter, and Eisenbrand (2000) concluded that HMF does not pose a serious health risk, even though the highest concentrations in specific foods approach the biologically effective concentration range in cell systems. Neither data from epidemiologic studies or case reports on potential association of HMF with cancer risk in humans nor chronic carcinogenicity studies are available.

Comparisons among indicators

GIM content increased during storage in all types of cereal, whereas HMF content only increased during storage in baby rice cereals and decreased in rice–corn and eight-cereals samples with caramel.

At 55 °C, high and significant correlation ($r^2 = 0.867$; $p = 0.021$) was obtained in baby rice cereals, however at high water activity (Aw = 0.65) very low and not significant correlations were obtained, probably because under drastic conditions HMF is generated both from the Maillard reaction and caramelization (Kroh, 1994) whereas GIM is produced only from the Maillard reaction (Resmini

et al., 1993). An inverse correlation was found in the other cereals (baby rice–corn and baby eight-cereals), which was high especially under drastic storage conditions ($r^2 = 0.667$; $p = 0.092$ and $r^2 = 0.557$; $p = 0.148$ at 25 and 55 °C respectively), which is logical since GIM increased and the HMF decreased. In our studies, gluosylisomaltol was found to be a better marker for heat damage than HMF.

Conclusion

GIM is a useful indicator of the extent of the browning reaction when wheat-based and rice-based baby cereals are stored under drastic conditions. The presence of GIM in baby cereals indicates inadequate storage. The GIM content was higher with greater protein and reducing sugar content in the baby cereals.

Supplementary material

The supplementary material for this article is available online at http://dx.doi.org/10.1080/19476337.2010.495788

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