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Evaluation of the risk of fungal spoilage when substituting sucrose with commercial purified Stevia glycosides in sweetened bakery products

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ABSTRACT

The objectives of this study were to compare the effect of different Stevia-based sugar substitutes (S1–S3), sucrose alone and a mixture of sucrose + S1 on: (a) humectant properties, (b) relative colonisation rates of sponge cake slices at 0.90 a_w by strains of *Aspergillus flavus*, *Eurotium amstelodami*, *Fusarium graminearum* and *Penicillium verrucosum* at 20 and 25 °C and (c) shelf-life periods in days prior to visible growth. Results showed that sucrose, S1 commercial sugar substitute and the mixture of sucrose + S1 in water solutions were able to reach water activity levels similar to those of glycerol and glucose mixtures. The S2 and S3 commercial sugar substitutes were unable to reduce a_w levels significantly. At 25 °C, colonisation of sponge cake slices by *E. amstelodami*, *A. flavus* and *P. verrucosum* occurred in all the treatments. Growth of *F. graminearum* only occurred on sponge cake slices containing S2 and S3 Stevia-based products at both temperatures. The best control of growth (30 days) was achieved in cake slices modified with sucrose or S1 Stevia treatments inoculated with *A. flavus* and in the sucrose treatment for *E. amstelodami* at 20 °C. *F. graminearum* growth was completely inhibited when sucrose alone, S1 or sucrose + S1 treatments were used at both temperatures. This study suggests that, as part of a hurdle technology approach, replacing sucrose with low calorie sugar substitutes based on Stevia glycosides needs to be done with care. This is because different products may have variable humectant properties and bulking agents which may shorten the potential shelf-life of intermediate moisture bakery products.

1. Introduction

Sucrose is currently one of the main ingredients in the food industry and is especially important in sweetened bakery products and confectionary for its preservation characteristics and as an important source of energy, providing 394 kcal/100 g of refined sugar. Its preservation properties are to act as a humectant by reducing the water activity (a_w) of bakery products as part of a hurdle technology strategy. Intermediate moisture bakery products have a relatively short shelf-life although this can be extended by addition of aliphatic acids and sometimes modified atmosphere packaging (Smith et al., 2004; Sozer et al., 2011). However, these intermediate moisture products are prone to colonisation by xerophilic and xerotolerant fungi when not stored properly. The most important spoilage moulds in bakery products are species from *Eurotium*, *Aspergillus* and *Penicillium* (Abellana et al., 1997; Arroyo et al., 2005; Guynot et al., 2005; Spicher, 1980; Williams, 1990).

There has been interest in the substitution of sucrose with alternative lower calorie sweeteners in bakery products. The most common high-intensity sweeteners in the world market (e.g. saccharin, aspartame, sucralose) are synthetic compounds (Abdalbasit et al., 2014). Other alternative plant-based products based on extracts from the plant *Stevia rebaudiana* (Bertoni) have received particular attention. The compounds of interest are known as steviol glycosides (Boileau et al., 2012). Stevia products have redefined the category of intense sweeteners globally, because, for the first time, food manufacturers have access to an effective non-calorific sweetener that has a "natural" image (Gibson-Moore, 2013). In 2011, the European Commission authorised the use of pure steviol glycosides (95%) in foods and beverages in the European Union.

Stevia products are being increasingly used (Chattopadhyay et al., 2014) by food companies that produce sweetened bakery products and recommended as a substitute for home baking. However, surprisingly there have been no studies on whether substituting sucrose with Stevia glycosides completely, or in combination with sucrose, will result in the required target a_w levels of products such as cakes and provide similar shelf-life properties as sucrose in terms of preventing fungal spoilage from being initiated.

The objectives of this study were to (a) evaluate the water binding capabilities of different commercial Stevia-based sweetener formulations by comparing them with sucrose, glucose and glycerol, (b) to examine the effect of three commercial Stevia products (S1-S3) alone or with sucrose on fungal colonisation rates on sponge cake slices at 20 and 25 °C by different spoilage fungi (*Aspergillus flavus*, *Penicillium verrucosum*, *Eurotium amstelodami* and *Fusarium graminearum*), and

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(c) relative shelf-life of different formulated sponge cake slices in terms of time (days) before visible spoilage was initiated.

2. Materials and methods

2.1. Commercially purified Stevia products

Three different commercially available products were bought from UK retail shops and identified as S1, S2 and S3. The information on the product labels about weight, composition, manufacturer dosage guidelines and energy per 100 g was recorded and are shown in Table 1.

2.2. Determination of the a_w of solutions of different sugars and additives

RQ-Water solutions containing 5, 10, 20, 30, 40, 80 and 100 g/100 mL of water were prepared using the different Stevia products. At higher concentrations, the Stevia products were close to their maximum solubility in water and where necessary the temperature was increased to facilitate complete dissolution.

For comparison, glycerol-water, sucrose-water and glucose-water solutions were prepared, according to the literature in the range 0.995 and 0.80 a_w (Dallyn and Fox, 1980; Scott, 1957). The a_w of all solutions was measured using an Aqualab 3TE instrument (Decagon, Pullman, WA, USA). All measurements were made with three replicates per treatment solution.

2.3. Preparation of the cake analogue

A sponge cake analogue similar to that used by Abellana et al. (1999) was prepared for these studies. The recipe consisted of the following basic ingredients: 275 g self-raising wheat flour, 250 mL vegetable oil and 4 medium sized eggs. In this study 5 different treatments were included. These were (i) sucrose-based cakes (control), (ii) 100% substitution of sucrose by the manufacturers recommended amounts of Stevia products for S1, S2 and S3 and (iii) 75% substitution of sucrose by the Stevia product S1 according to an online cake recipe by the manufacturer [1/4 sucrose + 1/4 S1 (as it is 3 times sweeter than sucrose)]. All substitutions where made according to the volume recommended by the manufacture as detailed in Table 1.

The ingredients were mixed in a multifunctional kitchen mixer to make the cake dough. The dough batches were placed in greased (vegetable oil) disposable aluminium tins and baked in an oven at 160-170 °C for 40–45 min.

After baking, the tins were covered with sterilised cooking foil and transferred to a laminar flow bench for further processing. The cooled cakes were cut into ≈4 mm thick slices and placed in sterile 9 cm Petri plates. The cake slices were then exposed to 254 nm UV light for 10 min in a Herolab CleneCab Plus (Herolab GmbH Laborgeräte, Germany) to eliminate any surface contamination. The a_w of the cake slices was checked using an Aqualab 3TE instrument (Decagon).

Subsequently, all the treatments were equilibrated at 0.90 a_w in order to carry out fungal growth experiments. The cake slices in Petri plates were divided into 4 groups and placed in plastic chambers together with two 500 mL beakers containing a glycerol-water solution with an equilibrium relative humidity value identical to the a_w treatments (= 0.90 a_w). Equilibration was achieved by incubating for 48 h at 4 °C. The experimental chambers were subsequently allowed to equilibrate at the target temperatures of 20 or 25 °C for 4 h. The equilibration at the target a_w was confirmed using an Aqualab 3TE instrument and found to be within ± 0.02 a_w. The cake slices of the treatments were then inoculated with spores of the fungal strains.

2.4. Strains used in these studies and inoculation method

Fungal strains of *A. flavus* (NRRL3357), *P. verrucosum* (OTA11), *E. amstelodami* (IMI229971) and *F. graminearum* (FgB (L1–2/2D) were grown on Malt Extract Agar (MEA; Oxoid Ltd, Basingstoke, U.K.) for 10 days, except for the *E. amstelodami* strain which was grown on MEA modified to 0.95 a_w using a glycerol-water solution. These species were selected based on previous studies and because they are common contaminant of bakery products (Abellana et al., 1997; Arroyo et al., 2005; Gerez et al., 2009). From these cultures, spores were collected using 10 mL sterile saline solution containing 0.05% Tween 80 (Acros Organics, USA) and rubbing the surface with a sterile glass rod in order to remove the conidia. The spore suspensions were counted using a haemocytometer (Fisher Scientific, United Kingdom) and adjusted to 1×10^6 spores/mL and directly used to inoculate the cake slices. Each cake slice was centrally inoculated with 3 μl of the spore suspension.

2.5. Incubation and measurement of growth and data analyses

Experiments were conducted at 20 and 25 °C. In all cases, observations were carried out every two days or as necessary, and the diameter of the colonies measured in two directions at right angles to each other. Growth was observed with the aid of a binocular magnifier (Olympus SZ, Olympus, Japan). Measurements were taken for a maximum of 30 days. All experiments were carried out with at least three replicates per treatment.

The temporal colony diameters were measured and subjected to primary modelling using the linear model. The maximum growth rate (μ_{max}) (mm diameter/day) of each fungal treatment on the different cake analogues was determined. Regression lines were made using the time points which represented the linear phase of the growth curves using Microsoft®Excel®:MAC 2011 (14.4.8) (Microsoft Corporation, Redmond, USA). The slope of the linear equation with an associated correlation coefficient of not $< R^2 = 0.98$ was considered the μ_{max}. Lag times (λ) were calculated by equalling the regression lines to the size of the inoculum point.

2.6. Statistical analysis

The statistical package JMP®12.1 Pro (SAS Institute Inc., 2015, Cary NC, USA) was used in the analysis.

The normality of the data sets was investigated using the Shapiro–Wilk test ($\alpha = 0.05$). This was followed by Levene's test ($\alpha = 0.05$) to determine variance homogeneity. Due to non-normality of the growth data and, analysis was performed using non-parametric tests for testing whether distributions across factor levels were centered at

Table 1
General specific information in the products label with regard to weight, composition manufacturer, use guidelines and energy.

Product	Weight of the package	Composition	Manufacturer dosage recommendations	Energy values per 100 g
S1	270 g	Bulking Agent: Erythritol Sweetener: Steviol Glycosides (1% Stevia Leaf Extract), Natural Flavourings	1/3 teaspoon sweetens like 1 teaspoon of sugar	0 kJ (0 kcal)
S2	75 g	Bulking Agent: Maltodextrin Sweetener: Steviol Glycosides (2%), Natural Flavourings	1 teaspoon (2 kcal) is equivalent in sweetness to one teaspoon of sugar (20 kcal)	1598 kJ (376 kcal)
S3	75 g	Bulking Agent: Maltodextrin Sweetener: Steviol Glycosides (2%)	1 teaspoon is equivalent in sweetness to one teaspoon of sugar	1656 kJ (390 kcal)