Food Chemistry 242 (2018) 162–168

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of (−)-epigallocatechin gallate (EGCG) extracted from green tea in reducing the formation of acrylamide during the bread baking process

Zhengjie Fu[a], Michelle J.Y. Yoo[a,*], Weibiao Zhou[b], Lei Zhang[a], Yutao Chen[a,e], Jun Lu[a,c,d,e,*]

[a] Centre for Food Science, School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand
[b] Food Science and Technology Program, c/o Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[c] School of Interprofessional Health Studies, Faculty of Health and Environmental Sciences, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand
[d] Institute of Biomedical Technology, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand
[e] College of Life and Marine Sciences, Shenzhen University, Shenzhen, Guangdong Province, China

ARTICLE INFO

Keywords:
Acrylamide
Green tea extract
Catechin
Bread
Baking
LC-MS

Chemical compounds studied in this article:
Acrylamide (PubChem CID: 6579)
Catechin (PubChem CID: 9064)

ABSTRACT

This is the first study to investigate the extent of reduction in acrylamide formation during baking with the addition of (−)-epigallocatechin gallate (EGCG) extracted from green tea, and to determine whether EGCG influences the texture and colour attributes of bread, or interacts with other ingredients. EGCG powders were added to white bread formulations at the concentrations of 3.3, 6.6 and 9.9 g·kg^{-1}. The amount of acrylamide in the bread was analysed using liquid chromatography-mass spectrometry. EGCG addition significantly reduced the acrylamide formation by 37% compared to the control and decreased the moisture content of the bread by 6%. It did not affect its texture attribute, but increased the lightness and the yellowness and decreased the redness of bread crust (with contrasting results in crumb). It also decreased granule size and porosity. In conclusion, EGCG fortification is a feasible method to decrease acrylamide formation in baked bread.

1. Introduction

Acrylamide is a potential carcinogen (Besaratinia & Pfeifer, 2007), and was first reported in food in 2002 (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002). Acrylamide in foods is formed predominantly via Maillard reactions, where a series of reactions between an amino acid, primarily asparagine (Stadler et al., 2002), and reducing sugars occur (Lingnert et al., 2002) at temperatures greater than 120 °C and peaks between 160 and 180 °C (Wilson, Rimm, Thompson, & Mucci, 2006) in starchy foods. According to Zyzak et al. (2003) the carbonyl group of a reducing sugar reacts with the amino group of an amino acid and forms a Schiff base. This can be decarboxylated and forms either acrylamide directly with elimination of imine or a product that can be hydrolysed to form 3-aminopropionamide, which is then thermally degraded to form acrylamide (Zyzak et al., 2003). Yet, the exact mechanism of acrylamide formation through the Maillard reaction is unknown.

Green Tea Extract (GTE) is composed of polyphenolic compounds from green tea, including catechins, which makes up 30% of the dry weight of green tea leaves. There are several polyphenolic catechins in green tea: (−) epicatechin (EC), (−) epicatechin-3-gallate (ECG), (−) epigallocatechin (EGC), (−) epigallocatechin-3-gallate (EGCG), (−) gallocatechin gallate (GCG), (+) catechin, and (+) gallocatechin (GC) (Zaveri, 2006). The most common GTE is usually a brown coloured powder and is composed of 10–60% of EGCG, about 30% of other catechins (e.g. ECG) and 2–5% of caffeine. Compared with the common GTE, Teavigo GTE is a highly purified extract from the leaves of *Camellia sinensis*. It is a fine, off-white to pale pink coloured powder with a higher amount of active substance (minimum 94% EGCG) and is free of caffeine. Beneficial and preventable effects of EGCG on cardiovascular and neurodegenerative disease (Higdon & Frei, 2003), type 2 diabetes (Wu et al., 2004) and cancer through epidemiological and animals studies (Minxing & Yaling, 2013) as well as its antioxidant activity (Lu, Lee, Mau, & Lin, 2010), are well documented in the literature. Through the study of *in vitro* digestibility of GTE-fortified baked and steamed bread, Goh et al. (2015) suggested that catechins could indirectly inhibit the acrylamide formation, accompanied by the reduced enzymatic activity of pancreatic α-amylase and glucosidase during digestion.

Attempts of adding GTE to foods have been trialled in the past, however, no detailed study has been performed to look at the effect of low dose fortification on inhibition or reduction of acrylamide formation. Sharma and Zhou (2011) reported on the positive potential of

* Corresponding authors at: Private Bag 92006, Auckland 1142, New Zealand (M. Yoo). Private Bag 92006, Box A-25, Auckland 1142, New Zealand (J. Lu).
E-mail addresses: michelle.yoo@aut.ac.nz (M.J.Y. Yoo), jun.lu@aut.ac.nz (J. Lu).

http://dx.doi.org/10.1016/j.foodchem.2017.09.050
Received 11 May 2017; Received in revised form 1 August 2017; Accepted 11 September 2017
Available online 12 September 2017
0308-8146/

adding GTE to foods, where high EGCG retention levels of about 83% in biscuit and bread, post baking, were shown. However, with the addition of GTE, changes in physicochemical and sensorial characteristics have not been widely reported. Wang, Zhou, and Isabelle (2007) showed that with the addition of GTE to bread, the brightness and sweetness of the bread decreased while its hardness (2.40–3.35), stickiness (2.02–2.82) and astringency (0.24–1.19) increased. Lu et al. (2010) reported that hardness, gumminess, chewiness, redness of the bread crumb (a^*), protein, total dietary fibre and ash content of baked cakes increased with increasing amounts of GTE addition. Cake volume, cohesiveness, adhesiveness, springiness, resilience, the colour of crust (L^*, a^*, b^*) and crumb (L^* and b^*) decreased with increasing amounts of GTE addition. With the addition of GTE, sweetness decreased due to the presence of bitterness naturally being present in the GTE. Pasrija, Ezhilarasi, Indrani, and Anandharamakrishnan (2015) attempted to improve the hardness and flavour of bread with the addition of microencapsulated green tea polyphenols in freeze-dried powder form. From their study, GTE addition was shown to improve the taste and antioxidant activity. The objective of this study was to determine the effects of low dose EGCG addition to white bread formulation on reduction of acrylamide formation during baking and to study the subsequent changes in physicochemical properties of bread.

2. Materials and methods

2.1. Reagents and chemicals

All reagents used were either Optima or LC-MS grade. Acetonitrile, Hexane, formic acid, GCG and acrylamide were purchased from Sigma-Aldrich (St Louis, MO, USA). Acrylamide-d3 (A191302) was purchased from Toronto Research Chemicals (TRC) Inc. (Toronto, Ontario, Canada). Milli-Q water was produced by Purite Fusion Milli-Q water purifying machine (Purite Limited, Thame, Oxon, UK).

2.2. Preparation of bread samples

Bread was made with 1000 g high grade flour (with ~10% protein content), 40 g white caster sugar, 20 g iodised table salt, 10 g active dry yeast and 30 g shortening, which were purchased from a local New Zealand supermarket. 620 g of deionised water was used to make the dough. DSM Teavigo® Green Tea Extract from Pharmachem Laboratories (Kearny, NJ, USA), containing 94% EGCG and up to 5% of other catechins (free of caffeine) was used. Teavigo GTE of 3.3, 6.6 or 9.9 $g{\cdot}kg^{-1}$ was added to the bread formulation, respectively. EGCG is defined as “generally regarded as safe” (a term used by FDA for food materials/ingredients), therefore it is suitable to be fortified directly into bakery products.

The ingredients were mixed using a bread maker (Breville®, Baker’s Oven, Alexandria, NSW, Australia) and kneaded for 10 min for the first knead and 20 min for the second knead, followed by 5 min of hand kneading. Each formulation was equally divided into four lots, into separate loaf tins and then the dough was proved (LineMiss XL195-B, Unox UK Ltd, UK) for 1 h, at 40 °C and 80% relative humidity. Then it was baked for 20 min at 200 °C, followed by 180 °C for another 20 min, in a conventional oven (Fisher & Paykel, OR61S2L, Auckland, New Zealand). Once baked, the bread was cooled to room temperature. The crust of the bread was removed, using a scalpel, and then freeze-dried (Christ Alpha2-4 LD-Plus, Osterode am Harz, Germany) at −80 °C for 24 h. The freeze dried samples were stored in a desiccator for later liquid chromatography-mass spectrometry (LC-MS) analysis. The fresh crumb samples were divided to two portions: one portion was ground by a coffee blender (Breville®, Alexandria, NSW, Australia) for moisture and colour analyses. The other portion was cut into 1 cm^3 cubes for texture analysis, and cut into slices for scanning electron microscope (SEM) analysis.

2.3. Extraction of acrylamide from bread crust

One gram of the ground and freeze-dried bread crust sample (particle size 69.7 ± 6.0 μm, measured by Mastersizer 2000, Malvern Instruments, Malvern, UK) was placed into a 50 ml tube from the Agilent Bond Elut QuEChERS Extraction kit for acrylamides (Agilent Technology, AGI-5982-5850, Santa Clara, CA, USA). The internal standard (acrylamide-d3) was added to the tube to make the final concentration at 100 $\mu g{\cdot}l^{-1}$. Then, 5 ml of hexane (Sigma-Aldrich 270504) was added, and the mixture was mixed using a vortex mixer for 20 s. Ten ml of Milli-Q water (Purite Fusion Milli-Q water purifying machine, Thame, Oxon, UK) and 10 ml of acetonitrile (Sigma-Aldrich 34967) were added followed by the Agilent Bond Elut QuEChERS extraction salt mixture. The sample tube was subject to vortex for 1 min and centrifuged at 5000*g* for 10 min.

After centrifuging, the hexane layer was discarded and 1 ml of the acetonitrile extract was transferred to a 2 ml tube from the Agilent Bond Elut QuEChERS AOAC Dispersive SPE kit (AGI-5982-5022). The tube contained 50 mg of primary secondary amine (PSA) and 150 mg of anhydrous $MgSO_4$. The tubes were subject to vortex for 1 min and then centrifuged at 5000*g* for 2 min. Supernatant of 250 μl was transferred to a 1.5 ml vial. Acetonitrile was evaporated with Centrivap centrifugal vacuum concentrator (Labconco, Kansas, MO, USA). Mill-Q water of 100 μl was added and mixed under 1-min vortex and then centrifuged at 10,000*g* for 2 min. Supernatant of 50 μl was taken and transferred into 100 μl vial with glass insert and stored in a −20 °C freezer until LC-MS analysis.

Extracted acrylamide was analysed using an Agilent 1200 Series high pressure liquid chromatography (HPLC) system linked with an Agilent 6460 Triple Quadrupole mass detector. Separation of the chemicals was achieved on a hypercarb column (2.1 mm × 100 mm, 3 μm, Phenomenex, Auckland, New Zealand). All data were processed by the Agilent Mass Hunter Quantity Analysis software.

2.4. Preparation of standard stock solutions and calibration

Acrylamide standard stock solution (1 $g{\cdot}l^{-1}$) was prepared by dissolving 100 mg of acrylamide in 100 ml of Milli-Q water. It was stored at 4 °C in a fridge until use. The internal standard acrylamide-d3 stock solution (1 $g{\cdot}l^{-1}$) was prepared by dissolving 10 mg of acrylamide-d3 in 10 ml of acetonitrile (Sigma-Aldrich 34967). It was stored in −20 °C freezer until use. All working solutions were prepared daily by serial dilution in Milli-Q water. The concentrations of the standard working solutions were 31.25, 50, 75, 125, 250, 375 and 500 $\mu g{\cdot}l^{-1}$. The calibration curves were linear with correlation coefficients (R^2) at 0.999.

2.5. LC-MS conditions

The LC-MS was operated in positive electrospray mode. The hypercarb column (2.1 × 100 mm, particle size 3 μm, Thermo Scientific, Waltham, MA, USA) was maintained at 30 °C. The mobile phase was comprised of 0.1% v/v formic acid (Sigma-Aldrich 94318-50ML-F) and the flow rate was maintained at 0.3 $ml{\cdot}min^{-1}$. The injection volume for each sample was 5 μl. The capillary voltage was 1.5 kV. The cone voltage was 20 V. The source temperature was 120 °C and the gas temperature was 325 °C. For the transitions, *m/z* 72 (acrylamide) and *m/z* 75 (acrylamide-d3), *m/z* 55.2 (acrylamide) and *m/z* 58.2 (acrylamide-d3) were used with the collision energies all set to 20 V. The column was washed for a minimum of 20 min with 50:50 methanol/acetonitrile after 12 samples or at the end of daily operations. Nitrogen gas was used at 6 $l{\cdot}min^{-1}$.

2.6. HPLC analysis for EGCG and GCG

The stability and fate of fortified EGCG were also investigated by measuring EGCG and GCG before (as in dough) and after baking (as in