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Stability and anti-glycation properties of intermediate moisture apple products fortified with green tea

Vera Lavelli [a,*], Mark Corey [b], William Kerr [b], Claudia Vantaggi [a]

[a] DISTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy
[b] Department of Food Science and Technology, University of Georgia, Food Science and Technology Building, 100 Cedar St., Athens, GA 30602, United States

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ABSTRACT

Intermediate moisture products made from blanched apple flesh and green tea extract (about 6 mg of monomeric flavan 3-ols added per g of dry apple) or blanched apple flesh (control) were produced, and their quality attributes were investigated over storage for two months at water activity (a_w) levels of 0.55 and 0.75, at 30 °C. Products were evaluated for colour (L^*, a^*, and b^* Hunter's parameters), phytochemical contents (flavan 3-ols, chlorogenic acid, dihydrochalcones, ascorbic acid and total polyphenols), ferric reducing antioxidant potential, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical-scavenging activity and ability to inhibit formation of fructose-induced advanced glycation end-products.

During storage of the fortified and unfortified intermediate moisture apples, water availability was sufficient to support various chemical reactions involving phytochemicals, which degraded at different rates: ascorbic acid > flavan 3-ols > dihydrochalcones and chlorogenic acid. Colour variations occurred at slightly slower rates after green tea addition. In the intermediate moisture apple, antioxidant and anti-glycoxidative properties decreased at similar rates (half-life was about 80 d at a_w of 0.75, 30 °C). In the green tea-fortified intermediate moisture apple, the antioxidant activity decreased at a slow rate (half-life was 165 d at a_w of 0.75, 30 °C) and the anti-glycoxidative properties did not change, indicating that flavan 3-ol degradation involved the formation of derivatives that retained the properties of their parent compounds. Since these properties are linked to oxidative- and advanced glycation end-product-related diseases, these results suggest that green tea fortification of intermediate moisture apple products could be a valuable means of product innovation, to address consumers' nutritional needs.

1. Introduction

Apples are one of the most important fruits in terms of production worldwide. The world's largest apple producer is China, followed by the US, then Turkey, Iran, Italy, France, Poland and Russia (http://www.fao.org/es/ess/top/commodity.html). Apples are rich in phytochemicals, such as flavan 3-ols (oligomeric procyanidins, epicatechin and catechin), hydroxycinnamic acids (chlorogenic acid and a *p*-coumaric acid derivative), dihydrochalcones (phloridzin and phloretin 2′*O*-xyloglucoside), flavonols (quercetin glycosides) and anthocyanins (cyanidin glycosides) (Vrhovsek, Rigo, Tonon, & Mattivi, 2004).

Most apple production is intended for industrial processing into juice, puree, nectar, cider, vinegar and dehydrated products. Two types of intermediate moisture (IM) apple products are manufactured. One is a concentrated juice and contains about 25% moisture (Spanos & Wrolstad, 1992). The second contains 10–25% moisture and is referred to as evaporated apple. These products are used either as ingredients for different food products (e.g. for pie filling) or are remoistened prior to use, or as IM foods (Brennan, 1994).

Processing of apples causes both a decrease in antioxidant content and browning (Spanos & Wrolstad, 1992). IM apple is stable from the stand point of fermentation, whereas major deteriorative quality problems are related to non-enzymatic browning (Burdurlu & Karadeniz, 2003; Torbido & Lozano, 1984; Vaikousi, Koutsoumanis, & Biliaderis, 2008) and phenolic oxidation (Lavelli & Vantaggi, 2009).

This study focused on the fortification of IM apple products with green tea (GT). The food industry has recently regarded GT as useful for food fortification, having the intention of improving the health-promoting properties of food products (Wang, Provan, & Helliwell, 2000). GT phytochemicals are associated with health-promoting effects. GT leaves are composed of about 10% (w/w) flavan 3-ols, which

Abbreviations: FRAP, ferric reducing/antioxidant power; DPPH, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical-scavenging activity; AGE, advanced glycation endproducts; GT, green tea; EGCG, (−)-epigallocatechin gallate; EC, (−)-epicatechin (EC); EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; GCG, (−)-gallocatechin gallate; GC, (−)-gallocatechin; CG, (−)-catechin gallate; C, (−)-catechin; BSA, bovine serum albumin; PPO, polyphenol oxidase.

* Corresponding author. Tel.: +39 2 50319172; fax: +39 2 50319191.
E-mail address: vera.lavelli@unimi.it (V. Lavelli).

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are referred to as catechins. The most prevalent compounds include (−)-epigallocatechin gallate (EGCG), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), and (−)-epicatechin gallate (ECG) (Wang, Provan, & Helliwell, 2003). GT catechins possess very high scavenging ability against reactive oxygen species. Indeed, various epidemiological studies have demonstrated that GT consumption decreases the occurrence of oxidative-related diseases, such as cancer and cardiovascular disease (Thielecke & Boschmann, 2009; Wang & Ho, 2009). In addition, GT catechins effectively trap carbonyl species, which are extremely reactive as they can modify lysine, arginine and cysteine residues of proteins, leading to formation of advanced glycation end-products (AGEs). Hence, GT catechins have the potential to inhibit the progression of symptoms associated with AGE-related diseases, such as diabetes (Wang, Sun, Cao, & Tian, 2009; Thielecke & Boschmann, 2009).

The intent of this study was to examine an intermediate moisture apple product that is not currently available to consumers and may be relevant to the functional foods market. This is a growing industry, and many food companies have seen a great potential for products that provide additional physiological benefits beyond their basic nutrition (Day, Seymour, Pitts, Konczak, & Lundin, 2009). Here, we have studied a new food product made from relatively cheap food ingredients, but that have somewhat valuable bioactive components in terms of human health protection. Challenges remain to ensure that functional ingredients remain stable after food processing and storage (Day et al., 2009).

In fact, during processing and storage of GT drinks, flavan 3-ols undergo degradation (Labbé, Têtu, Trudel, & Bazinet, 2008). GT flavan 3-ols also degrade during long-term storage in the dry state (Friedman, Levin, Lee, & Kozukue, 2009); therefore a new GT formulation should be evaluated for its stability. Intermediate a_w levels are particularly critical for product stability, since moisture content is sufficient to permit the occurrence of Maillard reactions and oxidation (Brennan, 1994). The rate of non-enzymatic browning is high in dehydrated foods, due to elevated concentrations of reagents, with maximum values in the IM foods with water activity (a_w) levels between 0.5 and 0.8. Below 0.5, the reaction is limited by low mobility of reactants whereas, above 0.8, the browning rate decreases with increasing a_w due to a dilution effect (Vaikousi et al., 2008). An increase in a_w above 0.3 increases the rate of antioxidant degradation in apples, most likely by increasing mobility of reactants and bringing catalysts into solution (Lavelli & Vantaggi, 2009). In addition, as the solid matrix swells, new surfaces for oxidation are exposed (Brennan, 1994).

Therefore, the GT flavan 3-ols incorporated into an intermediate moisture apple matrix are challenged by different reactive carbonyl species and free radicals derived from these degradation reactions.The purpose of this study was to investigate the effect of GT addition on IM apple quality and stability.

GT-fortified IM apple products were stored in the a_w range 0.56–0.75, at 30 °C. Kinetics of colour changes, phytochemical degradation and *in vitro* antioxidant and anti-glycoxidative properties were investigated in comparison to unfortified IM apple products.

2. Materials and methods

2.1. Materials

The reagents sodium chloride, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-*s*-triazine, $FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, Folin–Ciocalteu phenol reagent, bovine serum albumin (BSA, 97% fatty acid free), Chelex resin, fructose, sodium azide, sodium phosphate monobasic, sodium phosphate dibasic, and standards of ascorbic acid, caffeine, chlorogenic acid, phloridzin and gallic acid were purchased from Sigma Aldrich (Milan, Italy). Standards of C, EC, EGC, EGCG, ECG, GCG, and procyanidin B2 were purchased from Extrasynthese (Lyon, France).

2.2. Green tea extraction

Java Green Tea (Twinings, London, UK) was purchased at a local supermarket. In a half-litre Pyrex bottle, GT extract was prepared by extracting 25 g of dried leaves in 500 ml of deionised water, which had been pre-heated at 85 °C. The extraction proceeded for 5 min before the bottle was immersed in ice/water slurry. The extract was then filtered through Whatman No. 4 filter paper. A sample of extract was removed and analysed by HPLC for phenolic content.

2.3. Preparation of GT-fortified and unfortified IM apples

Fresh apples (*Malus domestica*) of the cultivar Golden Delicious were purchased from a local supermarket. Each apple was peeled, cored with seeds removed, and quartered. One half portion was designated for GT addition and the other for control. Two batches of apple portions (2 kg each) were placed into a wire mesh basket and immediately boiled in a deionised water pot at 100 °C for 4 min. After heating, the basket was rapidly removed from the boiling water, cooled by immersion in a 4 °C deionised water bath, and drained. Apple slices were then blended to a puree consistency in a K 3000 Braun Multisystem blender (Braun, Kronberg, Germany).

The amount of GT water extract to be added to the apple puree was chosen in order to obtain (in a single serving of the fortified apple product) an amount of GT flavan 3-ols equivalent to that present in a cup of GT infusion. Moisture content of the apple puree was determined. A 2 kg batch had 200 g of dry solids; therefore it corresponded to four single serving portions. In fact, a portion size of 50 g d.w. is consistent with a commercial single-serving of dehydrated apple products available in the marketplace. Preliminary trials were carried out to evaluate the average retention of GT catechins after freeze-drying, which was about 80%. Based on this information, a 2 kg batch of apple puree was added to 384 ml of GT extract (which was expected to provide an amount of GT catechins equal to four cups of GT) mixed thoroughly and freeze-dried to obtain the GT-fortified apple product. Control and GT fortified apple purees were then spread over stainless steel trays and freeze-dried in a Lyoflex Edwards freeze drier (Crawley, UK). The freeze-dried powders were ground in the food processor and sieved (800 μm pan sieve mesh size).

2.4. Storage study

For the storage study, powders of apple and apple fortified with GT were weighed into Petri dishes (0.141 g of powder per cm^2). The dishes were then placed onto wire mesh racks that were suspended within airtight plastic boxes above a saturated sodium chloride solution (a_w at 30 °C = 0.751 ± 0.001) or a saturated sodium bromide solution (a_w at 30 °C = 0.560 ± 0.004). The boxes were prepared in duplicate for each experimental treatment and stored at 30 °C over a period of 2 months in a thermostatted heating cabinet.

2.5. Water activity (a_w), moisture content, soluble solids, pH and titratable acidity

Water activity (a_w) of the samples and of the saturated salt solutions was measured in duplicate, every day from the incubation for 5 d, to confirm that the equilibrium a_w had been reached. An Aqualab water activity meter was used (Decagon Devices, WA, USA).