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Original Research Article

# Effects of cooking on *in vitro* sinigrin bioaccessibility, total phenols, antioxidant and antimutagenic activity of cauliflower (*Brassica oleraceae* L. var. *Botrytis*)

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ABSTRACT

Cauliflower (*Brassica oleraceae* L. var. *Botrytis*) is a good source of bioactive compounds, such as glucosinolates, phenolic compounds and vitamins. In this study, the effects of some processes (*i.e.* boiling, steaming) on the sinigrin bioaccessibility as a major glucosinolate found in cruciferous vegetables after *in vitro* digestion, also *in vitro* antimutagenic activities, total phenols and total antioxidant capacities of cauliflower were determined. The sinigrin content was reduced by approximately 9.6% and 29.1% in steamed and boiled cauliflower ($p > 0.05$), respectively. After *in vitro* simulated digestion, sinigrin content was decreased by 26.4% in raw samples, increased by 29.5% and 114.7% in steamed and boiled samples, respectively. In all samples, mutagenic effect to *Salmonella typhimurium* TA 100 was not seen. When samples were steamed, phenol content was increased by 14.83%. After boiling total phenol content of cauliflower was decreased by 1.8%. Total antioxidant capacities (TAC) measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3 ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods were increased by 47% and 39%, respectively ($p < 0.05$) in steamed samples and decreased by 8% and 7% with boiling, respectively ($p > 0.05$). TAC in raw sample of cauliflower, which was investigated in phosphomolybdenum assays, was determined as 18.7 mg ascorbic acid equivalents (AAE)/100 g. In all cases, the highest antioxidant activity was determined in the steamed samples, while the lowest antioxidant activity was in boiled samples.



## 1. Introduction

*Brassica* (cruciferous) vegetables such as broccoli, Brussels sprouts, cauliflower, cabbage and kale are substantially consumed throughout the world. They are very important in human nutrition and health because they contain phytochemicals such as vitamins, minerals, phenolic compounds and especially glucosinolates (b-thioglucoside-N-hydroxysulfates) (Cartea and Velasco, 2008; Gratacós-Cubarsí et al., 2010). Polyphenols are a large group of secondary metabolites distributed in plant species. Phenolic compounds induce a variety of physiological functions including antioxidant activity, enzyme regulation and apoptosis and the cell cycle control, but the most important of these is their antioxidant activity. In many *in vitro* studies, phenolic compounds demonstrated much higher antioxidant activity than antioxidant vitamins and carotenoids. They are categorized into classes depending on their structure and subcategorized within each class according to the number and position of the hydroxyl group and the presence of other substituents. Hydroxycinnamic acid derivatives are widely distributed in plants and are important biologically active constituents of the human diet. Cauliflower is a good source of ferulic acid, chlorogenic acid, gallic acid, catechin and also hydroxycinnamic acids (Bahorun et al., 2004; Podsedek, 2007; Raja et al., 2011; Lee et al., 2011).

*Abbreviations:* DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6- sulfonic acid); TEAC, Trolox equivalents antioxidant capacity; GAE, gallic acid equivalents; AAE, ascorbic acid equivalents; TAC, total antioxidant capacity; TPC, total phenolic contents; DW, dry weight; FW, fresh weight; SPE, solid phase extraction; DEAE, diethylaminoethyl; ONPG, o-nitrophenyl-b-D-galactopyranoside; TMACL, tetramethylammonium chloride; IQ, 2-amino-3-methyl-3H-imidazo (4,5-F) quinoline; NADP, β-nicotinamide adenine dinucleotide phosphate hydrate; G-6-P, D-glucose 6-phosphate disodium salt hydrate.

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Glucosinolates are an important group of phytochemicals and also a unique group of sulfur-containing plant secondary metabolite compounds which contain organic sulfur. They can be divided into three main groups as aliphatic, aromatic aryl and aromatic indole glucosinolates depending on the chemical structure of their amino acid-derived side chain (Kaushik and Agnihotri, 1999; Gross et al., 2000; Holst and Williamson, 2004; Eylen et al., 2009; Vig et al., 2009; Gratacós-Cubarsí et al., 2010).

Sinigrin (2-propenyl glucosinolate), a major glucosinolate found in cruciferous vegetables, is used as a precursor of the anticancer compound allyl isothiocyanate. This phytoconstituent has unique properties and various types of pharmacological activities such as antimicrobial and anticancer (Cartea and Velasco, 2008). Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) catalyzes the hydrolysis (breakdown) of glucosinolates that are physically separated from the glucosinolates in plant cells. Upon plant tissue disruption during food processing (*e.g.* by cutting, chewing), glucosinolates presumably stored in the cell vacuole are released and hydrolysed by the enzyme myrosinase located in the cytoplasm (Volden et al., 2009a; Al-Gendy et al., 2010). The myrosinase-mediated hydrolysis of glucosinolates generates an unstable aglycone intermediate, thiohydroxamate-*O*-sulfonate, which is immediately converted to a wide range of bioactive metabolites, including isothiocyanates, thiocyanates, nitriles (Holst and Williamson, 2004; Schouten et al., 2009).

Various features of the hydrolysis environment may influence the extent of hydrolysis of glucosinolates by myrosinase and the structure and concentration of the metabolites formed. Allyl isothiocyanate is produced from sinigrin by plant myrosinase or microbial myrosinase in gut microflora (Cartea and Velasco, 2008). Myrosinase is inactivated during cooking processes, especially wet-thermal treatment such as boiling, steaming or baking, and thereafter cannot produce allyl isothiocyanate from sinigrin. Humans thus ingest mainly sinigrin and not allyl isothiocyanate from cruciferous vegetable dishes after these cooking processes (Patel et al., 2012). On the other hand, processing can result in reduction of constituents through leaching or thermal destruction. Intrinsic factors such as coexisting plant myrosinase and its cofactors ascorbic acid, epithiospecifier protein or $Fe^{2+}$, and extrinsic factors such as pH and temperature, have been shown to affect the hydrolysis of glucosinolates *in vitro* (Krul et al., 2002; Rungapamestry et al., 2007; Volden et al., 2009b).

Naturally occurring isothiocyanates and other hydrolysis products of glucosinolates have been found to inhibit the development of cancers of the lung, liver, stomach, small intestine, colon and mammary gland in a variety of animal models. This association is often attributed to glucosinolates that induce a variety of physiological functions including antioxidant activity, enzyme regulation and apoptosis and cell cycle control (Francisco et al., 2009; Shen et al., 2010). The cancer-protective effect of isothiocyanates in animals has been attributed to their inhibitory action on phase I enzymes responsible for bioactivation of carcinogens and their activity as inducers of phase II detoxification enzymes (Gross et al., 2000; Wang et al., 2002; Tian et al., 2005; Cartea and Velasco, 2008; Kusznierewicz et al., 2008a,b).

The aim of this study was to determine the effects of boiling and steaming on *in vitro* sinigrin bioaccessibility, total phenols and *in vitro* antioxidant capacity and antimutagenic activity in cauliflower.

## 2. Materials and methods

### 2.1. Chemicals

(−) Sinigrin standard (85440) (≥99%), ONPG Standard (N1127) (≥98%), sulfatase (S9626) (≥300 unit/mg solid β-glucuronidase), DEAE Sephadex A-25 (A25120), bile extract (B8631), pepsin (P700), pancreatin (P8096), DPPH (D9132), Trolox standard (238813) (97%), L-histidine dihydrochloride (53340) (≥99%), G-6-P (G7250) (≥98%), NADP (N5755) (≥95%), Folin and Ciocalteu's phenol reagent (F9252), S9 from Liver (S2067), Sodium azide (S8032), L-ascorbic acid (A5960) (≥99%) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Polypropylene SPE column (57024) and D-Biotin (47868) were obtained from Supelco (Buchs, Switzerland). TMACL (87720) (≥98%), Nutrient Broth No.2, (70123), ammonium molybdate tetrahydrate (09880) (≥99%), ABTS (11557) (≥98%) and gallic acid (48630) (≥98%) were purchased from Fluka Chemie AG (Buchs, Switzerland). Agar-agar ultra pure (101613) and NaCl (106404) were obtained from Merck (Darmstadt, Germany). IQ (A6165) was obtained from Toronto Research Chemicals Inc (North York, Ontario, Canada). All reagents were of analytical grade. *Salmonella typhimurium* TA100 tester strain was kindly provided from Hacettepe University, Biology Department.

### 2.2. Samples

Cauliflower (*Brassica oleraceae* L. var. *Botrytis*) samples were purchased at different times from three different hypermarkets located in Izmir city (Turkey). They were harvested in January (winter time) and stored at 4 °C in the hypermarket. They were free from insects and mechanical damage. The samples were transported to the laboratory within 30 min of purchasing. They were placed in polyethylene bags at 4 °C until cooking and extraction. A random representative selection of each of the cauliflower was made. All heads were carefully stripped of their outer leaves and divided into individual florets and randomly mixed. All experiments were performed in triplicate and parallel. Six values for each sample were averaged ($n = 6$).

### 2.3. Cooking procedure

Steaming was carried out using a steam cooker (Braun GmbH, Frankfurter Straße, Kronberg, Germany) and with a single layer of the fresh cauliflower (300 g) samples suspended above 400 mL of boiling water for 10 min. Boiling was carried out with 1 L of water for 10 min. The fresh cauliflower florets (300 g) samples were added to already boiling water (98 °C). Samples were immediately cooled and lyophilized with Hetosicc GF-765 (Heto, Birkerød, Denmark). The freeze-dried samples were ground to a fine powder using a mill and stored at −20 °C until analyses.

### 2.4. Moisture analysis

All calculations were made according to dry matter basis. For determination of the dry matter content, 5 g of raw, boiled and steamed samples were homogenized with IKA Ultra-turrax T25 homogenizer (KA®-Werke GmbH & Co. KG, Staufen, Germany) at 13,000 rpm for 1 min. Then, homogenized samples were dried in a vacuum oven at 70 °C for at least 2 days until reaching their constant weight (AOAC, 1995).

### 2.5. Sinigrin analysis

#### 2.5.1. Extraction of sinigrin

Extraction was performed according to Schouten et al. (2009) and Eylen et al. (2009).

*Preparation of sulfatase solution:* the sulfatase was purified by dissolving the sulfatase powder (70 mg) in deionized water (3 mL) and adding ethanol (3 mL). This solution was centrifuged with Thermo Scientific CL31R (Germany) at 8000 g for 10 min at room temperature and ethanol (9 mL) was added and centrifuged again

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