Food Chemistry 156 (2014) 289–295

Contents lists available at ScienceDirect

Food Chemistry

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

Vegetable and fermented vegetable juices containing germinated seeds and sprouts of lentil and cowpea

Sebnem Simsek, Sedef Nehir El, Aslı Kancabas Kilinc, Sibel Karakaya *

Department of Food Engineering, Faculty of Engineering, Ege University, 35100 İzmir, Turkey

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form 10 December 2013
Accepted 27 January 2014
Available online 5 February 2014

Keywords:
In vitro digestion
Antioxidant activity
ACE inhibitory activity
Bile acid binding activity
Antidiabetic activity
Hemagglutinating activity

ABSTRACT

Health-promoting effects of vegetable juice (VJ) and fermented vegetable juice (FVJ) were determined. Both juices displayed antioxidant activity against DPPH radical, and ORAC values obtained for both juices were not statistically different. The α-glucosidase inhibitory activities of the VJ and FVJ were not different. However, α-amylase inhibitory effect of the VJ (IC_{50}: 41 μM) was higher than that of FVJ (IC_{50}: 149 μM) ($p < 0.05$). *In vitro* bile acid-binding capacity of FVJ was about 4.30 times higher than that of VJ ($p < 0.05$). Although *in vitro* ACE inhibitory activity of VJ was below 50%, FVJ displayed ACE inhibition (80.2%) with an IC_{50} value of 50 μg protein/ml. Even though ACE inhibitory activities of digested and undigested FVJ were similar, there was a 42-fold decrease in the IC_{50} value of FVJ after small intestinal digestion ($p < 0.05$). FVJ, diluted by one half, displayed hemagglutinating activity whilst VJ did not display any hemagglutinating activity.

1. Introduction

Today, increased awareness of nutrition leads to more consumer demand for healthier foods. This demand and the results of studies related to the health effects of foods have driven the food industry to produce healthy foods in a great variety. In a report of WHO/FAO (2003) high plasma LDL cholesterol level, obesity, lack of fruit and vegetable consumption and lack of physical activity have been considered as the principal risk factors for morbidity and mortality from certain diseases, including cardiovascular diseases and certain types of cancer. Therefore, nutrition scientists have stressed the importance of a healthy and nutritious diet and physical activity for maintaining health and/or preventing certain diseases (WHO/FAO, 2003).

To overcome diet-related diseases, WHO/FAO (2003) recommended that consumption of saturated fats, trans fats and free sugars should not exceed 10%, 1% and 10% of daily energy requirement, respectively. In addition, 400 g (or above) of fruits and vegetable consumption a day and less than 5 g of salt (NaCl) consumption a day are highly recommended.

The fruit and vegetable intake recommendations have been attributed to their nutrients and bioactive compounds. Fruits and vegetables are excellent sources of antioxidant vitamins, namely vitamin C, vitamin A and vitamin E, and trace elements, namely selenium, copper, zinc, and dietary fibre, and bioactive compounds, such as phenolics, glucosinolates, choline, carotenoids, phytoestrogens, alpha-lipoic acid and lectins (Cortés-Giraldo, Giròn-Calle, Alaiz, Vioque, & Megìas, 2012; Holst & Williamson, 2004; Smith, Shenvi, Widlansky, Suh, & Hagen, 2004).

Health-promoting functional characteristics of vegetables are mainly attributable to their functional bioactive constituents. These effects can be listed as antioxidant activity, cholesterol-binding capacity, antidiabetic activity and angiotensin-converting enzyme (ACE) inhibitory activity. Studies conducted in cell culture models and animal models have revealed that bioactive compounds of plant foods, including soybean, hawthorn fruit, tea, buckwheat, oats, onion, garlic, almond, and vegetable juice, composed of tomato watercress, parsley, carrots, celery, lettuce, beets and spinach, have cholesterol-lowering activity (El-Shatanovi, Ashoush, Ahmed, & Ali, 2012; Vadivel, Nandety, & Biesalski, 2011). Bioactive peptides can be present in foods containing protein. These peptides are not active in the intact protein, but can be released after enzymatic hydrolysis and act as regulatory substances, i.e. hormone-like activities, in several metabolic pathways. The best examples of these kinds of foods are milk and dairy products, egg and egg products, meats and legumes (Korhonen & Pihlanto, 2006). The ACE inhibitory activities of heat-processed lentils, common dry beans and common pinto beans were determined. It was also shown that *in vitro* digestion caused an increase in ACE inhibitory activity (Akillioglu & Karakaya, 2009). In recent years, studies on foods rich in α-amylase and α-glucosidase inhibitors, the so-called "hypoglycemic foods" have been receiving more attention. Commercially available enzyme inhibitors (acarbose, miglitol and metformin), used for the

* Corresponding author. Tel.: +90 232 3113012; fax: +90 232 3427592.
E-mail address: sibel.karakaya@ege.edu.tr (S. Karakaya).

http://dx.doi.org/10.1016/j.foodchem.2014.01.095
0308-8146/

treatment of type II diabetes, have several side effects, such as abdominal distention, flatulence and possibly diarrhea. Accordingly the search for an alternative, natural source of α-amylase and α-glucosidase inhibitors without any side effects is of considerable importance (Vadivel et al., 2011). Studies on α-amylase and α-glucosidase inhibitory activities have been intensified, mostly on tea catechins and to a lesser extent on soy (Ademiluyi & Oboh, 2013; Koh, Wog, Loo, Kasapis, & Huang, 2010).

Cereals and legumes have been regarded as nutritious foods since they are rich in proteins. However, they cannot be eaten without being cooked. Germination is one of the biochemical reactions that cause enhancement of nutritional value and health effects of cereals and legumes. During germination, the enzymatic breakdown of proteins and complex carbohydrates takes place to supply energy and nutrient needs of the embrionic axis. In addition, smaller fragments with enhanced functional properties can occur. For instance, germination of soybean causes an increase in the concentration of soluble proteins and isoflavone aglycones, and a decrease in lectin concentration and lipoxygenase activity (Paucar-Menacho, Berhow, Mandarino, Chang, & de Mejia, 2010).

The main goal of the present study was to produce a functional food, in the frame of optimal nutrition, that is mainly based on optimising the daily supply of both nutrients and bioactive compounds that favour the maintenance of health. In the light of the above knowledge, the production of vegetable juice (VJ) and fermented vegetable juice (FVJ) containing vegetables, seeds and sprouts of germinated lentils and cowpeas was planned. To evaluate beneficial effects, antidiabetic activity, cholesterol-lowering effect, ACE inhibitory activity, hemagglutinating activity, and total antioxidant activity of VJ and FVJ were determined. *In vitro* digestion was applied to understand how simulated digestion affected these activities. Additionally, total sugars, protein, vitamin C, total phenols, total anthocyanins, total flavonoids, and some physicochemical characteristics, such as pH, water-soluble dry matter and ash, of the products were determined.

2. Materials and methods

2.1. Materials

2-Azobis (2-methylpropionamidine) dihydrochloride (AAPH) (440914), fluorescein sodium salt (FL 46960), monobasic sodium phosphate, dibasic sodium phosphate, trolox (6-hydroxy-2,5,7,8 tetramethychroman-2-carboxylic acid) (23.881-3), angiotensin-converting enzyme from rabbit lung (ACE) (A6778), N-hippuryl-His-Leu hydrate (H1635), 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) (D9132), Folin–Ciocalteu's phenol reagent (F9252), catechin (C1251), 4-nitrophenyl α-D-glucopyranoside (PNPG) (N1377), α-glucosidase, type I: from bakers yeast (AG) (G5003), deoxycholic acid (D2510), cholic acid (C1129), taurocholic acid sodium salt hydrate (T4009), glycocholic acid hydrate (G2878), pepsin from porcine gastric mucosa (P7000), concanavalin A from *Canavalia ensiformis* (L7647) and pancreatin from porcine pancreas (P7545) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pancreatic α-amylase (Pancreatin, 3U/mg) was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). Bile Acid Diagnostic Kits (No. 450-11 and 450-100) were purchased from Trinity Biotech plc, Bray Co. (Wicklow, Ireland). All other chemicals were of analytical grade. All vegetables, cowpea, lentil, pomegranate and orange, were purchased from local markets in Izmir, Turkey.

2.2. Methods

2.2.1. Germination

Lentils (*Lens culinaris*) and cowpeas (*Vigna sinensis*) were germinated according to Kavas and El (1992) with slight modifications. Briefly, seeds were soaked in 3 volumes of water at room temperature for 8 h and then they were allowed to germinate in a refrigerated incubator (FOC 225E, Velp Scientifica, Italy) at 30 °C for 5 days and 3 days, respectively. Duration of germination was determined according to the length of coleoptiles (approximately 7 cm). Sprouts and seeds were used freshly in the preparation of fermented vegetable juice.

2.2.2. Preparation of vegetable juices (VJ)

Five different vegetable juices were produced, using tomato (*Lycopersicon esculantum*), carrot (*Daucus carota sativus*), beetroot (*Beta vulgaris*), celery (*Apium graveolens rapaceum*), fresh red pepper (*Capsicum annuum*), parsley (*Petroselinum crispum*), lettuce (Lactuca sativa), mushroom (*Agaricus bisporus*), lemon (*Citrus lemon*), and black cabbage (*Brassica oleracea* Acephala Group). First, vegetables were squeezed individually and then different amounts of the juices were combined to produce 5 different vegetable juices. Three of them (Nos.1, 4 and 5) were produced using all vegetables in different amounts. In addition to all vegetables, No.2 contained stem of celery as distinct from Nos.1, 4 and 5. Equal amounts of pomegranate and orange juices (3%) were added to No.3 in order to improve the taste of the vegetable juice. Then they were subjected to sensory analysis to determine the most preferred combination.

2.2.3. Fermented vegetable juice (FVJ) preparation

The composition of vegetables in FVJ was the same as the most preferred VJ. To obtain FVJ, freshly blended germinated seed and sprouts of lentils and cowpeas were added to the most preferred VJ and homogenised. Afterwards, VJ was subjected to pasteurisation at 90 °C for 10 min and cooled to 35 °C. *Lactobacillus plantarum* (VISBYVAC Serie 1000 Product Nr 40022951, Germany) was used as a starter culture to produce FVJ ($3–3.5 \times 10^8$ cfu microorganisms/300 ml of vegetable juice) after being sub-cultured in MRS broth (Oxoid). At the end of the fermentation period (19 h at 35 °C), FVJ was subjected to pasteurisation at 90 °C for 5 min and cooled to 25 °C. Samples were divided into small aliquots and stored at −20 °C prior to being analysed.

2.2.4. Sensory evaluation

Five coded VJ samples were given to ten panellists, and they were asked to rank the samples based on their preferences for taste and appearance. After ranking, germinated seed and sprouts of lentils and cowpeas were added in different amounts to the most preferred VJ and panellists again ranked them. The amounts of germinated seed and sprouts of lentils were 1%, 1.5% and 2.0% w/v. These amounts were 1.5%, 3% and 5% for germinated seed and sprouts of cowpea. The sample score sheet consisted of 5 scores (from 1:dislike to 5:like).

2.2.5. Proximate composition

Water-soluble dry matter of vegetable juices was determined by using a refractometer (Bellingham + Stanley Ltd RFM 330 Refractometer, England). Protein, total sugars and vitamin C contents of vegetable juices were determined by the Bradford procedure (Bradford, 1976), the Lane–Eynon method and titrimetric method (AOAC, 2000), respectively.

2.2.6. Total phenols (TP)

Total phenols of the samples were determined, using the Folin–Ciocalteau method (Xu & Chang, 2007). Results were expressed as mg (+)-catechin equivalents (CE) in 100 ml of sample.

2.2.7. Total flavonoids (TF)

A spectrophotometric method, proposed by Heimler, Vignolini, Dini, and Romani (2005), was used to determine TF of vegetable