Vegetable product containing caseinomacropeptide and germinated seed and sprouts

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Abstract In this study vegetable product containing germinated seed and sprouts of lentils and cowpeas, and caseinomacropeptide isolated from whey is produced. Three different forms of vegetable product namely puree (VP), freeze-dried (FD) and drum-dried (DD) are produced. Freeze-dried and DD forms are produced to diversify forms of utilization and to improve functionality such as increased shelf life and decreased storage space. Their beneficial effects on health are determined using *in vitro* methods. All forms displayed antioxidant activities against DPPH radical and oxygen radical, α -amylase inhibitory activities, bile acid binding capacities, and angiotension converting enzyme (ACE) inhibitory activities. Freeze-dried product exhibited the strongest inhibition on α -amylase and ACE with the IC_{50} value 0.09 μ M total phenolic and 0.82 mg protein/g sample, respectively when evaluated on the basis of serving size.

Keywords Caseinomacropeptide · Germination · ACE inhibitory activity · *in vitro* antidiabetic activity · *in vitro* bile acid binding activity

Introduction

Due to rapid changes in nutrition concept in recent years, non-nutrient substances having beneficial effects beyond basic provision of energy and essential nutrients have received ample interest from academic and research institutes, from

governmental authorities as well as from the food industry. Functional foods that are being developed by all major food companies have been anticipated that can contribute to an overall better state of health for the people (Verhagen et al., 2004). According to the World Health Organization and the Food and Agriculture Organization, dietary habits and lifestyle constitute major modifiable risk factors in relation to the development of chronic diseases including cancer, cardiovascular diseases, type 2 diabetes, obesity, osteoporosis and periodontal disease (WHO/FAO 2003). Especially, fruit and vegetable consumption has been strongly associated with a reduced risk of chronic diseases. Health-promoting functional characteristics of vegetables are mainly due 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 (Kahlon et al. 2008; Vadivel et al. 2011; Ademiluyi and Oboh 2013).

Epidemiological and clinical studies have indicated that legumes have health benefits including lipid homeostasis control, hypocholesterolemic effect, glycemic control, anticarcinogenic effects (protease inhibitors and lectins), therapeutic effects (α -amylase and protein inhibitors on obesity and diabetes) and antioxidant activities (Duranti 2006; Akillioglu and Karakaya, 2010). 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 in order to supply energy and nutrient needs of the embryonic 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 (Pauca-Menacho et al. 2010).

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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 (Akillioglu and Karakaya, 2010). One of the bioactive peptides liberated from κ -casein during cheese manufacturing is caseinomacropeptide (CMP) or glycomacropeptide (GMP). Next to β -lactoglobulin and α -lactalbumin, CMP is the most abundant protein/peptide in whey proteins. Recently, extensive research has highlighted the beneficial effects of CMP including interactions with toxins, viruses, and bacteria, modulation of immune system responses, promotion of bifido-bacterial growth, suppression of gastric secretions and regulation of blood circulation (Thomä-Worringer et al. 2006). CMP has been reported to stimulate the release of cholecystokinin, the satiety hormone involved in controlling food intake and digestion in the duodenum of animals and humans. Therefore CMP is considered a potential ingredient for functional food concepts (Tolkack and Kulozik 2005).

The main goals of this study are to produce vegetable product in the forms of purée, freeze-dried and drum-dried as a functional food containing germinated seed and sprouts of lentil and cowpea, and CMP, and to determine its beneficial effects including antioxidant activity, in vitro antidiabetic activity, in vitro ACE-inhibitory activity and in vitro bile acid binding capacity besides its proximate composition, and total phenols, total flavonoids and total anthocyanins.

Materials and methods

Materials

2,2'-Aziobis (2-methylpropionamidine) dihydrochloride (AAPH), fluorescein sodium salt, trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carbonsaure), angiotensin converting enzyme from rabbit lung (ACE), N-Hippuryl-His-Leu hydrate, 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), Folin & Ciocalteu's phenol reagent, (+) – catechin hydrate, 4-Nitrophenyl α -D-glucopyranoside (PNPG), mammalian α -glucosidase (intestinal acetone powders from rat), deoxycholic acid, cholic acid, taurocholic acid sodium salt hydrate, glycocholic acid hydrate, pepsin from porcine gastric mucosa, and pancreatin from porcine pancreas were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bile Acid Diagnostic Kits were purchased from Trinity Biotech plc, Bray Co. (Wicklow, Ireland). All other chemicals were of analytical grade. All vegetables, cowpea and lentil were purchased from local markets in İzmir, Turkey. Whey was kindly supplied by Yasar Holding Food and Beverage Group, İzmir Turkey.

Germination

Green lentils (*Lens culinaris* M.) and cowpeas (*Vigna sinensis* L.) were steeped in tap water containing 0.5 % hypochlorite (v/v) for 2 min. After soaking (room temperature for 8 h), germination was performed in a refrigerated incubator (FOC 225E, Velp Scientifica Italy) at 30 °C for 5 days and 3 days, respectively. Sprouts and seeds were used freshly in the preparation of vegetable mix.

Isolation of caseinomacropeptide (CMP) from whey

Caseinomacropeptide was isolated from whey with a modified method described by Martín-Diana et al. (2002). After pH adjustment (5.2), rennet sweet whey was heated at 90–95 °C for 1 h. Then mixture was centrifuged (10,000 X g and 4 °C for 20 min) to remove denaturated proteins. Supernatant was concentrated by ultrafiltration (Sartocon Slice Ultrafiltration System, Sartorius Stedim Biotech GmbH, Germany) with MWCO 10 kDa at 50 °C. Then diafiltration was applied to eliminate the excess of lactose and minerals. The retentate containing CMP was then concentrated, lyophilized (–45 °C, 0.045 bar-Labconco 2.5 FreeZone, USA) and stored at –20 °C.

Preparation of the product

Vegetable product was prepared by combining pureed vegetables including tomatoes (*Lycopersicon esculentum*), sweet red pepper (*Capsicum annuum*), purple carrot (*Daucus carota*), red cabbage (*Brassica oleracea* var. *Capitata rubra*), carrot (*Daucus carota*), beetroot (*Beta vulgaris conditiva* Alef.), lemon (*Citrus lemon*), and germinated seed and sprouts of lentils (*Lens culinaris* L.), and cow pea (*Vigna unguiculata* (L.) Walp.) and CMP. Briefly all vegetables were chopped separately after washing with tap water. Germinated seed and sprouts were added to the vegetables and the vegetable mix was blended. After addition of CMP, the vegetable mix was filled into the glass jars and sterilized at 121 °C for 15 min. The amounts of the ingredients in the formulation were determined by sensory analysis. Three vegetable products containing different amounts of vegetables, seed and sprouts and CMP were given to ten panelists and they were asked to rank the samples based on their preferences for taste, appearance and overall quality. The sample score sheet consisted of 5 scores (from 1: dislike to 5: like).

The most preferred formulation filled in the glass jar in the form of puree and sterilized at 121 °C for 15 min (VP) or freeze-dried, (FD) or drum-dried (DD). Vegetable puree without CMP and, seed and sprouts of germinated lentils and cowpeas was prepared as control (CP) and sterilized at 121 °C for 15 min.

Proximate composition

Moisture contents of the samples were determined gravimetrically in vacuum oven. Total sugars, protein (factor of 6.25 was used for converting the total nitrogen into total protein) and vitamin C contents of the samples were determined by Lane-Eynon, Kjeldahl and titrimetric methods, respectively according to AOAC (2000).

Total phenols (TP)

The extraction of phenolic compounds was carried out according to Vinson et al. (1995). Total phenols of the samples were determined using Folin-Ciocalteu's method (Vinson et al. 1995). Absorbance readings were taken at 765 nm. TP of the samples were expressed as g (+)-catechin equivalents (CE) in 100 g sample.

Total flavonoids (TF)

Flavonoids were extracted by cold extraction procedure (Diamanti 2010). A method proposed by Heimler et al. (2005) was used to determine TF of the samples. Absorbance was read at 510 nm against reagent blank. TF of the samples were expressed as g (+)-catechin equivalents (CE) in 100 g sample.

The total non-flavonoid phenolics (n-FP) content of the samples was calculated by subtracting TF from TP.

Total anthocyanins (AC)

Total monomeric anthocyanins of the samples were determined by the pH differential method (Giusti et al. 1999). Absorbance readings were taken at 520 nm and 700 nm, respectively. Absorbance value was corrected according to Eq. (1). Then AC content of the samples was calculated by using Eq. (2).

$$A = (A_{520} - A_{700})_{\text{pH}1.0} - (A_{520} - A_{700})_{\text{pH}4.5} \quad (1)$$

Monomeric anthocyanins (mg cyanidine-3 glycoside/kg)

$$= \frac{(A)(MW)(D_f)1000}{(\epsilon)l} \quad (2)$$

Where; A: Corrected absorbance value, MW: Molecular weight of cyanidine-3- glycoside (449.2), D_f : dilution factor, ϵ : Molar extinction (26,900), l : (1-cm-path-length).

Antioxidant activity (AA)

The free radical scavenging activity was tested using DPPH radical according to Brand-Williams et al. (1995). This method is based on electron transfer mechanism. Oxygen

radical absorbance capacity (ORAC) method based on hydrogen atom transfer was applied as a second assay according to the method proposed by Ou et al. (2001). Antioxidant activity (AA) results were given as Trolox equivalents (mM TEAC/100 g sample).

In vitro antidiabetic activity

Antidiabetic activities of the samples were determined by measuring expected glycemic index and inhibitory effects of the samples on α -glucosidase and α -amylase enzymes in vitro.

Expected glycemic index (eGI)

Expected glycemic index was determined by measuring in vitro starch digestibility of the samples (Englyst et al. 2000). The rate of starch digestion was expressed as the percentage of total starch hydrolyzed at different times (20, 60, 90, 120, and 180 min). Hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the samples by the area obtained for white bread. The eGI was calculated using the Eq. (3) described by Goni et al. (1997).

$$\text{eGI} = 39.71 + 0.549(\text{HI}) \quad (3)$$

α -glucosidase inhibition Assay

α -glucosidase (AG) inhibition assay was conducted according to Koh et al. (2010) with slight modifications. Briefly, 340 μL of the sample, at different concentrations, were incubated with 20 μL of AG solution (25 mg/mL). Then, 40 μL of PNPG solution (30 mM) was added to initiate the digestion. After 15 min, p-nitrophenol and 10 μL of EDTA (0.1 mM) were added to prevent interaction between iron, which vegetable juice could possibly contain. For reaction termination 1 mM Na_2CO_3 (190 μL) was added. Absorbance at 400 nm was measured by using a Microplate reader (Thermo Scientific Varioskan Flash, Finland). Percentage of AG inhibition was calculated with Eq. (4). The concentration of the sample required to produce a 50 % inhibition of the initial rate of reaction was calculated by interpolation of percentage inhibitions against different sample concentrations.

$$\text{Inhibition}(\%) = 100 \times \frac{[(A_{\text{control}} - A_{\text{control blank}}) - (A_{\text{sample}} - A_{\text{sample blank}})]}{(A_{\text{control}} - A_{\text{control blank}})} \quad (4)$$

Where; A_{control} , $A_{\text{control blank}}$, A_{sample} , $A_{\text{sample blank}}$ refer to absorbance reading of reaction mixture containing active enzyme and buffer, inactive enzyme and buffer, active enzyme and sample and inactive enzyme and sample, respectively. Substrate was present in all mixtures.

α-amylase inhibition assay

α-amylase inhibition assay was conducted according to Koh et al. (2010) and Yang et al. (2012). 820 μL of the sample, at different concentrations, were incubated with 100 μL α-amylase. Then 80 μL of the potato starch solution in PBS (1 %) were added, and the whole incubated at 37 °C. Reaction was terminated with 500 μL of HCl (10 %). Retained starch in the medium was determined by 150 μL of iodine solution (0.0025 M I₂/0.0065 M KI). Absorbance was measured at 620 nm by using a Microplate reader (Thermo Scientific Varioskan Flash, Finland). Percentage of HSA inhibition was calculated by using Eq. (4). IC₅₀ of the sample was determined by interpolation from the curve.

***In vitro* angiotensin-converting enzyme (ACE) inhibitory activity**

ACE-inhibitory activities of the samples were determined by the method proposed by Oboh et al. (2012) with minor modifications. Briefly, 100 μL ACE (25 U/mL) solution and 40 μL sample in different concentrations were incubated at 37 °C for 15 min. The enzymatic reaction was initiated by adding the substrate Hip-His-Leu (100 μL of 8.33 mM) and 0.3 M NaCl in 50 mM sodium borate buffer (pH 8.3) to the mixture. Mixture incubated for 15 min at 37 °C, and then 150 μL 1 M HCl was added. Following 1000 μL ethyl acetate addition, tubes were centrifugated (1500 X g for 15 min). Then 750 μL of ethyl acetate layer was transferred into a clean test tube and evaporated. The residue was redissolved in distilled water and its absorbance was measured at 228 nm. The control included all other reagents and the enzyme with the exception of the test sample. The ACE-inhibitory activities were expressed as percentage inhibition by using Eq. (5). In addition IC₅₀ values of the samples were calculated.

$$\text{Inhibition(\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (5)$$

Where; A_{control} : Absorbance value recorded for control, A_{sample} : Absorbance value recorded for sample.

***In vitro* bile acid binding capacity**

The *in vitro* bile acid binding procedure was performed as described by Kahlon et al. (2008) with slight modifications. After adjustment of pH to 2.0, reaction mixture was incubated for 1 h in a 37 °C shaker water bath. Then pH was adjusted to 6.3 with 0.1 M NaOH. Bile acid mixture working solution (4 mL, 0.72 μM) was added to each test sample, whereas only PBS (4 mL, 0.1 M, pH 6.3) was added to the individual substrate blanks. After the addition of 5 mL of porcine pancreatin, tubes were incubated for 1 h in a 37 °C shaker bath. Mixtures were centrifuged at

9000 X g for 10 min. Cellulose and cholestyramine were used as the negative and positive controls, respectively. Bile acids were analyzed using Trinity Biotech bile acids procedure No. 450 (Trinity Biotech Distribution, St. Louis, MO) using Microplate reader (Thermo Scientific Varioskan Flash, Finland). Values were determined from a standard curve obtained by analyzing Trinity Biotech bile acid calibrators (No. 450–11) at 5, 25, 50, 100 and 200 μM.

Statistical analysis

All analyses and the vegetable product preparations were performed in triplicate. Differences between the variables were tested by analysis of variance (One-Way ANOVA). Duncan and LSD tests were applied to determine significant differences ($P < 0.05$) using SPSS for Windows (Version 15.0, SPSS Inc., Chicago, IL, USA). The results of sensory evaluation were analyzed and interpreted using Friedman Analysis, a simple ranking test by using Kramer's tables.

Results and discussion

Isolation of CMP from whey

Whey has been considered a novel raw material for many purposes recently. Next to the whey proteins, the CMP present in sweet whey is a valuable constituent for both its functional properties as well as its specific nutritive value (Tolkack and Kulozik 2005). The protein fractions of bovine sweet whey obtained from kashkaval cheese production as waste and CMP isolate were shown in Fig. 1. CMP peaks (GMP and nGMP) were compared with the RP-HPLC chromatogram of CMP (Davisco Food International), which is used as food supplement. Since the main goal was to isolate CMP from sweet cheese whey, the other whey proteins, proteose peptone (PP), α-lactalbumin (α-lac), bovine serum albumin (BSA), β-lactoglobulin (β-Ig), immunoglobulin (IgG) were identified by comparing retention times reported in the previous studies and in the study of Elgar et al. (2000).

Sensory evaluation

The most preferred formulation was the product containing 25 % tomato, 12.5 % sweet red pepper, 12.5 % purple carrot, 10 % red cabbage, 10 % carrot, 12.5 % beetroot, 5.5 % lemon juice, 12 % germinated seeds and sprouts and 2.5 % CMP ($p < 0.05$).

Proximate analyses and content of TP, TF, n-FP and AC

Total sugar, protein, TP, TF, n-FP and AC of CP, VP, FD and DD were shown in Table 1. There were significant differences

Fig. 1 **a** Protein profile of whey; **b** Protein profile of CMP isolate obtained from whey (GMP: Glucomacropeptit, nGMP: non-glycosylated GMP, CMP: GMP + nGMP)

in total sugar contents of dried samples (FD, DD) and non-dried samples (CP, VP) ($p < 0.05$). The higher protein content of VP than that of CP can be explained by its CMP content. Approximately n-FP consisted of 56.7 %, 78.2 %, 78.8 % and 91.8 % of TP in CP, VP, FD and DD respectively. The difference between CP and VP can be expected due to germinated seed and sprouts content of VP. Troszyńska et al. (2011) reported that germination of lentil caused significant decrease in proanthocyanins and catechin glycosides.

As can be seen from Table 1, DD contains the highest amount of TP, although there are no significant difference between the moisture contents of FD and DD. As is known Folin-Ciocalteu method is an electron transfer based assay and measures reducing capacity of the samples. Therefore, the reagent can react with any reducing substances such as

Maillard reaction products (Prior et al. 2005). Maillard reaction can easily take place due to samples are exposed severe heat conditions during drum-drying process. The percentages of TF and AC in TP of VP were 21 % and 3.70 % respectively. These percentages were 19.0 % and 14.8 % for FD, and 7.21 % and 4.14 % for DD, respectively. These values indicate the changes in phenolic profile during drying processes.

Antioxidant activity, *in vitro* antidiabetic, bile acid binding and ACE-inhibitory activities

Antioxidant activities of CP and VP against DPPH radical were lower than those of against oxygen radical (Table 2). Contrary to, DPPH radical scavenging activities of FD and DD were higher than those of ORAC value. Different

Table 1 Moisture, total sugars, protein, TF, TP, n-FP and AC contents of vegetable products¹

	CP	VP	FD	DD
Total sugars (%)	5.6 ± 0.7 ^a	5.7 ± 0.3 ^a	32.6 ± 1.0 ^b	28.1 ± 1.4 ^c
Protein (%)	1.17 ± 0.05 ^a	4.03 ± 0.58 ^b	10.5 ± 0.25 ^c	16.1 ± 0.07 ^d
TF (mg CE/100 g)	47 ± 3 ^a	31 ± 3 ^b	123 ± 13 ^c	87 ± 6 ^d
TP (mg CE/100 g)	108 ± 16 ^a	142 ± 21 ^a	581 ± 38 ^b	1090 ± 12 ^c
n-FP (mg CE/100 g)	61 ± 16 ^a	111 ± 22 ^b	458 ± 28 ^c	1001 ± 17 ^d
AC (mg cyanidine-3 glycoside/100 g)	7.44 ± 2.4 ^a	5.25 ± 2.6 ^a	96 ± 12 ^b	50 ± 13 ^c

CP, control puree; VP, vegetable puree; FD, freeze-dried puree; DD, drum-dried puree; TF, total flavonoids; TP, total phenols; n-FP, total non-flavonoids phenolics; AC, total anthocyanins

^{a-d} Different letters in the same rows indicate statistically significance ($p < 0.05$)

¹ Values were given as mean ± SD ($n = 6$)

behaviors on the antioxidant activities against different oxidation mechanisms obtained for non-dried and dried samples can be explained by the changes in phenolic profile during drying processes. In addition, Maillard reaction products (MRP) can have antioxidant activities (Yu et al. 2013). Therefore, MRP that is supposed to be produced during drying process can enhance the antioxidant activity of dried vegetable products. It should be noted that evaporation of water during drying process is another factor for enhanced antioxidant activity. Antioxidant activities of VP determined by both methods were lower than those of CP ($p < 0.05$). This decrease was 18 % for DPPH and 22 % for ORAC. This indicates that addition of germinated seed and sprouts and CMP has no profounding effect on antioxidant activity.

The glycemic index (GI) is a scale that has been introduced to enable comparison of carbohydrate-rich foods based on their glycemic response. Foods are classified according to the reference material, glucose (GI = 100) as: low glycemic index foods ($GI \leq 55$), medium glycemic index foods ($56 < GI < 69$), and high glycemic index foods ($GI \geq 70$). Low GI foods produce a more gradual rise in blood glucose and insulin levels, and is therefore associated with reduced incidence of insulin resistance and type 2 diabetes (Schakel et al. 2008).

Expected glycemic indexes of FD and DD are 34.1 ± 1.49 , and 50.0 ± 6.82 , respectively. Glycemic index of VP is not determined by *in vitro* method because it seemed not to be proper approach due to its low starch (1.57 ± 0.69 %) content. Glycemic index of VP should be determined by *in vivo* method. According to above classification FD and DD are belonging to low GI foods.

The presence of α -glucosidase and α -amylase inhibitors in the diet is recognized as a proper approach in the retardation of digestion of dietary carbohydrates and accordingly, controlling postprandial hyperglycemia. Pancreatic α -amylase is involved in the breakdown of

starch into disaccharides and oligosaccharides before intestinal α -glucosidase catalyzes the breakdown of disaccharides to liberate glucose, which is later absorbed into the blood circulation. Inhibition of these enzymes would slow down the breakdown of starch in the gastrointestinal tract, thus reducing postprandial hyperglycemia (Kwon et al. 2007). Inhibition results were expressed as IC_{50} , and acarbose, which is commonly used oral hypoglycemic agent in diabetic patients, was used as a positive control.

IC_{50} values for the α -amylase inhibitory activities were determined on the basis of total phenol concentrations of the samples (Table 3). There were no significant differences between α -amylase inhibitory activities of CP and VP. This result showed that CMP addition and germination seemed not having any effect on the α -amylase inhibitory activity of the VP. Freeze-dried product displayed the highest α -amylase inhibitory activity among the samples analyzed when evaluated on the basis of serving size ($p < 0.05$). We could not calculate IC_{50} values for the α -glucosidase inhibitory activities of all the samples due to no dose-response relationship (linear, logarithmic, binomial etc.) was found between the concentration of total phenols of the samples and inhibition percentages of α -glucosidase. Control product displayed 63.7 % α -glucosidase inhibition at the concentration of 308 μ M TP and VP displayed 47.7 % α -glucosidase inhibition at the concentration of 469 μ M TP. This inhibition percentage obtained for DD was 57.0 % at the concentration of 587 μ M TP. Freeze-dried product inhibited α -glucosidase activity by 51.2 % at the concentration of 496 μ M TP. All of the products are relatively strong α -amylase inhibitors when compared to their α -glucosidase inhibitory activities. Among them the strongest α -amylase inhibitor is FD with the IC_{50} value 0.09 μ M TP. Gonçalves et al. (2011) reported that increasing procyanidin concentration caused increased α -amylase inhibition. The degree of polymerization of the procyanidin fractions was the determinant in increased inhibition. Previous research reported that phenolic-linked ingredients of different eggplant varieties have the potential for the α -glucosidase inhibition indicating the potential to reduce glucose absorption in the intestine (Kwon et al. 2008). It was reported that an effective strategy in the management of type 2 diabetes is the strong inhibition of intestinal α -glucosidases and mild inhibition of pancreatic α -amylase. Excessive inhibition of α -amylase could result in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Obboh et al. 2012). Ademiluyi and Obboh (2013) reported that phenolic rich extracts of soy inhibited α -amylase and α -glucosidase activities in a dose dependent manner, and bound phenolic extract exhibited significantly higher α -amylase activity (IC_{50} : 526.32 μ g/mL) than that of the free phenolic extract. They indicated that the

Table 2 Antioxidant activities of CP, VP, FD and DD

Samples	Antioxidant activity (mM TEAC/100 g sample)	
	DPPH	ORAC
CP	21 ± 1^a	65 ± 7^a
VP	17 ± 5^a	50 ± 8^a
FD	1157 ± 21^b	501 ± 29^b
DD	964 ± 156^c	555 ± 46^b

Values were given as mean $\pm$ SD ($n = 6$)

CP, control puree; VP, vegetable puree; FD, freeze-dried puree; DD, drum-dried puree

^{a-b} Different letters in the same column indicate statistically significance ($p < 0.05$)

Table 3 *In vitro* antidiabetic and bile acid binding activities of CP, VP, FD, and DD

Samples	Antidiabetic activity		Bile acid binding activity
	α -glucosidase inhibition IC ₅₀ (μ M TP)	α -amylase inhibition IC ₅₀ (μ M TP)	Bile acid binding relative to cholestyramine (%)
CP	392 $\pm$ 131 ^a	0.24 $\pm$ 0.04 ^a	17.6 $\pm$ 2.23 ^a
VP	515 $\pm$ 59 ^a	0.25 $\pm$ 0.09 ^a	14.2 $\pm$ 1.24 ^a
FD	-	0.09 $\pm$ 0.07 ^b	68.1 $\pm$ 5.78 ^b
DD	-	0.31 $\pm$ 0.03 ^a	36.4 $\pm$ 2.07 ^c

Values were given as mean $\pm$ SD ($n = 6$)

CP, control puree; VP, vegetable puree; FD, freeze-dried puree; DD, drum-dried puree

^{a-c} Different letters in the same column indicate statistical significance ($p < 0.05$)

(-) can not be calculated

activities of α -amylase and α -glucosidase inhibitors in fruits and vegetables were relatively lower than those of synthetic ones.

Relative to cholestyramine, bile acid binding capacities of CP and VP are 17.61 % and 14.23 % respectively (Table 3). These capacities are relatively higher than the relative bile acid binding capacities of steam cooked kale (13.1 %), broccoli (8.1 %), spinach (5.2 %), cabbage (4.1 %), green bell pepper (4.6 %) and Brussels sprouts (10.4 %) reported by Kahlon et al. (2008). Bile acid binding capacities of foods have been associated to a large extent with dietary fiber they contain (Kahlon et al. 2008). It is clear that vegetable products contain dietary fiber, and adding CMP to vegetable mix (CP) seems not cause any effect on bile acid binding capacity in a positive manner. The binding values of FD and DD were significantly different ($p < 0.05$). During freeze-drying and drum-drying processes VP concentrated approximately 5.95 times. However, bile acid binding capacities of FD and DD increased 4.79 and 2.56 times, respectively. Lower bile acid binding capacity of DD can be due to the heat that may cause changes in the amount and composition of dietary fiber (Borchani et al. 2011). Although reduced fat absorption and cholesterol reduction can have a beneficial effect on human health, it is important whether the degree of bile acid binding is high enough to impair lipophilic micronutrients' absorption. The combination of bile salts and pancreatic enzymes is essential for the efficient micellarization of lipophilic compounds. Bile salt concentration in the small intestine ranged from 4 to 20 mM, depending on whether the subject was in the fasted or fed state. Bile acid binding of the vegetable products relative to cholestyramine changed from 14 % to 68 % in this study. In the case of 20 mM bile salt concentration in the small intestine FD could bind 13.6 mM bile salt. Even in this condition, 6.4 mM bile salt would still be available for micellarization. It should also be noted that FD and DD are very concentrated products, and consequently their consumption as a portion of

100 g cannot be a realistic approach. Therefore, consumption of vegetable products cannot cause any interference on digestion and/or absorption of lipophilic compounds.

Angiotensin converting enzyme inhibitory activities based on IC₅₀ values of the samples were given on the basis of mg protein/g sample in the Table 3. Accordingly, if these values were calculated on the amount of vegetable products needed to provide 50 % ACE inhibition, the results were found as about 48 mg, 8 mg, and 6.5 mg for VP, FD, and DD, respectively. Park and Jhon (2010) reported that methanol extracts of two different kinds of bamboo shoot displayed ACE-inhibitory activity with the IC₅₀ value 3.5 and 6.0 mg/mL. While an abundance of ACE-inhibitory activity has been associated with dietary peptides, the rennin-angiotensin regulatory activity of dietary plants is less well understood since they are not a good source of protein. Recent report underlined the significant correlation between ACE-inhibitory activity and polyphenols that dietary plants contain (Patten et al., 2012). Further work is needed to reveal relationship between ACE-inhibitory activity and polyphenols of vegetable mixes.

Conclusion

In conclusion, this study revealed that all forms of vegetable products had potential beneficial effects on health due to their bile acid binding capacity, ACE-inhibitory, α -amylase and α -glucosidase inhibitory, and antioxidant activities. Therefore they can be recommended to people who are suffering from chronic diseases such as cardiovascular diseases, type II diabetes and obesity. Besides they can contribute to optimal nutrition pattern of healthy people.

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