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ORIGINAL ARTICLE

Efficiency of selected food ingredients on protein efficiency ratio, glycemic index and in vitro digestive properties

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Abstract The human body on exposure to high-altitude, undergoes many physiological challenges. The cardiopulmonary reserves are favoured against the digestive system. Hence, the efficiency of digestion is compromised to a great extent, which leads to anorexia, hypophagia, epigastralgia, dyspepsia, nausea, and peptic ulcers. The present study was focused on in vitro digestive influence of selected food ingredients viz. cardamom, carom, cumin, coriander, fennel, fenugreek, ginger, pepper, star anise, turmeric, papaya, orange, pineapple, liquorice, valerian, and tarragon on the activities of digestive enzymes of rat pancreas, duodenum, and small intestine. In-vitro antioxidant activities of the above food ingredients were also carried out with respect to their radical scavenging activity against $DPPH^{\cdot}$, $NO^{\cdot}$, and ferrous reducing antioxidant power. All the studied food ingredients showed a comparative range of free radical scavenging activity. Further, pineapple has shown enhanced enzymatic activity of pancreatic amylase, trypsin and chymotrypsin among the tested samples with 432, 252, and 86%, respectively. However, all food ingredients showed inhibitory effect towards maltase activity, while the sucrose activity was enhanced in tarragon compared to control. Almost all the selected food ingredients have been observed to have low glycemic index and low protein efficiency ratio except pineapple. The results suggested that ample merit in the use of pineapple extract can be carried forward for the formulation of highly digestible foods for extreme environmental conditions.

Keywords Digestive enzymes · Protein efficiency ratio · Glycemic index · Antioxidant activity · Spices · Herbs

Introduction

People travelling to extreme environmental conditions like high altitude, for example, the Trans-Himalayan regions exposes the human body to a variety of stresses; the most prominent hypoxia with the increase in altitude due to the reduced partial pressure of oxygen (Chawla and Saxena 2014). The diets of humans differ in quantity and composition in different climatic conditions. Appetite suppression may persist even after the symptoms of acute mountain sickness have disappeared, or at an altitude where acclimatisation is incomplete. Symptoms related to digestive system disorders such as anorexia, epigastric discomfort, epigastralgia, heartburn, dyspepsia, nausea, vomiting, diarrhoea, hematemesis, piles and peptic ulcers are frequently found in mountaineers and altitude sojourners (Wu et al. 2007).

Food ingredients have a significant role in human life as they serve us with valuable components such as beverages, and medicines. Some of the Indian herbs, fruits and spices are being claimed to aid digestion, flavouring agents and food preservatives since ages (Shan et al. 2007). Spices and herbs are believed to aid in digestion by intensifying salivary flow and gastric juice secretion. There are reports which state that certain spices or their active principles stimulate bile flow and increase the secretion of bile acids, which have an important role in digestion and absorption of dietary fat (Platel and Srinivasan 2000a, b). It has been

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shown that several common spices have a stimulatory influence on the activities of pancreatic lipase, amylase, and proteases (Prakash and Srinivasan 2012). Some medicinal herbs contain phytochemicals characterized by bitter and pungent properties exert their digestive action through endocrine and paracrine release (Valussi 2012). Few fruits such as pineapple, papaya, fig, and kiwi contain proteolytic enzymes such as bromelain, papain, ficin, and actinidin, respectively have been reported with digestive property (Nam et al. 2016). Protease enzymes are known to break down or change the composition of proteins/peptides and helps in digestion process. Moreover, these proteases are clinically proved to have a role in strengthening the immune system of the intestine by its anti-inflammatory property (Ketnawa et al. 2012).

The antioxidant activities of the food ingredients are due to the presence of phenolic compounds, which scavenge reactive oxygen species and reduce the process of lipid peroxidation and protein oxidation (Shobana and Naidu 2000). The glycemic index (GI) is carried out to know the effect of carbohydrate in food sample to the blood glucose level. It gives a ranking based on how quickly the carbohydrates are digested and the release of glucose (Monro and Shaw 2008). The protein quality of food can be measured by protein efficiency ratio (PER)which is one of the oldest and simplest methods reported (Takruri and Dameh 1998).

Newer generation foods are required to be developed to reduce the digestive problems in high-altitudes. In the present study, the experiments have been categorized into two parts i.e.in vitro antioxidant status of selected food ingredients and further their effect on digestive enzymatic activity on rat pancreas, intestine and duodenum and in vivo studies to determine the efficacy of the selected food ingredients on PER and GI.

Materials and methods

Collection, preparation, and extraction of the food ingredients

A total of sixteen fruit/spice/herbal materials were procured from the local market, Mysore, India. The list of materials selected for the study is given in Table 1. Spices were procured in dry form; fruits and herbal materials were procured in fresh form. Fresh fruits were shade dried and further ground into paste using pestle and mortar, and dried spices were made into powdered form by lab mill grinder (Cyclone Sample Mill, India), and processed further overnight extraction with Milli-Q water (1:10 w/v). The extracted materials were centrifuged at 3000 rpm for a period of 20 min. The supernatant was collected and further utilized for in vitro antioxidant and digestive enzyme activity studies. The samples were freshly prepared for each assay to avoid nutrient losses.

Determination of in vitro antioxidant activities

DPPH radical scavenging activity

The free radical scavenging activity of the food ingredients was determined by in vitro DPPH (1, 1 diphenyl 2, picryl hydrazyl) assay (Xu and Chang 2007). DPPH in methanol (0.1 mM) was prepared and 2.9 ml of this solution was added to 20 µl of food ingredient and made up to 100 µl with methanol. The absorbance was measured at 517 nm after incubation for 30 min at room temperature. Percent inhibition was calculated as follows: [A control − A sample]/A control × 100, where A is the absorbance. The antioxidant activity of the extract was expressed as IC_{50}, which the concentration (µg/ml) of extract inhibits the formation of DPPH radicals by 50%.

Ferrous reducing antioxidant power (FRAP) assay

FRAP reagent consisted a mixture of 50 ml acetate buffer (300 mM; pH 3.6), 5 ml TPTZ (2, 4, 6-tripyridyl-s-triazine; 10 mM) in HCl (40 mM) and 5 ml $FeCl_3.6H_2O$ (20 mM). 20 µl of the food ingredient made up to 100 µl was then allowed to react with 2900 µl of the FRAP solution for 30 min in dark and then measured at 593 nm for the coloured product of ferrous tripyridyltriazine complex (Re et al. 1999). FRAP results were expressed in units of µmole Fe(II)/g of the sample.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured by Griess reagent (1% sulphanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid). Briefly, sodium nitroprusside (in PBS) was mixed with 100 µl of food ingredient and incubated for 120 min at room temperature. Further, Griess reagent was added to the mixture and absorbance was measured at 546 nm (Green et al. 1982). Percentage inhibition = [A control − A sample]/A control × 100, where A is the absorbance. The antioxidant activity of the extract was expressed as IC_{50}, which the concentration (µg/ml) of extract inhibits the formation of NO^* radicals by 50%.

Preparation of rat pancreas, duodenum, and small intestine homogenates

Male Wistar rats (180–200 g) from the stock colony of the central animal house, DFRL, Mysore were used for enzyme

Table 1 Antioxidant activity of selected food ingredients

Food ingredients	Parts used	DPPH[a]	FRAP[b]	NO[a]
Cardamom (*Elettaria cardamomum*)	Seed	322.419 ± 10.15	184.41 ± 13.75	16.53 ± 1.66
Carom (*Trachyspermum ammi*)	Seed	63.89 ± 1.75	1796.52 ± 41.76	13.38 ± 1.02
Cumin (*Cuminum cyminum*)	Seed	73.85 ± 1.82	1463.85 ± 386.09	15.36 ± 0.22
Coriander *(Coriandrum sativum*)	Seed	121.8 ± 8.78	662.92 ± 28.98	14.06 ± 0.90
Fennel (*Foeniculum vulgare*)	Seed	186.57 ± 6.12	587.59 ± 20.28	15.58 ± 1.60
Fenugreek (*Trigonella foenum-graecum*)	Seed	1050.46 ± 73.73	128.97 ± 8.70	9.90 ± 0.53
Ginger (*Zingiber officinale*)	Rhizome	236.97 ± 8.23	735.24 ± 11.70	10.56 ± 0.33
Liquorice (*Glycyrrhiza glabra*)	Root	107.92 ± 8.77	3525.54 ± 664.78	10.37 ± 0.28
Orange (*Citrus reticulata*)	Peel	160.94 ± 6.90	138.01 ± 19.60	10.70 ± 0.72
Papaya (*Carica papaya*)	Fruit	366.63 ± 10.22	144.64 ± 18.18	9.18 ± 0.43
Pepper (*Piper nigrum*)	Fruit	151.17 ± 8.10	896.75 ± 22.41	16.27 ± 0.77
Pineapple (*Ananas comosus*)	Fruit	113.68 ± 15.64	235.64 ± 5.97	11.85 ± 0.23
Star Anise (*Illicium verum*)	Fruit	33.82 ± 1.04	2724.01 ± 253.98	14.58 ± 0.79
Tarragon (*Artemisia dracunculus*)	Root	167.12 ± 6.50	2010.46 ± 178.39	15.25 ± 0.35
Turmeric (*Curcuma longa*)	Rhizome	356.26 ± 17.77	355.57 ± 4.32	16.30 ± 0.59
Valerian (*Valeriana officinalis*)	Root	156.24 ± 14.26	1134.20 ± 69.89	11.91 ± 0.41

DPPH - 1 1 diphenyl 2, picryl hydrazy, *FRAP* Ferrous reducing antioxidant power, *NO* nitric oxide radical scavenging activity

[a]Expressed in IC_{50} values in μg/ml

[b]Expressed in units of μmol Fe(II)/g

assays. Pancreas, duodenum, and small intestine were excised after scarifying under anaesthetic condition. The small intestine was flushed with ice-cold saline to remove excess food debris. The organs were independently homogenised with 0.9% ice-cold saline and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and stored at − 20 °C for further studies.

Enzyme assays

Rat pancreas, duodenum, and small intestine homogenates were used as an enzyme source for amylase, trypsin, chymotrypsin, disaccharidases, and carboxypeptidases.

Amylase activity

The pancreatic homogenate was used for the determination of amylase activity (1, 4-α-D-Glucan glucanohydrolase, EC 3.2.1.1) by 3, 5-dinitro salicylic acid (DNS) method (Ramakrishna et al. 2003). Briefly, 0.05 ml enzyme solution and an equal amount of food ingredient were treated with 1% starch solution (substrate) prepared with phosphate buffer (20 mM; pH-6.9) and then the mixture was incubated at room temperature for a period of 10 min. To the above mixture, DNS reagent (1% w/v) was added and kept for incubation at 100 °C for a period of 5 min. The mixture was cooled to room temperature and the absorbance was recorded at 546 nm to measure the concentration of nitroaminosalicylic acid formed and directly correlated to the pancreatic-amylase activity and expressed as nmol maltose liberated/min/mg protein.

Trypsin activity

Trypsin (EC 3.4.21.4) is formed in the pancreas and is secreted into the lumen of the intestine. The proenzyme trypsinogen is activated by incubating pancreatic homogenate with 2% enterokinase for 24 h at 5 °C. Briefly, trypsin solution (0.2 ml), triethanolamine buffer (1.8 ml; 0.2 M; pH 8.1), pancreatic homogenate (0.1 ml) and the food ingredients were mixed together and incubated for 5 min at room temperature. To the mixture, N-benzoy-larginine-p-nitroanilide (BApNA; 1 ml) was added as a substrate to determine trypsin activity (Platel and Srinivasan 2000a, b). The release of p-nitroaniline was observed with the increase in the extinction at 405 nm. Trypsin activity was expressed as nmol p-nitroaniline released/min/mg protein.

Chymotrypsin activity

Chymotrypsin (EC 3.4.21.1) has chymotrypsinogen as its precursor, which is activated by trypsin. N-Benzoyl-L-Tyrosine Ethyl Ester (BTEE) was used as a substrate. The substrate used is specific for chymotrypsin assay as trypsin does not hydrolyze BTEE (Ramakrishna et al. 2003).

Briefly, 1.5 ml Tris buffer (80 mM; pH 7.8), 1.4 ml BTEE (1.07 mM) and 0.1 ml of sample were added and the extinction was recorded for 3 min. The release of p-nitroaniline was observed at 256 nm and expressed as nmol p-nitroaniline released/min/mg protein.

Carboxypeptidase activity

Carboxypeptidase A (EC 3.4.12.2) N-(Carbo-β-naphthoxy)-DL-phenylalanine was used as the substrate which is converted to β-naphthol (Falguera et al. 2011). Briefly, 4 ml of Tris buffer (50 mM, pH 7.8), 0.1 ml calcium chloride (250 mM) and the 0.4 ml of the enzyme and the sample are mixed and incubated for 10 min. To this 0.5 ml of N-(Carbo-β-naphthoxy)-DL-phenylalanine (6 mM) is added and incubated for 25 min. One ml of chromogen solution is added and exactly after 1 min, the sample mixture is extracted with 1 ml 70% perchloric acid and ethyl acetate to measure the absorbance at 546 nm. The activity was calculated by measuring the amount of β-naphthol released using a β-naphthol standard curve and the values were expressed as μmol β-naphthol released/mg protein.

Disaccharidases activity

Disaccharidases such as sucrase and maltase activities were carried out using glucose oxidase method. Briefly, 10 μl of disaccharide solution, 10 μl of enzyme solution and food ingredient were mixed together. The mixture was incubated at room temperature for a period of 1 h. To this mixture, 300 μl of glucose reagent was added and incubated for one hour at 37 °C (Ramakrishna et al. 2003). The glucose released was measured at 450 nm. The activity was calculated using a glucose standard curve and expressed as μg glucose hydrolysed/mg protein. The protein content in the duodenum, intestine, and pancreatic homogenates was measured by modified Lowry's method (Hartree 1972).

In-vivo method

Protein efficiency ratio (PER)

Male weanling Wistar rats (35–50 g) from the stock colony of the Animal House, DFRL, Mysore were used for this experiment. After 7 days of adaptation, the rats were subjected to a feeding trial for 28 days. The rats were placed in individual stainless steel cages and randomly distributed into seventeen groups (n = 6). The control group was fed with casein diet, other groups were fed casein diet along with food ingredients individually (1 g/kg body weight). During this period, water and food were fed ad libitum. The casein diet (1 kg) consisted of casein (240 g), vitamin mixture (10 g), mineral mixture (40 g), groundnut oil (40 ml), cod liver oil (10 ml), and cornstarch (658 g). The food ingredients were incorporated into the diet at the expense of corn starch to give the various diets. The food intake was monitored every day and the weight of the rats was recorded weekly (Mensa-Wilmot et al. 2001). The PER was calculated using the formula:

PER = Increase in body weight (gram)/Weight of protein consumed (gram)

Glycemic index (GI) studies

Before the start of the experiment, rats fasted overnight. Fasting blood glucose was measured and glucose (3 g/kg body weight) was orally administered along with the food ingredients (100 mg/ml). Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 min (Shivanna et al. 2013). Glycemic index(GI) was calculated using the formula:

$GI = (AUC_{sample}/AUC_{glucose}) \times 100\%$, where AUC = area under the blood glucose curve

Statistical analysis

Results were expressed as mean value ± standard deviation (n = 6) for experiments. Linear regression analysis was conducted to find out the correlation coefficient. Statistical significance was evaluated by employing *t* test and $p < 0.05$ was considered to be significant.

Results

In the present study, a total of sixteen food ingredients were analysed for their (a) antioxidant and digestive properties by in vitro methods to understand their influence on digestive enzymes such as pancreatic amylase, trypsin, chymotrypsin, carboxypeptidases and disaccharidases, and (b) PER and GI by in vivo methods. Among the sixteen food ingredients, ten belong to spices, three belong to medicinal herbs and three belong to fruits. Different parts of the food ingredients were used in the study depending upon their medicinal usage (Table 1).

Antioxidant activity

Results of DPPH, FRAP and NO free radical scavenging activities of the food ingredients screened are placed in Table 1. Star anise, carom, and cumin showed highest DPPH activity with IC_{50} value 33.82 ± 1.04, 63.89 ± 1.75 and 73.85 ± 1.82 μg/ml respectively. Total antioxidant activity with respect to ferrous reducing antioxidant power (FRAP) was expressed as μmol Fe(II)/g sample. Fenugreek

showed highest FRAP activity with 128.97 ± 8.70 µmol Fe(II)/g (IC_{50}); whereas, liquorice showed the lowest activity. The comparative study of the food ingredients was best in scavenging nitric oxide radical with IC_{50} ranging from 9 µg/ml to 17 µg/ml.

Enzyme assays

Effect of food ingredients on amylase activity

The amylase activity was significantly enhanced by most of the studied food ingredients. Pineapple showed maximum stimulatory effect 186.3 ± 3.8 nmol maltose liberated/min/mg protein, which is 432% higher than the control (Table 2). Valerian, ginger, papaya, fennel, orange peel, carom, coriander, cumin, turmeric, star anise, pepper, and cardamom showed increased levels of pancreatic amylase activity when compared to the control ($p \leq 0.05$). The food ingredients like liquorice, fenugreek, and tarragon showed decreased pancreatic amylase activity when compared to the control (Table 2).

Effect of food ingredients on trypsin activity

The maximum activity for trypsin enzyme was shown by pineapple (252%) followed by papaya, cumin, carom, fennel, pepper, star anise, coriander, cardamom, orange peel powder, turmeric, ginger, liquorice, valerian, and tarragon. Fenugreek and cardamom showed reduced pancreatic trypsin activity (Table 2).

Effect of food ingredients on chymotrypsin activity

All studied food ingredients were able to increase chymotrypsin activity when compared to the control (except fenugreek). Chymotrypsin activity was ranged between 3.15 and 6.96 nmol p-nitroaniline released/min/mg protein. Whereas, pineapple showed highest chymotrypsin activity, which is 86% more than control (Table 2).

Effect of food ingredients on carboxypeptidase activity

Enhanced carboxypeptidase activity was observed by tarragon i.e. 149% more when compared to the control (Table 3). While, other food ingredients showed the following carboxypeptidase activities when compared with

Table 2 Effect of selected food ingredients on amylase, trypsin, and chymotrypsin

Sample	Amylase[a]	Trypsin[b]	Chymotrypsin[b]
Control	35.0 ± 2.2	2.74 ± 0.85	3.75 ± 0.05
Cardamom	38.5 ± 2.5 (9%)*	2.04 ± 0.13 (26%)**	5.75 ± 0.03 (53%)*
Carom	110.3 ± 5.5 (215%)*	9.06 ± 0.02 (230%)*	5.11 ± 0.01 (36%)*
Coriander	108.3 ± 2.2 (210%)*	7.26 ± 0.87 (165%)*	4.98 ± 0.09 (33%)*
Cumin	103.7 ± 5.5(196%)*	9.19 ± 0.02 (236%)*	4.84 ± 0.02 (29%)*
Fennel	164.6 ± 5.5 (370%)*	8.59 ± 0.92 (213%)*	4.39 ± 0.01 (17%)*
Fenugreek	18.3 ± 0.4 (48%)**	1.93 ± 0.73 (30%)**	3.15 ± 0.14 (16%)**
Ginger	180.9 ± 2.7 (417%)*	6.47 ± 0.12 (136%)*	6.48 ± 0.10 (73%)*
Liquorice	33.5 ± 2.2 (4%)**	5.32 ± 0.81 (94%)*	5.58 ± 0.05 (49%)*
Orange	112.6 ± 2.2 (222%)*	6.88 ± 0.02 (151%)*	5.98 ± 0.01 (59%)*
Papaya	165.2 ± 10.2 (372%)*	9.38 ± 0.20 (242%)*	6.90 ± 0.09 (84%)*
Pepper	72.3 ± 3.8 (106%)*	7.67 ± 0.02 (180%)*	5.82 ± 0.02 (55%)*
Pineapple	186.3 ± 3.8 (432%)*	9.64 ± 0.62 (252%)*	6.96 ± 0.12 (86%)*
Star Anise	85.5 ± 4.7 (144%)*	7.41 ± 0.61 (170%)*	4.44 ± 0.05 (18%)*
Tarragon	31.5 ± 1.4 (10%)**	4.68 ± 0.20 (71%)*	5.35 ± 0.05 (43%)*
Turmeric	86.0 ± 5.2 (146%)*	6.74 ± 0.36 (146%)*	5.28 ± 0.01 (41%)*
Valerian	183.0 ± 4.1 (423%)*	5.21 ± 0.70 (90%)*	5.97 ± 0.15 (59%)*

Values are mean ± SD of six independent determinations. Values in parentheses indicate % difference compared to control value

[a]Expressed in nmol maltose liberated/min/mg protein

[b]Expressed in nmol p-nitroaniline released/min/mg protein

*Significant increase over the control value ($p \leq 0.05$)

**Significant decrease compared to control value ($p \leq 0.05$)

Table 3 Effect of selected food ingredients on carboxypeptidase and disaccharidase

Sample	Carboxypeptidase[a]	Maltase[b]	Sucrase[b]
Control	17.3 ± 1.0	205.97 ± 1.21	215.70 ± 2.84
Cardamom	13.1 ± 0.6 (25%)[#]	205.31 ± 13.43[#]	204.42 ± 1.01 (5%)#
Carom	9.1 ± 0.3 (47%)[#]	204.84 ± 0.40 (1%)[#]	155.37 ± 2.43 (28%)[#]
Coriander	20.9 ± 0.9 (21%)*	201.68 ± 10.94 (2%)[#]	190.96 ± 4.25 (12%)[#]
Cumin	15.2 ± 0.4 (12%)[#]	166.85 ± 2.47 (19%)[#]	128.62 ± 1.46 (40%)[#]
Fennel	25.6 ± 1.1 (48%)*	162.23 ± 1.94 (21%)[#]	250.57 ± 9.70 (16%)*
Fenugreek	18.1 ± 0.4 (120%)*	202.38 ± 0.70 (2%)[#]	145.38 ± 3.21 (33%)[#]
Ginger	41.7 ± 0.2 (141%)*	173.89 ± 6.98 (16%)[#]	158.37 ± 3.03 (27%)[#]
Liquorice	11.4 ± 0.8 (34%)[#]	75.18 ± 5.16 (64%)[#]	164.81 ± 7.28 (24%)[#]
Orange	25.3 ± 0.7 (46%)*	167.98 ± 6.5 (18%)[#]	216.69 ± 5.46 (0.3%)*
Papaya	34.1 ± 1.0 (97%)*	194.08 ± 1.62 (6%)[#]	215.92 ± 2.52 (0.03%)*
Pepper	29.9 ± 1.5 (73%)*	192.60 ± 3.65 (7%)[#]	178.80 ± 1.60 (17%)[#]
Pineapple	35.1 ± 1.4 (103%)*	185.20 ± 2.41 (10%)[#]	220.11 ± 0.51 (2%)*
Star Anise	1.1 ± 0.1 (94%)[#]	147.138 ± 3.63 (29%)[#]	131.79 ± 5.46 (39%)[#]
Tarragon	43.1 ± 0.3 (149%)*	127.47 ± 3.22 (38%)[#]	261.72 ± 8.49 (21%)*
Turmeric	36.8 ± 0.1 (113%)*	201.45 ± 0.40 (2%)[#]	136.77 ± 0.60 (37%)[#]
Valerian	38.6 ± 0.2 (123%)*	75.44 ± 1.45 (63%)[#]	196.52 ± 10.31 (22%)[#]

Values are mean ± SD of six independent determinations. Values in parentheses indicate % difference compared to control value

[a]Expressed in μmol β-naphthol released/mg protein

[b]Expressed in μg glucose hydrolysed/mg protein

*Significant increase over the control value ($p \leq 0.05$)

[#]Significant decrease compared to control value ($p \leq 0.05$)

control: cumin (12%), cardamom (25%), carom (47%), liquorice (34%) and star anise (94%).

Effect of food ingredients on disaccharidases activity

To understand the disaccharidase activity, maltase and sucrase activities were carried out. All food ingredients showed inhibitory activity towards maltase assay; while fennel (16%), orange (0.3%), papaya (0.03%) and pineapple (2%) elevated sucrase activity when compared to the control (Table 3).

In-vivo methods

Protein efficiency ratio

The PER of casein diet fortified with all the selected food ingredients in same concentrations are tabulated in Table 4. Pineapple extract fortified casein diet gets a 1.97 PER rating when compared to 1.37 of the control and hence can be used in many nutritional supplements.

Table 4 Protein efficiency ratio (PER) of the selected food ingredients

Food ingredients	PER
Casein diet	1.37 ± 0.24
Cardamom	0.68 ± 0.03
Carom	0.53 ± 0.08
Coriander	0.66 ± 0.01
Cumin	0.65 ± 0.02
Fennel	0.61 ± 0.05
Fenugreek	0.60 ± 0.01
Ginger	0.58 ± 0.03
Liquorice	0.93 ± 0.22
Orange	1.09 ± 0.20
Papaya	0.92 ± 0.36
Pepper	0.64 ± 0.11
Pineapple	1.95 ± 0.19*
Star anise	0.53 ± 0.09
Tarragon	0.92 ± 0.32
Turmeric	1.02 ± 0.21
Valerian	0.98 ± 0.23

Values are mean ± SD of six independent determinations

*Significant increase in the control value ($p \leq 0.05$)

Table 5 Glycemic index (GI) of the food ingredients

Food ingredients	GI
Control	97.0 ± 0.9
Cardamom	94.0 ± 3.4
Carom	86.7 ± 0.05
Coriander	88.4 ± 1.9
Cumin	85.8 ± 5.6
Fennel	78.4 ± 1.5
Fenugreek	93.3 ± 1.0
Ginger	84.0 ± 2.7
Liquorice	98.2 ± 1.4
Orange	95.8 ± 0.9
Papaya	94.8 ± 1.4
Pepper	97.5 ± 1.8
Pineapple	98.0 ± 0.5*
Star anise	89.3 ± 2.9
Tarragon	87.2 ± 0.5
Turmeric	85.4 ± 1.8
Valerian	88.9 ± 3.7

Values are mean ± SD of six independent determinations

*Significant increase in the control value ($p \leq 0.05$)

Glycemic index

GI of plain glucose was measured in absence and presence of all the food ingredients. Almost all food ingredients showed reduced glycemic index (GI) compared to the control except pineapple with GI of 98 and was significantly different from that of the control ($p < 0.05$) (Table 5).

Discussion

Gastrointestinal-related problems are of common occurrence in people who ascend high altitudes (HA) like Ladakh and north-east India. Various high altitude environmental factors that influence gastrointestinal (GI) function, such as the physiological effects of hypobaric hypoxia, changes in dietary habits due to a limited variety of food materials and other environmental conditions (Anand et al. 2006). Hypoxia is the major contribution of oxidative stress at high altitudes. Certain environmental factors, such as sunlight, cold, diet and reduced partial pressure of oxygen in the atmosphere, also add to the cumulative burden of oxidative stress at high altitudes (Askew 2002). A limited amount of research suggests that antioxidant supplementation at high altitude may be beneficial to reduce the symptoms of acute mountain sickness (AMS), reduce muscle soreness and improve red blood cell membrane fluidity.

Oxidative stress at the cellular and molecular level is considered to be a major culprit in disease processes like cardiovascular diseases, intestinal inflammation, cancer, and aging. Many bioactive components of medicinal herbs/fruits/spices are known to have free radical scavenging activity by the activation of antioxidant phase II enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) (Kumar et al. 2015). These bioactive components mainly belong to phenolics i.e. polyphenols, flavonoids, hydrolysable tannins, anthocyanins, phenolic acids and vitamins viz., tocopherol, ascorbic acid, and β-carotene (Brewer 2011).

The food ingredients studied in this investigation are commonly used in Indian culinary system. For instance, cumin is a common ingredient in seasoning; ginger is being used in a variety of foods/drinks as an appetizer since ages and as a remedy for nausea; carom is an ingredient of many dishes and savouries; piperine is an active component of pepper known to influence on membrane fluidity of the intestinal brush border; fenugreek is well known hypoglycaemic activity due to the presence of 4-hydroxy isoleucine; turmeric has an anti-ulcerative agent due to presence of curcumin; cardamom and coriander are known flavouring agents with digestive properties; fennel has been used for dyspepsia, bloating, flatulence and poor appetite; star anise is said to possess carminative, stimulant, stomachic, diuretic properties; papain from unripe papaya is used as digestive and softening agent; bromelain is a proteolytic enzyme derived from pineapple has an antimicrobial effect, as well as displaying anti helminthic activity to vanish gastrointestinal nematodes; tarragon is used as an aromatizing agent with carminative property; liquorice is used as a laxative, antidiabetic, anti-inflammatory, immunomodulatory, antitumour; valerian is also used to treat insomnia and anxiety; orange is used to treat anorexia and aging apart from being choleretic (Aglarova et al. 2008; Frankic et al. 2009).

Gastrointestinal (GI) tract diseases include infection of mucosal surface, colon cancers and chronic inflammatory conditions example Crohn's disease, ulcerative colitis etc., all these diseases will involve reactive oxygen species and oxidative damage. Sometimes, oxidative responses are beneficial, for example during invasion by pathogens whereas if it is not controlled can cause tissue destruction due to the production of peroxides and free radicals that will damage proteins, lipids and DNA. Also in GI tract endogenous and exogenous antioxidants can counteract reactive species and maintain a balance between oxidative and an antioxidant response, which is critical for maintaining good intestinal health (Cheli and Baldi 2011).

DPPH free radical Scavenging activity is one of the common antioxidant assay carried out (Xu and Chang 2007). FRAP assay is one of the in vitro antioxidant studies performed in terms of chelation power on ferrous ions, which is main cause of oxidative damage in GI tract (Re et al. 1999). Nitric oxide (NO) also plays an important role in physiology of GI tract and high concentrations of NO are related to numerous pathological processes of GI tract (Salzman 1995). Hence, it is essential to evaluate the antioxidant properties in the food ingredients selected for support of digestion. In the present study, all the selected food ingredients have shown antioxidant activity against DPPH radicals, nitric oxide radicals, and ferrous reducing property. Star anise, carom, and cumin have shown maximum free radical scavenging activity against DPPH radical ($IC_{50} < 100$ µg/ml); whereas, cardamom, fenugreek, orange, and papaya have shown best FRAP activity among the selected food ingredients (> 200 µmol Fe(II)/g).

Nitric oxide is classified as a free radical because of the presence of unpaired electron. NO radical displays an important reactivity with various proteins and other free radicals. NO is very unstable and produces intermediates like nitrogen dioxide (NO_2), dinitrogen tetroxide (N_2O_4), dinitroazanide (N_3O_4). NO radicals may produce genotoxic peroxinitrities by reacting with superoxide (Wink et al. 1991). Further, in high altitude conditions (hypoxic exposure) NO radical synthesis may occur including acute mountain sickness and high altitude pulmonary and cerebral oedema situations (Askew 2002). Therefore, foods having NO radical scavenging activity may be useful to rectify these hypoxic conditions. Moreover, all the selected food ingredients have shown best nitric oxide radical scavenging activity. Previous studies on lipid peroxidation activity have shown that these food ingredients are capable of inhibiting lipid peroxidation through enhancing the activity of endogenous enzymes such as SOD, CAT, GPx, and GR (Shobana and Naidu 2000). Further studies have proved that these biochemicals have the capacity to reduce the arachidonate metabolites (PGE2, leukotrienes) and also shown inhibiting activity on the secretion of lysosomal enzymes such as elastase, collagenase, and hyaluronidase (Srinivasan 2005). Antioxidant reports of the present study are in line with the previous investigations on spices (Shobana and Naidu 2000).

Any food additive which has a digestive stimulant action is known to stimulate the digestive secretions (gastric, bile, pancreatic) and/or stimulate the digestive enzymes such as amylase, protease, and lipases. Therefore, the present study was designed to know the digestive properties of selected food ingredients on pancreatic, duodenum and small intestine homogenates by in vitro methods. Previous animal studies have documented the beneficial effects of turmeric, pepper, ginger, carom and fennel on the activity of digestive enzymes (Platel and Srinivasan 2000a, b, 2001). Further, it is confirmed that these digestive enzymes hydrolyse macromolecules of the food such as protein, starch, and triglycerides into smaller molecules and induce digestive stimulant action (Platel and Srinivasan 1996). The observations of the present study indicate that the tested samples have a favourable influence on the activity of pancreatic amylase, trypsin, and chymotrypsin. Pineapple has shown the best enzymatic activity of pancreatic amylase, trypsin and chymotrypsin among the tested samples with 432, 252, and 86%, respectively. In the present study, fenugreek has shown a negative effect on all pancreatic digestive enzymes. It may be due to the presence of protease inhibitors such as bitter saponins (Weder and Haubner 1991). The digestive enzymatic activity also helps in stimulation of bile flow and bile acid secretion. Carboxypeptidase is a protease enzyme that hydrolyzes a peptide bond at the carboxy terminal of protein. In the present study, fenugreek, ginger, pineapple, tarragon, turmeric, and valerian have shown higher duodenum carboxypeptidase activity when compared to other tested samples. However, maltase activity was inhibited by the food ingredients taken; fennel, orange, papaya, pineapple, and tarragon showed higher sucrose activity. By in vivo studies of PER and GI, it can be concluded pineapple extract was found to be promising based on the studies carried out. This can be used in many nutritional supplements in the form of munch, with both appetizing and digestive properties for improved digestion capacity for mountaineers and infants as the active component of pineapple i.e. bromelain remains active in acidic and alkaline environment of stomach and small intestine respectively which makes it an efficient oral digestive aid (Pavan et al. 2012). Gastrointestinal problems are common for infants and home remedies are being practiced since ages in India. Fennel water extract has the property to correct flatulence in infants and being practiced in Indian system of medicine. The stomach lining of infants and children are susceptible for ulcer and other small reasons; however, ginger and fenugreek seeds are commonly used as treatment (Platel and Srinivasan, 1996). However, while using these food ingredients as medicine, there should be a prescribed dose and duration; otherwise, this may lead to other adverse effects.

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References

Aglarova AM, Zilfikarov IN, Severtseva OV (2008) Biological characteristics and useful properties of tarragon (*Artemisia dracunculus* L.). Pharm Chem J 42(2):81–86. https://doi.org/10.1007/s11094-008-0064-3

Anand AC, Sashindran VK, Mohan L (2006) Gastrointestinal problems at high altitude. Trop Gastroenterol 27(4):147–153

Askew EW (2002) Work at high altitude and oxidative stress: antioxidant nutrients. Toxicol 180(2):107–119. https://doi.org/10.1016/S0300-483X(02)00385-2

Brewer MS (2011) Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev Food Sci Food Saf 10(4):221–247. https://doi.org/10.1111/j.1541-4337.2011.00156.x

Chawla S, Saxena S (2014) Physiology of high-altitude acclimatization. Resonance 19(6):538–548. https://doi.org/10.1007/s12045-014-0057-3

Cheli F, Baldi A (2011) Nutrition-based health: cell-based bioassays for food antioxidant activity evaluation. J Food Sci. https://doi.org/10.1111/j.1750-3841.2011.02411

Falguera V, Miarnau O, Pagan J, Ibarz A (2011) Inhibitory effect of melanins from *Agaricus bisporus* polyphenol oxidase and two different substrates on carboxypeptidases A and B activity. Eur Food Res Technol 233(6):1075–1079. https://doi.org/10.1007/s00217-011-1595-5

Frankic T, Voljc M, Salobir J, Rezar V (2009) Use of herbs and spices and their extracts in animal nutrition. Acta Agric Slov 94(2):95–102.http://aas.bf.uni-lj.si/zootehnika/94

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS et al (1982) Analysis of nitrate, nitrite, and [15 N] nitrate in biological fluids. Anal Biochem 126:131–138

Hartree EF (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal Biochem 48(2):422–427

Ketnawa S, Chaiwut P, Rawdkuen S (2012) Pineapple wastes: a potential source for bromelain extraction. Food Bioprod Process 90:385–391. https://doi.org/10.1016/j.fbp.2011.12.006

Kumar GP, Anilakumar KR, Naveen S (2015) Phytochemicals having neuroprotective properties from dietary sources and medicinal herbs. Pharmacogn J 7(1):1–17. https://doi.org/10.5530/pj.2015.7.1

Mensa-Wilmot Y, Phillips RD, Hargrove JL (2001) Protein quality evaluation of cowpea-based extrusion cooked cereal/legume weaning mixtures. Nutr Res 21(6):849–857. https://doi.org/10.1016/S0271-5317(01)00302-5

Monro JA, Shaw M (2008) Glycemic impact, glycemic glucose equivalents, glycemic index, and glycemic load: definitions, distinctions, and implications. Am J Clin Nutr 87(1):237S–243S. https://doi.org/10.1093/ajcn/87.1.237S

Nam SH, Walsh MK, Kim SH, Yang KY (2016) Identification and functional characterization of cysteine protease from nine pear cultivars (*Pyrus pyrifolia*). Int J Food Prop 19(7):1631–1644. https://doi.org/10.1080/10942912.2015.1107576

Pavan R, Jain S, Kumar A (2012) Properties and therapeutic application of bromelain: a review. Biotechnol Res Int. https://doi.org/10.1155/2012/976203

Platel K, Srinivasan K (1996) Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. Int J Food Sci Nutr 47(1):55–59. https://doi.org/10.3109/09637489609028561

Platel K, Srinivasan K (2000a) Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. Mol Nutr Food Res (Nahrung) 44(1):42–46. https://doi.org/10.1002/(SICI)1521-3803(20000101)44:1<42:AID-FOOD42>3.0.CO;2-D

Platel K, Srinivasan K (2000b) Stimulatory influence of select spices on bile secretion in rats. Nut Res 20:1493–1503. https://doi.org/10.1016/S0271-5317(00)80030-5

Platel K, Srinivasan K (2001) A study of the digestive stimulant action of select spices in experimental rats. J Food Sci Technol 38(4):358–361

Prakash UN, Srinivasan K (2012) Fat digestion and absorption in spice-pretreated rats. J Sci Food Agric 92(3):503–510. https://doi.org/10.1002/jsfa.4597

Ramakrishna RR, Platel K, Srinivasan K (2003) In vitro influence of spices and spice-active principles on digestive enzymes of rat pancreas and small intestine. Mol Nutr Food Res 47(6):408–412. https://doi.org/10.1002/food.200390091

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Riceevans C (1999) Antioxidant activity applying an improved ABTS radical decolorization assay. Free Radic Bio Med 26:1231–1237

Salzman AL (1995) Nitric oxide in the gut. New Horiz 3(1):33–45

Shan B, Cai YZ, Brooks JD, Corke H (2007) The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol 117(1):112–119. https://doi.org/10.1016/j.ijfoodmicro.2007.03.003

Shivanna N, Naika M, Khanum F, Kaul VK (2013) Antioxidant, antidiabetic and renal protective properties of Stevia rebaudiana. J Diabetes Complic 27(2):103–113. https://doi.org/10.1016/j.jdiacomp.2012.10.001

Shobana S, Naidu K (2000) Antioxidant activity of selected Indian spices. Prostaglandins Leukot Essent Fat Acids 62(2):107–110. https://doi.org/10.1054/plef.1999.0128

Srinivasan K (2005) Spices as influencers of body metabolism: an overview of three decades of research. Food Res Int 38(1):77–86. https://doi.org/10.1016/j.foodres.2004.09.001

Takruri HR, Dameh MA (1998) Study of the nutritional value of black cumin seeds (*Nigella sativa* L). J Sci Food Agric 76(3):404–410. https://doi.org/10.1002/(SICI)1097-0010(199803)76:3<404:AID-JSFA964>3.0.CO;2-L

Valussi M (2012) Functional foods with digestion-enhancing properties. Int J Food Sci Nutr 63(1):82–89. https://doi.org/10.3109/09637486.2011.627841

Weder JK, Haubner K (1991) Inhibitors of human and bovine trypsin and chymotrypsin in fenugreek (*Trigonella foenum-graecum* L.) seeds. Z Lebensm-Untersuch Forsch 193(3):242–246. https://doi.org/10.1007/BF01199974

Wink OA, Kasprzak KS, Maragose M (1991) DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. Science 254:1001–1003

Wu TY, Ding SQ, Liu JL, Jia JH, Dai RC, Zhu DC, Sun YF (2007) High-altitude gastrointestinal bleeding: an observation in Qinghai-Tibetan railroad construction workers on Mountain Tanggula. World J Gastroenterol 13(5):774. https://doi.org/10.3748/wjg.v13.i5.774

Xu BJ, Chang SKC (2007) A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. J Food Sci 72(2):160–161. https://doi.org/10.1111/j.1750-3841.2006.00260.x