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Enhancement of digestive enzymatic activity by cumin (*Cuminum cyminum* L.) and role of spent cumin as a bionutrient

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Abstract

Cuminum cyminum L., commonly known as cumin, belongs to the apiacea family. The effects of different cumin extracts, e.g. saline, hot aqueous, oleoresin and essential oil were studied for various enzymatic activities. Results showed maximum increases in amylase, protease, lipase and phytase activities in the presence of saline and hot aqueous extracts, along with high antioxidant activity. The study was aimed at utilizing the deoiled cumin or spent cumin available from spice industries. The saline and hot aqueous extracts of spent cumin showed enzymatic activities similar to that of native cumin. The spent cumin had a carbohydrate content of 23%, protein 19%, fat 10% and soluble dietary fibre 5.5%, along with vitamins such as thiamine (0.05), riboflavin (0.28) and niacin (2.7) (mg/100 g). It was also a rich source of minerals, having Fe^{2+} (6.0) and Zn^{2+} (6.5) (mg/100 g). Different concentrations of phytase were used to improve the bioavailability of iron and zinc. Results showed that phytase (ratio of 1:1000), in the presence of 20 mM citric acid, increased iron and zinc bioavailability significantly. Thus, the spent cumin can find potential use in various health food formulations, showing improved digestibility and a good nutrient composition.

Keywords: Hot water cumin extract; Saline cumin extract; Essential oil; Oleoresin; Protease activity; Amylase activity; Lipase activity; Bioavailability

1. Introduction

Recently, spices are gaining importance as bionutrients, both as functional food ingredients and nutritional supplements. The use of spices as food additives has been widely practised since ancient times. Spices have a definite role to play in enhancing the taste and flavour of any food. Apart from this, spices are believed to have medicinal value. They form an important part of the Ayurvedic Pharmacopoeia (Indian System of Medicine). They have been used in a large number of medicinal preparations for the treatment of several disorders, particularly of the digestive system (Sreenivasamurthy & Krishnamurthy, 1959).

Several reports have appeared in the literature in the past 50 years, on the bactericidal, bacteriostatic, fungistatic, antifertility, antihelminthic and other medicinal properties of spices. Spices are also believed to aid digestion. Thus, in this era of nutraceuticals and functional foods, spices and herbs have an increasingly larger role to play in Indian recipes. Among the large number of spices used to flavour foods and beverages in India, cumin occupies a place of prominence. Spices are primarily used in the food industry for improving the quality of the product. These powdered spices suffer disadvantages, e.g. quality variations from batch to batch caused by uneven distribution of flavour, loss of flavour strength, quality during storage, insect infestation, bacterial contamination, unhygienic nature and inconveniency in bulk handling. To overcome these problems, spice essential oil has come into use in the food industries, thereby leaving behind deoiled spent cumin after oil extraction.Here, an attempt has been made to assess the role of cumin extracts, namely hot water, saline, oleoresin and essential oil in various enzymatic activities (amylase, protease, lipase, phytase) and antioxidant activity. Information on this aspect of spices is inadequate. Therefore, the

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objective of this study was to investigate the effect of cumin extracts and spent cumin (deoiled), on various enzymatic activities. Nutritional components of spent cumin were analysed to explore its potential in various food formulations.

2. Materials and methods

2.1. Plant materials

Cumin (*Cuminum cyminum* L.) was procured from a local supermarket. The seeds were milled into fine powder and used for extraction of water-soluble, saline-soluble, essential oil and oleoresin.

2.2. Chemicals

Pepsin (1020 U/mg protein), pancreatin (lipase activity 8 U/mg protein, amylase activity 100 U/mg protein, protease 100 U/mg protein) and bile extracts from porcine sources, used for *in vitro* digestibility, were purchased from Sigma–Aldrich (St. Louis, USA). Enzymes used were papain (580 TU/mg protein) from *Carica papaya*, from Enzochem private limited (Mumbai, India), lipase from wheat germ(10 U/mg protein) and α-amylase from *Bacillus amyloliquefaciens* (1500 U/mg protein), procured from Sigma–Aldrich (St. Louis, USA). Exogenous phytase from wheat (0.04 U/mg solid) used for dephytinization and 2,2-diphenyl-1-picrylhyrazyl (DPPH·) were procured from Sigma–Aldrich (St. Louis, USA). α-Amylase, from *Bacillus licheniformis* (Termamyl SC DS), was from Novozymes (Australia). Vitamins, iron and zinc standards, used for measuring the vitamin and mineral content, were purchased from Sigma–Aldrich (St. Louis, USA). Folin–Ciocalteau reagent was procured from Sisco research lab (Mumbai, India). Soluble starch and dinitrosalicylic acid were obtained from Merck (Mumbai, India). Dialysis tubing having 10 kDa molecular weight cut off (MWCO) was purchased from Spectrum, USA.

2.3. Preparation of various extracts of cumin

2.3.1. Material

Cumin seeds were ground in a domestic grinder and sieved to obtain a particle size less than 420 microns. The ground cumin was stored in polythene bags in desiccators prior to further use. The various enzymatic activities (amylase, protease, lipase, phytase) and antioxidant activity for different extracts were calculated, based on weight equivalence of cumin present per ml of the extract.

2.3.2. Hot water extract

Ground cumin, weighing 100 g, was extracted with 600 ml of hot water at 50 °C for 24 h in a thermostat-controlled shaking water bath. The extract was then centrifuged at 3000 rpm for 20 min, decanted and the volume of supernatant was made up to 600 ml with distilled water.

2.3.3. Saline extract

Ground cumin, weighing 100 g, was extracted with 600 ml of 0.5 N NaCl in a shaker incubator at 30 °C for 24 h. The extract was then centrifuged at 3000 rpm for 20 min, decanted and volume of the supernatant was made up to 600 ml with distilled water.

2.3.4. Essential oil

Oil was prepared by hydro-distillation of ground cumin (100 g) using a Clevenger distillation method, as previously described (AOAC, 1997).

The essential oil thus obtained was dried using anhydrous sodium sulphate and stored at 4 °C.

2.3.5. Oleoresin

The cumin oleoresin was prepared as previously described (AOAC, 1997). It was prepared by successive extraction of ground cumin (100 g) with acetone. The acetone extract obtained was then desolventized and the oleoresin was weighed and stored at 4 °C prior to further use.

2.3.6. Spent cumin (deoiled cumin)

After extraction of essential oil, the left over material, called the spent cumin, was stored at 4 °C for further work. The spent cumin was evaluated for various enzymatic activities (amylase, protease, lipase, phytase) and nutrient analysis.

2.4. Total phenolic compounds

The total phenolic contents in various extracts of cumin were determined spectrophotometrically using Folin–Ciocalteu reagent as reported by Zainol, Abd-Hamid, Yusuf and Muse (2003) and results were expressed as tannic acid equivalents. All the experiments were carried out in triplicate and variations in the values expressed as standard deviations.

2.5. Antioxidant activity

The antioxidant activity, measured as the radical-scavenging activity of various cumin extracts towards DPPH radical, was carried out using the method given by Ramadan, Lothar and Morsel (2003), calculating the % of DPPH radical inhibition. The IC_{50} value was defined as the concentration of samples required to decrease the activity by 50%. To determine the free radical-scavenging potential, the extracts were made to react with DPPH; a N-centered free radical with a characteristic absorbance at 517 nm, which gets discoloured by abstracting a hydrogen atom from the phenolic hydroxyl groups, thereby intercepting the free radical chain reaction and forming stable free radicals. All the experiments were carried out in triplicate and the variations in the values expressed as standard deviations.

2.6. Effect of various cumin extracts on digestive enzymatic activities (in vitro)

2.6.1. Amylase activity

The effect of different cumin extracts on amylase activity was determined by the (DNS) dinitrosalicylic acid method described by Rick and Stegbauer (1974). The method involves spectrophotometric measurement of reducing sugar liberated from 1% soluble starch by the action of amylase using dinitrosalicylic acid reagent at pH 6.9 at room temperature. One unit of activity was defined as the amount of enzyme required to release a reducing group in 3 min from 1% soluble starch, corresponding to 1 mg of glucose. All the experiments were carried out in triplicate and the variations in the values expressed as standard deviations.

2.6.2. Proteolytic activity

The effect of various cumin extracts on the proteolytic activity was measured using the spectrophotometeric method described by Sierecka (1998). The method was based on the enzymatic hydrolysis of denatured hemoglobin at pH 6.2 at 37 °C, followed by precipitation of unhydrolysed substrate with trichloroacetic acid. Enzyme activity was expressed in terms of hemoglobin units on a tyrosine basis (HUT). One HUT is the amount of enzyme that, under assay conditions, produces a hydrolysate whose absorbance at 275 nm is same as that of a solution containing 1.1 μg/ml tyrosine in 0.006 N HCl. All the experiments were carried out in triplicate and the variation in the values expressed as standard deviations.

2.6.3. Lipase activity

The effect of various cumin extracts on lipase activity was determined using the method described by Tietz and Fiereck (1966). This method was based on titrimetric estimation of free fatty acids liberated from an emulsified substrate by lipase action at pH 7.4 at 37 °C by titration with standard alkali. One unit of enzyme activity was expressed as micro equivalents of alkali consumed per mg of protein per h of incubation. All the experiments were carried out in triplicate and the variations in the values expressed as standard deviations.

2.6.4. Phytase activity

The effect of different cumin extracts on phytase activity was determined by the spectrophotometric method given by De Angelis et al. (2003), involving measurement of phosphate liberated from 3 mM sodium phosphate by the action of phytase at pH 5.2 at 50 °C. Phytase activity was measured in terms of inorganic orthophosphate released from the phytic acid by phytase. One unit of phytase activity was defined as the amount of enzyme required to liberate 1 nanomole of phosphate per min under the assay conditions. All the experiments were carried out in triplicate and the variations in the values expressed as standard deviations.

2.7. Nutritional composition of spent cumin

Official methods (AOAC method, 1990) were used to determine nutritional composition of spent cumin. The phenol–sulfuric method was used for estimating total carbohydrate, Kjeldahl for protein (N × 6.25), Soxhlet apparatus using petroleum ether (b.p. 60–80 °C) for fat, drying of the sample at 105 °C for 24 h for moisture, incineration at 550 °C for 5 h for ash. Mineral content was estimated by the atomic absorption spectroscopy method described by Guohua, Yanhua and Dongzhi (2006). Dietary fibre was estimated by the enzymatic gravimetric method (Leon, Nils-Georg, Thomas, Jonathan, & Wan, 1992). All the experiments were carried out in triplicate and the variations in the values expressed as standard deviations.

2.8. Vitamin analysis

Riboflavin and thiamine were estimated using fluorescence (AOAC, 2000). Niacin was estimated by HPLC method (Tyler & Genzale, 1990). All the experiments were carried out in triplicate and the variations in the values expressed as standard deviations.

2.9. Antinutritional factors

Tannin content was determined colorimetrically (AOAC, 1990). Oxalic acid was determined by a titrimetric method (Wilson, Shaw, & Knight, 1982). Phytate was determined by a colorimetric method (Wheeler & Ferrel, 1971). All the experiments were carried out in triplicate and the variations in the values expressed as standard deviations.

2.10. Bioavailablity of iron and zinc

Bioavailabilities of iron and zinc were estimated by the method of equilibrium dialysis (Luten et al., 1996). In order to dephytinize, exogenous phytase and citric acid were used. All the experiments were carried out in triplicate and the variations in the values expressed as error bars.

3. Results and discussion

3.1. Weight equivalence of cumin in different extracts

The weight equivalence of cumin extracts in different solvents is shown in Table 1. In terms of weight equivalence of cumin in hot water and saline extracts (per ml of extract) the solubles were minimum (0.16 g), whereas the weight equivalence of essential oil was maximum (10.66 g), with more solubles per ml.

Table 1
Weight equivalence of cumin in various cumin extracts

Extracts	Quantity of cumin used for extraction (g)	Quantity of extract obtained (ml)	Weight equivalence of cumin extract (g/ml)
Hot water extract	100	600 ± 5	0.16 ± 0.02
Saline extract	100	600 ± 5	0.16 ± 0.02
Essential oil	100	3.4 ± 0.5	29.4 ± 1
Oleoresin	100	9.2 ± 1	10.66 ± 1

3.2. Total polyphenol content

The polyphenol contents in different cumin extracts are shown in Table 2. Comparing the extractability of phenolic compounds per mg of cumin in the extracts, the results show maximum phenol content present in hot water extract, followed by saline extract, then oleoresin, with least in the essential oil. The results showed that aqueous solutions were more efficient than organic solvents for extracting phenolic compounds from cumin. The results showed that extraction of phenolic compounds in steam-distilled essential oil fraction was much less. This could be due to the polar nature of phenolic compounds, resulting in their poor extractability in essential oil.

3.3. Antioxidant activity

The IC_{50} values of antioxidant activity for various cumin extracts were estimated. The highest antioxidant activity was exhibited by saline extract, followed by hot water extract and oleoresin as shown in Table 2. The essential oil exhibited less antioxidant activity. This shows that the salt-water fraction of cumin finds possible use as an antioxidant in a variety of processed foods, wherein cumin and salt are common ingredients, which are generally involved. Moreover, the higher antioxidant activity exhibited by the saline extract shows that the antioxidant principles of cumin, or its synergists, are better extracted in aqueous media containing salt. The higher osmotic pressure of the saline solution might be responsible for the higher extraction of antioxidant or synergistic compound, resulting in better antioxidant activity by the saline extract. The polyphenol contents of different extracts were also examined in an attempt to broadly identify the functional group responsible for the antioxidant activity of these extracts.

Table 2
Phenol content and antioxidant activity of various cumin extracts

Extracts	Phenol content per mg of cumin[a] (μg)	IC_{50} value for antioxidant activity[a] (g)
Hot water extract	13.1 ± 2.2	0.124 ± 0.03
Saline extract	9.5 ± 1.2	0.09 ± 0.01
Essential oil	0.21 ± 0.01	54.7 ± 2
Oleoresin	2.76 ± 0.01	2.65 ± 0.02

[a] Values are averages of triplicate determinations.

3.4. Effect of various cumin extracts on the digestive enzymatic activities (in vitro)

3.4.1. Amylase activity

The effects of various cumin extracts on amylase activity were assayed and are shown in Table 3. The water-soluble cumin extracts showed increase in the amylase activity with a maximum in the hot water extract, followed by saline extract. However, the amylase activity was not affected by the presence of essential oil and oleoresin from cumin.

3.4.2. Proteolytic activity

The effect of different cumin extracts on proteolytic activity is shown in Table 3. Both hot-water and saline extracts of cumin showed a significant increase in the proteolytic activity. Cumin oleoresin and essential oil did not show any enhancing effect, (similar to the amylase activity). In order to eliminate the effect of inherent proteolytic enzymes in these extracts, the hot-water and saline extracts were boiled and again checked for protease activity. However, with the boiled hot-water and saline, extracts also showed similar increase in the proteolytic activity. Thus it can be concluded that hot-water and saline extracts of cumin causes an enhancement of proteolytic activity at pH 6.2 at 37 °C. The compound responsible for this increase in activity needs to be elucidated.

3.4.3. Lipase activity

The effects of various cumin extracts on lipase activity were assayed, and are shown in Table 3. There is an increase in the lipase activity in the presence of water-soluble and saline-soluble extracts, with no effect shown by the presence of essential oil and oleoresin extracts.

3.4.4. Phytase activity

The effects of various cumin extracts on phytase activity were assayed, and are shown in Table 3. The results showed maximum increase in the phytase activity in presence of hot water and saline extracts, oleoresin and essential oil did not

Table 3
Effects of different cumin extracts on amylase, protease, lipase and phytase activity

Extracts[a] specific activity	Control	Hot water extract	Saline extract	Essential oil	Oleoresin
Amylase U/mg protein/g cumin/min	4.0 ± 0.1	300 ± 63	269 ± 32	1.80 ± 0.1	3.7 ± 0.5
Protease HUT/mg protein/g cumin/min	183.6 ± 1.2	7250 ± 331	8450 ± 380	13.0 ± 0.4	4.3 ± 1.8
Lipase U/mg protein/g cumin/min	2.94 ± 0.5	37.15 ± 5	36.98 ± 5	0.21 ± 0.01	0.58 ± 0.1
Phytase U/mg protein/g cumin/min	1.66 ± 0.5	196 ± 20	150 ± 15	0.73 ± 0.01	1.66 ± 0.5

[a] Values are averages of triplicate determinations.

show any significant effect. Thus the increase in phytase activity in the presence of the cumin extracts would play a great role in degrading the phytic acid present and thereby increasing the bioavailability of iron.

Our results have shown that hot water and saline extracts of cumin show significant increase in amylase, proteolytic, lipase and phytase activities. Also, the spent cumin showed similar effects on these enzymatic activities.

The digestive stimulant action of spices has long been recognized. Several commonly used spices have stimulatory influences on digestive enzymes of pancreas and intestinal mucosa (Glatzel, 1968). Earlier studies have shown that spices have active principles to stimulate bile flow and increase bile acids that have an important role in digestion and absorption of food lipids. The digestive stimulatory action of spices may be through stimulation of activities of enzymes that participate in digestion (Platel & Srinivasan, 1991).

3.5. Nutritional composition of spent cumin

The total protein, fat and carbohydrate contents of the spent cumin were estimated and are shown in Table 4. Protein was found to be 19% and fat 10%. They were high compared with other cereals, e.g. ragi (7.3% protein, 1.3% fat), wheat flour (11.8% protein, 1.7% fat), milled rice (6.8% protein, 0.5% fat) and jowar (10.4% protein, 1.9% fat) (Gopalan, Rama Sastri, & Balasubramanian, 1989). Spent cumin is a rich source of iron and zinc, as shown in Table 4. These values are also high compared to other cereals, e.g. ragi (3.9 mg iron, 2.3 mg zinc), wheat flour (4.9 mg iron, 2.7 mg zinc), milled rice (0.7 mg iron, 1.4 mg zinc), jowar (4.1 mg iron, 1.6 mg zinc) per 100 g. Having the above composition, the spent cumin may have a high potential as a basic ingredient for various food formulations. Total dietary fibre in spent cumin is very high: insoluble fibre 60% and soluble fibre 5.5%. Insoluble fibre is high compared with other commonly consumed cereals, e.g. ragi (9.9% insoluble fibre, 1.6% soluble fibre), wheat flour (9.6% insoluble fibre, 2.9% soluble fibre), rice (3.2% insoluble fibre, 0.9% soluble fibre) and jowar (8.0% insoluble fibre, 1.7% soluble fibre). Riboflavin, niacin and thiamine were estimated in the spent cumin and the results are shown in Table 4. Vitamins are organic substances present in small amounts in many foods. They are required for carrying out many vital functions of the body. Spent cumin has significant amounts of all vitamins compared with other cereals, e.g. bajra (0.25 mg riboflavin, 2.3 mg niacin), ragi (0.19 mg riboflavin, 1.1 mg niacin), wheat flour (0.17 mg riboflavin, 5.5 mg niacin), milled rice (0.06 mg riboflavin, 1.9 mg niacin), jowar (0.13 mg riboflavin, 3.1 mg niacin), per 100 g.

Some part of the daily requirement of niacin, thiamine and riboflavin can be met with the spent cumin. The antinutritional factors, such as tannin, oxalic acid and phytate, are present in low quantity, which is shown in (Table 4), thus making it a good candidate as a bionutrient. Phytate, a known potent inhibitor of iron absorption, binds with iron and zinc and forms insoluble complexes, rendering the minerals unavailable (Hurrell, 2004). Dephytinization can therefore be one of the tools for improving the bioavailability of iron.

3.6. Bioavailability of iron and zinc

In order to increase the iron bioavailability, the spent cumin was treated with the enzyme phytase in different ratios (1:2000, 1:1000, and 1:50) in order to degrade phytic acid. There was a gradual increase in bioavailability of iron and zinc with increase in enzyme concentration, with an optimum enzyme substrate ratio of 1:1000 (Fig. 1). There was 1.1-fold increase in bioavailable iron and 1.06-fold increase in bioavailable zinc. After optimizing the phytase concentration, the spent cumin was treated with different concentrations of citric acid (2 mM, 5 mM, and 20 mM), in combination with the phytase, to further enhance min-

Table 4
Nutritional composition of spent cumin

Components	Content[a] (per 100 g)
Moisture (g)	10.0 ± 1.0
Ash (g)	9.0 ± 0.05
Carbohydrate (g)	23.0 ± 0.5
Protein (g)	19.0 ± 0.5
Fat (g)	10.0 ± 0.5
Dietary fibre (Soluble)	5.5 ± 0.5
Iron (mg)	6.0 ± 0.5
Zinc (mg)	6.5 ± 0.5
Riboflavin (mg)	0.28 ± 0.05
Thiamine (mg)	0.05 ± 0.005
Niacin (mg)	2.7 ± 0.05
Tannin (g)	0.01 ± 0.007
Oxalic acid (g)	0.08 ± 0.006
Phytic acid (g)	1.7 ± 0.03

[a] Values are averages of triplicate determinations.

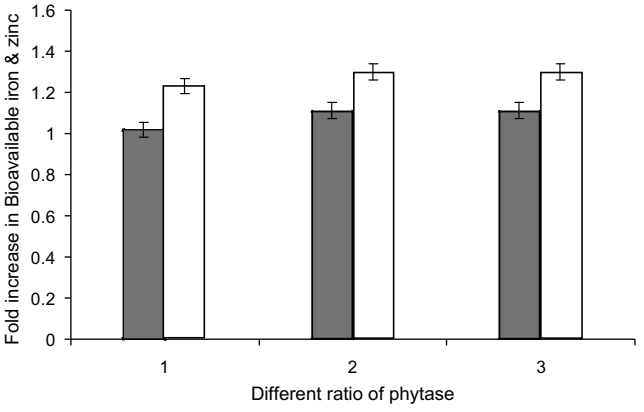

Fig. 1. Effects of different concentrations of phytase on bioavailability of iron and zinc present in the spent cumin. The ratios of enzyme to substrate are shown as 1.1:2000, 2.1:1000, 3.1:50 ■ iron □ zinc.

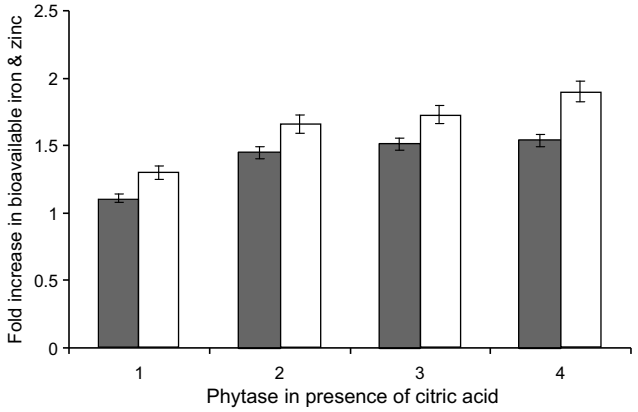

Fig. 2. Effects of different concentrations of citric acid in the presence of phytase (1:1000) on the bioavailability of iron and zinc in the spent cumin. 1. phytase, 2. phytase + 2 mM citric acid, 3. phytase + 5 mM citric acid, 4. phytase + 20 mM citric acid ■ iron □ zinc.

eral absorption (Fig. 2). The results showed 1.4–1.5-fold increase in bioavailable iron and zinc in the presence of a 2–20 mM concentration of citric acid, however, further increase in the concentration of citric acid had no beneficial effect on dialyzability of iron and zinc.

4. Conclusion

The analytical data on saline and hot water extracts of cumin showed higher protease, lipase and amylase activities thereby showing a potential role in enhancing the digestive enzymatic activities. These extracts enhance the phytase activity, thereby improving the bioavailability of micronutrients. The enhanced enzymatic activity is retained, even after boiling the saline and hot water extracts, indicating the possible presence of bioactive components in these extracts. Our results also show that saline and hot aqueous extracts have higher polyphenol content and antioxidant activities than have essential oil and oleoresin, along with increased capacity for digestive enzymes. Similar results were observed with the spent cumin (results not shown), with respect to increased enzymatic activity. The underutilized spent cumin, with good nutrient composition, can be a good substitute for the conventional foodstuffs in health food formulations and can find application in therapeutic foods. Thus, spent cumin has great potential for producing healthy and functional products that might also strengthen its industrial use as protein supplement.

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