In vitro influence of spices and spice-active principles on digestive enzymes of rat pancreas and small intestine

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In vitro influence of 14 individual spices (curcumin, capsaicin, piperine, garlic, onion, ginger, mint, coriander, cumin, ajowan, fennel, fenugreek, mustard, and asafoetida) on the activities of digestive enzymes of rat pancreas and small intestine was examined by including them in the reaction mixture at two different concentrations. A majority of spices enhanced the activity of pancreatic lipase and amylase when they are directly in contact with the enzyme. It is inferred that this positive influence on the activity of enzymes may have a supplementary role in the overall digestive stimulant action of spices, besides causing an enhancement of the titres of digestive enzymes in pancreatic tissue.

1 Introduction

Spices, which are used as food additives to enhance the taste and flavour of food, are also understood to exhibit a wide range of beneficial physiological effects and are used as ingredients of several medicinal preparations in the indigenous system of medicine [1]. In India, spices are important commercial crops from the point of view of both domestic consumption and export. Most of the spices are consumed in seasoning, curry powders, pickles, meat and meat products and commercial bakery products. Studies in the past three decades have experimentally documented several health-beneficial physiological effects of spices. The digestive stimulant action of spices is probably the most commonly experienced beneficial physiological effect of these food additives. The earliest report on the effect of spices on digestion is that of Glatzel [2], documenting an enhanced secretion of saliva and activity of salivary amylase by spice treatment in human subjects. It is reported that chilli has a great salivary stimulating capacity. Salivary and gastric secretions are increased when the sense of smell and presence of certain stimulants in foodstuffs stimulate the nerve centers [3]. Recent studies have revealed that spices or their active principles stimulate bile flow and enhance bile acid secretion, which play an important role in the digestion of dietary lipids [4–7]. In addition to an effect on bile secretion, spices also stimulate activities of digestive enzymes of pancreas and small intestine [8–11].

We report on the *in vivo* influence of 14 individual spices, *viz*., curcumin, capsaicin, piperine, garlic, onion, ginger, mint, coriander, cumin, ajowan, fennel, fenugreek, mustard, asafoetida, and three spice combinations on the activities of digestive enzymes of the pancreas [8, 10–12]. These studies have evidenced that several of the test spices produce pronounced stimulatory influence on these enzymes as a result of continued dietary intake or a one-time oral exposure. Such a stimulation of pancreatic digestive enzymes could be a major mechanism by which spices exert their digestive stimulant action. It is of interest to know if the mere presence of relevant spice or spice principles in proximity to the digestive enzymes would have a similar desirable effect on their activity. The present investigation was therefore undertaken to examine the *in vitro* influence of 14 individual spices (curcumin, capsaicin, piperine, garlic, onion, ginger, mint, coriander, cumin, ajowan, fennel, fenugreek, mustard, and asafoetida) on the activities of digestive enzymes of rat pancreas and small intestine. In this context, lipase, amylase and chymotrypsin of rat pancreas and the disaccharidases of small intestinal mucosa have been examined with each spice included in the reaction mixture at two different concentrations.

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2 Materials and methods

2.1 Materials

Fresh spices (garlic, onion, ginger, mint, coriander, cumin, ajowan, fennel, fenugreek, mustard, and asafoetida) were procured locally, dried then finely powdered and stored in sealed containers till their use. The three spice principles – capsaicin, curcumin and piperine – were procured from Fluka Chemie (Buchs, Switzerland). The spice powders were thoroughly homogenized with 0.9% saline to form a uniform suspension. These spice suspensions were added to the enzyme reaction mixtures to give two different spice concentrations, namely 0.1 mg/mL and 1.0 mg/mL. The spice principles (capsaicin, curcumin, piperine) were dissolved in acetone to obtain a stock solution. Suitable aliquots of these stock solutions were added to the reaction mixtures to deliver 0.5 and 5.0 µg/mL as the two concentrations for capsaicin, 5.0 and 50 µg/mL for curcumin, and 7.5 and 75 µg/mL in the case of piperine. These concentrations corresponded to 0.1 and 1.0 mg/mL of the respective parent spices.

2.2 Preparation of rat pancreatic homogenate and intestinal mucosal scrapings

Adult female Wistar rats (200–220 g) from the stock colony of the Experimental Animal Production Facility of this Institute were used for obtaining pancreas and small intestine. The animals were sacrificed under ether anaesthesia. Pancreas and small intestine (20–25 cm long segments between jejunum and caecum leaving about 5 cm on either side) were excised immediately and collected in ice-cold saline. Intestines were flushed with ice-cold saline and cut open longitudinally. Mucosa was scraped with a glass microscopic slide. The pancreas and intestinal mucosal scrapings were then homogenised in a Potter-Elvehjem homogeniser with physiological saline to give approximately 10% homogenate and were used for the enzyme assays in the presence of various spices. For the sake of convenience, the *in vitro* studies with 14 different spice materials were divided and carried out in four different sets of experiments performed on different days using entirely different batches of animals.

Table 1. Influence of spice principles (capsaicin, curcumin and piperine) and spices (garlic, onion, ginger and mint) on pancreatic digestive enzymes *in vitro*

		Enzyme activity		
Spice/principle	Conc. (mg/mL)	Lipase[a)]	Amylase[b)]	Chymotrypsin[c)]
Control		12.4 ± 1.21	0.302 ± 0.016	3.33 ± 0.37
Capsaicin (µg)	0.5	10.6 ± 1.15	0.343 ± 0.043	3.05 ± 0.44
	5.0	11.8 ± 1.88	0.361 ± 0.046* (20%)	3.57 ± 0.51
Curcumin (µg)	5.0	12.5 ± 2.30	0.364 ± 0.043* (21%)	
	50	28.0 ± 3.30* (126%)	0.380 ± 0.029* (26%)	
Piperine (µg)	7.5	20.6 ± 2.28* (66%)	0.378 ± 0.040* (25%)	4.05 ± 0.61
	75	17.1 ± 2.85* (38%)	0.395 ± 0.032* (31%)	3.84 ± 0.42
Control		11.7 ± 1.05	0.249 ± 0.020	3.52 ± 0.36
Garlic (mg)	0.1	15.6 ± 0.86* (33%)	0.250 ± 0.027	3.54 ± 0.49
	1.0	15.2 ± 1.19* (29%)	0.296 ± 0.021* (19%)	2.55 ± 0.31** (27%)
Onion (mg)	0.1	16.3 ± 1.32* (39%)	0.276 ± 0.040	3.19 ± 0.33
	1.0	13.9 ± 1.34	0.310 ± 0.027* (24%)	3.04 ± 0.26
Ginger (mg)	0.1	23.5 ± 1.79* (100%)	0.258 ± 0.006	3.17 ± 0.19
	1.0	23.3 ± 2.43* (99%)	0.385 ± 0.013* (55%)	1.08 ± 0.10** (69%)
Mint (mg)	0.1	23.8 ± 1.35* (103%)	0.225 ± 0.009	3.45 ± 0.45
	1.0	18.0 ± 1.28* (54%)	0.320 ± 0.024* (31%)	1.38 ± 0.28** (61%)

Values are mean ± SEM of six independent determinations. Values in parentheses indicate % difference compared to control value.
Specific activity units:
a) µmol FFA released/min/mg protein
b) µmol maltose liberated/min/mg protein
c) nmol *p*-nitroaniline released/min/mg protein
* Significant increase over the control value ($P < 0.05$)
** Significant decrease compared to control value ($P < 0.05$)

2.3 Enzyme activity determinations

The activities of lipase, amylase and chymotrypsin were determined using standard procedures [13–15]. Aliquots of pancreatic homogenates for various enzyme activity determinations were pre-equilibrated with the different spices added at two concentrations for 5 min at the reaction temperature. Lipase was assayed by aerobically incubating the pancreatic homogenate with olive oil suspension in 0.9% saline in 0.2 M Tris-HCl buffer, pH 8.5, and reacting the released free fatty acids with copper nitrate; the copper-bound free fatty acids extracted into chloroform were then determined by a colour reaction with diethyl dithiocarbamate [13]. Amylase activity was determined using starch as substrate and measuring the maltose released by reacting with 3,5-dinitrosalicylic acid according to Bergmeyer [14]. Chymotrypsin in pancreatic tissue samples was assayed using succinyl phenylalanine *p*-nitroanilide as substrate by following the release of *p*-nitroaniline [15] after pre-activation with enterokinase, according to the procedure of Lewis *et al.* [16]. The enzyme activity was computed using the molar extinction coefficient of *p*-nitroaniline (10.2). Disaccharidases (sucrase, lactase and maltase) were assayed by incubating the mucosal preparation with the respective disaccharide in maleate buffer (pH 6.0) and measuring the released glucose by the glucose oxidase method [17]. Protein content in the pancreatic homogenate and intestinal mucosal samples was estimated by the modified method of Lowry *et al.* [18]. The analytical data were subjected to statistical analysis by the Student's *t*-test, as described by Snedecor and Cochran [19].

3 Results and discussion

In the present study, 14 spices or their active principles have been examined for their *in vitro* influence on digestive enzymes of the pancreas by including them at two concentrations in the reaction mixture, namely, 0.1 and 1.0 mg/mL. In the case of the three spice principles – capsaicin, curcumin and piperine – the concentrations tested were equivalent to the above two concentrations of the respective parent spices, *i.e.*, red pepper, turmeric, and black pepper. The influence of the three spice principles – capsaicin, curcumin and piperine – and the spices – garlic, onion, ginger and mint – on the pancreatic digestive enzymes is shown in Table 1. While capsaicin did not have any influence on the activity of pancreatic lipase, curcumin at 50 µg/mL concentration, and piperine at both concentrations tested significantly enhanced it. The extent of stimulation was as high as 126% in the case of curcumin and 66 and 38% in the case of the two concentrations of piperine, respectively. All the three spice principles enhanced the activity of pancreatic amylase by 20–31%. The activity of chymotrypsin remained unaltered in the presence of capsaicin and piperine at both the concentrations. Due to interference by the colour of curcumin in the method employed, the effect of this compound on the activity of pancreatic chymotrypsin could not be measured.

All four spices generally stimulated the activity of lipase at both the concentrations tested. The maximum effect was produced by ginger, which was about 100% at both the concentrations, and mint, which produced 103 and 54% stimulation at the two concentrations, respectively. The stimulation of this

Table 2. Influence of spices (coriander, cumin, ajowan, fennel, fenugrek, mustard and asafoetida) on pancreatic digestive enzymes *in vitro*

Spice	Conc. (mg/mL)	Enzyme activity		
		Lipase[a)]	Amylase[b)]	Chymotrypsin[c)]
Control		11.4 ± 1.32	0.343 ± 0.050	2.98 ± 0.38
Coriander	0.1	8.18 ± 1.09** (28%)	0.561 ± 0.048* (64%)	2.91 ± 0.22
	1.0	14.3 ± 0.20* (26%)	0.468 ± 0.028* (36%)	3.02 ± 0.41
Cumin	0.1	10.2 ± 1.66	0.503 ± 0.027* (46%)	2.20 ± 0.28** (26%)
	1.0	12.0 ± 2.29	0.368 ± 0.032	2.89 ± 0.30
Ajowan	0.1	15.5 ± 1.04* (36%)	0.455 ± 0.008* (32%)	3.11 ± 0.22
	1.0	21.0 ± 2.90* (84%)	0.451 ± 0.017* (31%)	2.68 ± 0.21
Fennel	0.1	15.6 ± 1.02* (37%)	0.390 ± 0.058	2.86 ± 0.21
	1.0	16.5 ± 2.18* (45%)	0.407 ± 0.050	2.62 ± 0.23
Control		12.3 ± 0.87	0.250 ± 0.044	4.12 ± 0.36
Fenugreek	0.1	5.73 ± 0.49** (53%)	0.314 ± 0.032* (25%)	4.33 ± 0.62
	1.0	6.19 ± 0.92** (50%)	0.430 ± 0.062* (72%)	2.95 ± 0.38** (28%)
Mustard	0.1	10.9 ± 2.04	0.224 ± 0.013	3.79 ± 0.26
	1.0	17.8 ± 0.92* (45%)	0.831 ± 0.079* (232%)	3.90 ± 0.23
Asafoetida	0.1	8.90 ± 1.27** (28%)	0.420 ± 0.062* (68%)	3.49 ± 0.58
	1.0	9.97 ± 1.45** (19%)	0.500 ± 0.092* (100%)	3.84 ± 0.57

Values are mean ± SEM of six independent determinations. Values in parentheses indicate % difference compared to control value.
Specific activity units:
a) µmol FFA released/min/mg protein
b) µmol maltose liberated/min/mg protein
c) nmol *p*-nitroaniline released/min/mg protein
* Significant increase over the control value ($P < 0.05$)
** Significant decrease compared to control value ($P < 0.05$)

enzyme was about 30 and 40% by garlic and onion, respectively. All four spices enhanced the activity of amylase only at the higher concentration tested. As in the case of lipase, ginger produced the highest stimulation of amylase (55%), followed by mint (31%), onion (24%), and garlic (19%). Contrary to the positive influence of these spices on lipase and amylase, ginger, mint and garlic decreased the activity of chymotrypsin when included at a concentration of 1 mg/mL.

The influence of coriander, cumin, ajowan, fennel, fenugreek, mustard, and asafoetida on pancreatic digestive enzymes is presented in Table 2. Both ajowan and fennel significantly enhanced the activity of lipase (36 and 84% by ajowan and 37 and 45% by fennel, depending on the concentrations tested). Coriander had a similar effect (26% increase) only at the higher concentration, while the lower concentration produced the opposite effect. Mustard also stimulated lipase activity (45% increase) when present at the higher concentration. Fenugreek and asafoetida, on the other hand, decreased the activity of lipase independently of the tested concentrations. This decrease was in the range of 50–53% in the case of fenugreek and 19–28% in the case of asafoetida depending on the concentration used. Cumin did not influence the activity of this enzyme. The activity of amylase was generally stimulated by the presence of the tested spices, the extent of stimulation being 68 and 100% for asafoetida, 25 and 72% for fenugreek, 64 and 36% for coriander, 32 and 31% for ajowan at the two concentrations tested and as high as 232% for the higher concentration of mustard. Cumin added at the highest concentration and fennel at the two concentrations used had no effect on amylase activity. The activity of chymotrypsin was not affected by any of these spices except for cumin at the lower concentration and fenugreek at the higher concentration, which reduced the activity by 26 and 28%, respectively.

The *in vitro* influence of various test spices and spice principles on intestinal disaccharidases is presented in Tables 3 and 4. Unlike their influence on pancreatic lipase and amylase, these spices either had no influence or negatively influenced the activities of disaccharidases – sucrase, lactase and maltase. Onion, ginger, mint, ajowan and fennel decreased the activities of all the three disaccharidases especially at the higher concentration (1 mg/mL). Piperine, cumin and coriander did not have any influence on these enzymes, whatever the tested concentrations. Among the disaccharidases, maltase was inhibited by most of the spices (excluding piperine, cumin and coriander) at the higher concentration.

The observations of the present study indicate that the physical presence of the tested spices at the site of enzyme action generally has a favourable influence on the activity of both pancreatic lipase and amylase. Our earlier animal studies have documented the positive influence of dietary intake of capsaicin, curcumin, piperine, ginger, ajowan, fennel and asafoetida, and of a single oral dose of mint on the activity of pancreatic lipase [8, 10, 11]. Continued intake of capsaicin, curcumin, piperine, ginger, cumin, asafoetida and onion and single oral dose of capsaicin, piperine and fennel are also evidenced to produce a beneficial stimulatory influence on pancreatic amylase [10, 11]. Dietary capsaicin, curcumin, piperine, ginger, coriander, cumin, fenugreek, asafoetida and onion reportedly stimulate the activity of chymotrypsin [10, 11].

While the earlier *in vivo* studies have documented the beneficial influence of these spices essentially on the titres of digestive enzymes in pancreatic tissue, the current study showed a favourable influence of a majority of these tested spices on the activity *per se* of these enzymes when added to the reaction mixture. This beneficial stimulatory influence of the presence of these spices on the activities of pancreatic

Table 3. Influence of spices principles (capsaicin, curcumin and piperine) and spices (garlic, onion, ginger and mint) on intestinal disaccharidases *in vitro*

		Enzyme activity		
Spice/principle	Conc. (mg/mL)	Sucrase	Lactase	Maltase
Control		72.2 ± 2.54	17.0 ± 0.94	372.7 ± 15.5
Capsaicin (µg)	0.5	69.2 ± 0.83	16.4 ± 1.07	304.4 ± 16.5** (18%)
	5.0	73.1 ± 2.98	16.2 ± 1.69	296.2 ± 20.8** (21%)
Curcumin (µg)	5.0	68.0 ± 1.21	16.4 ± 0.51	377.5 ± 10.3
	50.0	45.1 ± 2.08** (38%)	16.8 ± 0.33	232.2 ± 35.1** (38%)
Piperine (µg)	7.5	70.8 ± 2.02	16.1 ± 1.42	348.0 ± 10.1
	75.0	71.3 ± 2.53	16.1 ± 1.27	353.3 ± 10.8
Control		70.2 ± 3.16	19.5 ± 1.15	411.1 ± 7.43
Garlic (mg)	0.1	71.8 ± 1.72	14.9 ± 0.65** (24%)	345.5 ± 11.8** (16%)
	1.0	76.6 ± 0.81	16.2 ± 0.47** (17%)	344.7 ± 7.18** (16%)
Onion (mg)	0.1	77.9 ± 2.47	17.0 ± 0.98** (13%)	344.3 ± 9.13** (16%)
	1.0	51.5 ± 2.05** (27%)	11.7 ± 0.86** (40%)	223.6 ± 7.10** (46%)
Ginger (mg)	0.1	69.0 ± 2.34	22.1 ± 1.52	314.6 ± 18.5** (23%)
	1.0	60.8 ± 2.63** (13%)	16.4 ± 1.62** (16%)	268.0 ± 20.5** (35%)
Mint (mg)	0.1	72.1 ± 1.69	15.2 ± 1.17** (22%)	349.6 ± 14.2** (15%)
	1.0	48.7 ± 1.55** (31%)	14.5 ± 1.10** (26%)	150.8 ± 9.21** (63%)

Values are mean ± SEM of six independent determinations. Values in parentheses indicate % difference compared to control value.
Specific activity units: nmol glucose released/min/mg protein
** Significant decrease

Table 4. Influence of spices (coriander, cumin, ajowan, fennel, fenugrek, mustard and asafoetida) on intestinal disaccharidases *in vitro*

		Enzyme activity		
Spice	Conc. (mg/mL)	Sucrase	Lactase	Maltase
Control		74.0 ± 3.82	17.1 ± 0.92	376.4 ± 12.3
Coriander	0.1	73.3 ± 2.41	15.8 ± 1.07	383.5 ± 16.1
	1.0	71.9 ± 1.36	14.6 ± 1.14	351.2 ± 11.2
Cumin	0.1	75.8 ± 1.72	16.5 ± 0.53	397.9 ± 25.4
	1.0	68.9 ± 0.99	14.7 ± 0.83	356.7 ± 13.2
Ajowan	0.1	66.6 ± 4.93	14.2 ± 1.74	368.3 ± 14.3
	1.0	55.0 ± 5.73** (26%)	12.7 ± 1.14** (26%)	279.7 ± 12.2** (26%)
Fennel	0.1	55.2 ± 6.90** (26%)	15.7 ± 1.26	391.0 ± 12.4
	1.0	64.9 ± 5.28** (12%)	13.7 ± 1.38** (20%)	313.9 ± 7.84** (17%)
Control		65.7 ± 3.70	17.1 ± 0.76	374.8 ± 15.3
Fenugreek	0.1	63.3 ± 1.64	16.9 ± 1.86	336.5 ± 15.1
	1.0	67.4 ± 1.47	10.0 ± 0.90** (42%)	325.4 ± 9.19** (13%)
Mustard	0.1	66.1 ± 2.15	18.3 ± 2.12	355.2 ± 21.2
	1.0	51.9 ± 3.24** (21%)	18.9 ± 1.54	311.3 ± 4.56** (17%)
Asafoetida	0.1	60.3 ± 2.38	17.9 ± 2.72	258.6 ± 15.5** (31%)
	1.0	62.6 ± 2.15	20.7 ± 2.34	230.1 ± 6.05** (39%)

Values are mean ± SEM of six independent determinations. Values in parentheses indicate % difference compared to control value.
Specific activity units: nmol glucose released/min/mg protein
** Significant decrease

digestive enzymes, especially lipase and amylase, could be an additional factor contributing to the digestive stimulant action of spices through a stimulation of enzymes involved in digestion. Thus, spices, in addition to causing an enhancement of the titres of lipase, amylase and proteases in pancreatic tissue [10, 11], are also likely to further the beneficial effect by amplifying the activity of lipase and amylase when the pancreatic juice encounters them in the gastro-intestinal tract.

The pancreatic digestive enzymes hydrolyse proteins, starch and triglycerides in food into smaller molecules that are assimilable. Since digestion of fat is vital to the digestion of other macromolecules of food, the role of pancreatic lipase assumes greater significance. The significant enhancing *in vitro* influence of curcumin, piperine, ginger, garlic, onion, mint and mustard on pancreatic lipase in addition to a favourable *in vivo* influence on the enzyme titre suggests that these spices/spice principles have a beneficial stimulatory effect on digestion through stimulation of lipase. Stimulation of amylase also assumes greater significance in the Indian context, in view of the higher cereal content in Indian dietary, starch being their major ingredient. Similar to their influence on lipase, a majority of the tested spices have shown an enhancing *in vitro* influence on pancreatic

amylase. This, in addition to their positive effect on the enzyme titres *in vivo*, contributes to the digestive stimulant action of spices additionally through stimulation of amylase.

The decreased activity of chymotrypsin observed in the presence of a few of the tested spices could be attributed to the presence of protease inhibitors in these spices. This fact, however, needs to be verified. While the tested spices had a general stimulatory effect on pancreatic lipase and amylase *in vitro*, such a beneficial effect was not demonstrated in the case of intestinal disaccharidases. However, these latter enzymes may not contribute to the overall process of digestion as much as pancreatic enzymes; and hence the *in vitro* influence of spices on digestive process may still be considered positive.

In summary, the present investigation shows that a majority of spices enhance the activity of pancreatic lipase and amylase when they are present at the site of enzyme action. This *in vitro* positive influence on the activity of these enzymes may have an additional role in the overall digestive stimulant action of spices which are known to enhance the titres of digestive enzymes in pancreatic tissue.

4 References

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