<u>ANEXO IV</u>: ARTÍCULO DE INVESTIGACIÓN EMPLEADO EN LA PROPUESTA METODOLÓGICA.

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Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats

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A few common spices or their active principles, were examined for their possible influence on digestive enzymes of intestinal mucosa in experimental rat. The animals were fed the following diets for 8 weeks: control, curcumin (0.5%), capsaicin (15 mg%), piperine (20 mg%), ginger (50 mg%), cumin (1.25%), fenugreek (2%), mustard (250 mg%) and asafoetida (250 mg%). Dietary curcumin, capsaicin, piperine and ginger prominantly enhanced intestinal lipase activity and also the disaccharidases sucrase and maltase. Dietary cumin, fenugreek, mustard and asafoetida brought about decreases in the levels of phosphatases and sucrase. The positive influences of a good number of spices on these terminal enzymes of digestive process could be an additional feature of spices that are generally well recognized to stimulate digestion.

Introduction

The use of spices as food additives is widely practised since ancient times. Spices have a definite role to play in enhancing the taste and flavour of any food. Even a nutritious food is not acceptable if it is not adequately spiced. Besides, spices also exhibit several beneficial physiological effects, and have been used in the indigenous system of medicine in India (Sreenivasamurthy & Krishnamurthy, 1959; Anon, 1987). Spices have been generally believed to intensify salivary flow and gastric juice secretion, and hence aid in digestion (Glatzel, 1968).

Earlier studies from our laboratory have revealed that certain spices and their active principles stimulate bile flow and influence bile composition (Ganesh Bhat *et al.*, 1984, 1985; Ganesh Bhat & Chandrasekhara, 1987; Sambaiah & Srinivasan, 1991). Since spices are claimed to aid digestion, it is of interest to examine if they have any stimulatory effect on the digestive enzymes. Information on this aspect of spices available in literature is inadequate. The present investigation was therefore undertaken to study the influence of certain common spices / their active principles on the digestive enzymes of the rat intestinal mucosa. The spices studied in this context are: asafoetida (*Ferula asafoetida* Linn.), cumin (*Cuminum cyminum* Linn.), fenugreek (*Trigonella foenumgraecum* Linn.), ginger (*Zingiber officinale*), mustard (*Brassica nigra* Linn.), black pepper (*Piper nigrum* Linn.), red pepper (*Capsicum annum* Linn.) and turmeric (*Curcuma longa* Linn.).

Asafoetida is mainly used as a condiment and is known to exert carminative, antispasmodic and laxative properties (Chopra *et al.*, 1958; Ramachandran & Ambasta, 1986). Cumin and fenugreek are common ingredients of curry powders and are also used for seasoning. As an

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ingredient of indigenous medicine cumin is a digestive stimulant and carminative, while fenugreek possesses carminative, tonic and galactogogue properties (Chopra *et al.*, 1958; Ramachandran & Ambasta, 1986). Ginger is used in a variety of foods both in fresh and dry forms and also in carbonated drinks. It is used in medicine as a carminative and stimulant and also for flatulence and colic (Anon, 1987; Ramachandran & Ambasta, 1986). Mustard is largely used in seasoning and in pickles. Medicinally mustard is vermicidal, antihelmenthic and gastric stimulant (Krithikar & Basu, 1935; Ramachandran & Ambasta, 1986). Black pepper, the source of piperine, is a common ingredient of curry powders. It is also prescribed for cholera and various other gastric ailments (Chopra *et al.*, 1958). Red pepper, the source of capsaicin is widely used in pickles, sauces, ketchups, etc. besides being used for seasoning. Apart from this, red peppers are used in Indian medicine against typhus, dropsy, gout, dyspepsia, etc. (Anon, 1987). Turmeric, which is the source of curcumin, is a versatile commodity with innumerable uses. It is an important constituent of curry powder; is used as a colouring agent in food preparations and in pickles as a preservative. In the Indian system of medicine, it is used in a number of gastrointestinal disorders (Anon, 1987).

Materials and methods

Female Wistar rats weighting 120 ± 10 g were used in this study. Spice principles capsaicin and piperine were obtained from Fluka Chemie, Switzerland, while curucumin was purchased from M/s Flavours & Essences Pvt. Ltd, Mysore. Spices ginger, cumin, fenugreek, mustard and asafoetida were procured from the local market, cleaned and powdered to pass through a 50-mesh sieve. All the chemicals and fine chemicals used were of analytical grade.

Groups of 8 rats were housed in individual cages and the animals had free access to food and water. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; refined peanut oil, 10; vitamin mixture, 1; and salt mixture, 4. The various spice diets consisted of respective spice/spice principle incorporated into this basal diet substituting an equivalent amount of corn starch at the following levels (%): Ginger, 50 mg; cumin, 1.25 g; fenugreek, 2 g; mustard, 250 mg; asafoetida, 250 mg, curcumin, 0.5 g; capsaicin, 15 mg; and piperine, 20 mg. The animals were thus maintained on *ad libitum* spice diets for 8 weeks.

At the end of the feeding trial, the animals were sacrificed under light ether anaesthesia. Small intestine (20–25 cm long segment between jejunum and caecum leaving about 5 cm on either side) was immediately excised and flushed with ice-cold 0.9% saline. The intestinal segments were then cut open longitudinally, and mucosa was scraped with a microscopic slide. The mucosal scrapings were homogenized in 0.9% saline and used for various enzyme assays.

Standard methods were employed for various enzyme activity determinations. Lipase was assayed by aerobically incubating the mucosal preparation with olive oil suspension in saline in a buffered medium of 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 color reaction with diethyl dithiocarbamate (Bergmeyer, 1974a). Activity of amylase was estimated by incubating the mucosal preparation with starch solution at pH 6.9 and reacting the released maltose with dinitrosalicylic acid (Bergmeyer, 1974b).

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 (Bergmeyer, 1974c). Alkaline and acid phosphatases in the mucosal preparations were measured using sodium-β-glycerophosphate as the substrate at appropriate pH and measuring the released inorganic phosphorus by the method of Hubscher & West (1965). Protein content of the mucosal preparation was determined by the phenol method of Lowry *et al.* (1951) using bovine serum albumin as standard. Statistical analysis of the analytical data was done using Students' *t*-test and a P-value < 0.05 was considered significant (Snedecor & Cochran, 1976).

Results and discussion

In the present study, spices/spice active principles have been fed to animals at levels corresponding to about 5 times the average human

Table 1. Effect of dietary spices on the activities of lipase, amylase and phosphatases of intestinal mucosa

Group	Lipase[a]	Amylase[b]	Alkaline phosphatase[c]	Acid phosphatase[c]
Control-I	25.7 ± 3.12	0.024 ± 0.0030	20.7 ± 1.50	1.07 ± 0.04
Curcumin	61.0 ± 5.17*	0.021 ± 0.0025	21.2 ± 0.72	1.35 ± 0.03*
Capsaicin	67.2 ± 6.90*	0.020 ± 0.0024	24.1 ± 1.08	1.53 ± 0.11*
Piperine	62.6 ± 3.87*	0.024 ± 0.0011	19.2 ± 1.40	1.09 ± 0.07
Ginger	44.8 ± 2.97*	0.025 ± 0.0013	22.4 ± 2.99	1.31 ± 0.11*
Control-II	20.5 ± 0.69	0.024 ± 0.0018	22.8 ± 1.29	1.14 ± 0.09
Cumin	24.7 ± 3.27	0.022 ± 0.0006	15.3 ± 0.42**	0.84 ± 0.03**
Fenugreek	18.0 ± 1.69	0.021 ± 0.0013	15.2 ± 0.74**	0.66 ± 0.05**
Mustard	13.3 ± 1.63**	0.016 ± 0.0017**	15.3 ± 0.88**	0.80 ± 0.03**
Asafoetida	19.1 ± 2.74	0.024 ± 0.0015	17.5 ± 1.07**	0.84 ± 0.05**

Values are mean ± SEM of 8 animals per group.
[a]nmol free fatty acids liberated/min/mg protein.
[b]μmol maltose liberated/min/mg protein.
[c]μmol inorganic phosphorus released/hr/mg protein.
*Significantly higher than corresponding control value ($P < 0.05$).
**Significantly lower than corresponding control value ($P < 0.05$).

dietary intake of these spices. The levels used here are based on calculated dietary intake of spices in the form of curry powder and on dietary survey conducted in India (Thimmayamma *et al.*, 1983). The food intake was essentially similar in various spice fed groups and corresponding control groups. Similarly the gain in body weights during 8 weeks spice treatment was comparable to controls (data not given).

The effects of selected spices and their active principles on intestinal mucosal lipase, amylase and phosphatases are presented in Table 1. It is evident that the spice principles curcumin, capsaicin and piperine and ginger prominently enhanced the activity of lipase. The stimulation of this enzyme activity was more than 100% of the control value particularly in the case of curcumin, capsaicin and piperine fed animals. While capsaicin produced maximum stimulation (161%) of this digestive enzyme, spices cumin, fenugreek, mustard and asafoetida did not produce any stimulation. On the otherhand, mustard produced about 35% decrease in lipase activity. None of the spices or spice principles

Table 2. Effect of dietary spices on the activities of disaccharidases of intestinal mucosa

Group	Sucrase	Lactase	Maltase
Control-I	71.5 ± 3.31	32.8 ± 1.14	555.3 ± 19.8
Curcumin	86.7 ± 1.76*	35.0 ± 1.18	618.5 ± 10.8*
Capsaicin	98.6 ± 2.59*	33.9 ± 0.81	670.5 ± 35.3*
Piperine	97.3 ± 5.05*	35.5 ± 2.29	720.8 ± 11.8*
Ginger	75.8 ± 5.45	29.4 ± 1.39	619.2 ± 15.0*
Control-II	77.6 ± 2.64	29.5 ± 2.37	513.5 ± 28.4
Cumin	63.4 ± 2.38**	27.4 ± 2.34	642.2 ± 40.8*
Fenugreek	54.1 ± 2.29**	26.2 ± 1.74	533.3 ± 33.8
Mustard	50.4 ± 1.20**	27.4 ± 1.09	466.2 ± 22.3
Asafoetida	55.0 ± 3.24**	29.1 ± 1.51	654.3 ± 46.8*

Values are Mean ± SEM of 8 animals per group.
Activity Units: nmol disaccharide hydrolysed/min/mg protein.
*Significantly higher than corresponding control value ($P < 0.05$).
**Significantly lower than corresponding control value ($P < 0.05$).

studied here produced any influence on intestinal amylase except that mustard again produced about 35% decreased activity of this enzyme.

The activity of acid phosphatase was significantly enhanced by curcumin, capsaicin and ginger, while these spices/spice principles did not influence alkaline phosphatase. The spices cumin, fenugreek, mustard and asafoetida brought about significant decreases in the activities of both alkaline and acid phosphatases.

Table 2 reveals the effect of dietary spices on disachharidases – sucrase, lactase and maltase of intestinal mucosa. Spice principles curcumin, capsaicin and piperine stimulated the activity of sucrase by 21, 38 and 36% respectively. On the other hand, spices – cumin, fenugreek, mustard and asafoetida brought about significant decreases in the activity of intestinal sucrase (decreases being 18, 30, 35 and 29% respectively). Lactase level of intestinal mucosa was unaffected by any of the spices/spice principles studied here. Maltase activity was significantly higher in animals fed spice principles – curcumin, capsaicin, piperine and in those fed spices – ginger, cumin and asafoetida. Dietary fenugreek and mustard did not have any effect on intestinal maltase activity.

An earlier study on the effect of asafoetida on digestive enzymes revealed an enhanced activity of pancreatic amylase and no significant effect on intestinal dehydrogenases, esterases, phosphatases and peptidases *in vitro* (Patwardhan & Sastry, 1957). Our study has revealed that dietary asafoetida had no effect on lipase, amylase and lactase; while it decreased the activities of phosphatases and sucrase, the level of maltase was enhanced. Sambaiah *et al.* (1978) have observed that red pepper or its active principle capsaicin lowers liver lipid accumulation with simultaneous elevation of serum total lipids. Here in our current study, capsaicin had a significant stimulatory effect on intestinal mucosal lipase, the enzyme that aids in digestion of fat. Stimulation of intestinal mucosal lipase by these spice principles curcumin, capsaicin, piperine and the spice ginger would probably mean a higher rate of fat digestion and absorption. Stimulation of sucrase and maltase by the spice principles curcumin, capsaicin and piperine could be a beneficial factor in the digestion of carbohydrates.

Glatzel (1968) has reported stimulation of secretion of saliva and the activity of salivary amylase by chilli, ginger, capsicum, pepper and mustard in human subjects. Salivary and gastric secretions are increased when the nerve centres are stimulated by the sense of smell and by the presence of certain irritants in the food stuff (Sreenivasamurthy & Krishnamurthy, 1959). Spices, by virtue of certain irritants they contain, and by imparting flavour to food stuffs, may also enhance salivary and gastric secretions. The role of spices in digestion is not limited to a single effect, but is a combination of their influences on salivary, gastric, pancreatic and biliary secretions and the terminal digestive enzymes present on the mucosa of small intestines.

Recent studies in our laboratory on experimental animals have established a positive influence of several common spices/or their active principles on bile juice production and secretion by the liver as well as on the lipid composition of the bile, especially bile acid secretion (Ganesh Bhat *et al.*, 1984; Ganesh Bhat *et al.*, 1985; Ganesh Bhat & Chandrasekhara, 1987; Sambaiah & Srinivasan, 1991). Higher rate of biliary secretion of bile acids have been documented in rats fed curcumin, capsaicin, ginger and fenugreek (Ganesh Bhat *et al.*, 1984, 1985). The role of bile and biliary bile acids in fat digestion and absorption is well known. Hence, stimulation of bile secretion and especially secretion of the bile with higher levels of bile salts is probably a major mechanism by which spices aid in digestion.

In summary, the present study has provided supplementary information on the possible influence of some selected spices commonly consumed on the terminal enzymes of small intestine that play a role in digestion. Among those screened, a good number of spices/spice active principles, *viz.*, curcumin, capsaicin, piperine and ginger have been found to generally stimulate activities of enzymes lipase and disaccharidases – sucrase and maltase. This could be an additional feature of spices that are well recognised to have a role in stimulation of digestion. A complete understanding of the mechanisms of stimulation of digestion by the food adjunct spices would however necessitate screening of the spices for their influence, if any, on digestive enzymes secreted by pancreas also as the latter play a major role in digestion of food.

References

Anon (1987): Spices – for what they are. *Indian Spices* **24**, 3–8.

Bergmeyer HU, ed. (1974a): *Methods of Enzymatic Analysis*, Vol. 2, p. 819. New York: Academic Press.

Bergmeyer HU, ed. (1974b): *Methods of Enzymatic Analysis*, Vol. 2, p. 885. New York: Academic Press.

Bergmeyer HU, ed. (1974c): *Methods of Enzymatic Analysis*, Vol. 2, p. 916. New York: Academic Press.

Chopra RN, Chopra IC, Handa KL & Kapur LD, ed. (1958): *Chopra's Indigenous Drugs of India*, 2nd edn. Calcutta: Dhur & Sons.

Ganesh Bhat B & Chandrasekhara N (1987): Effect of black pepper and piperine on bile secretion and composition in rats. *Die Nahrung* **9**, 913–916.

Ganesh Bhat B, Srinivasan MR & Chandrasekhara N (1984): Influence of curcumin and capsaicin on the composition and secretion of bile in rats. *J. Food Sci. Technol.* **21**, 225–227.

Ganesh Bhat B, Sambaiah K & Chandrasekhara N (1985): The effect of feeding fenugreek and ginger on bile composition in the albino rat. *Nutr. Rep. Int.* **32**, 1145–1153.

Glatzel H (1968): Physiological aspects of flavour compounds. *Indian Spices* **5**, 13–21.

Hubscher C & West GR (1965): Specific assays of some phosphatases in subcellular fractions of small intestinal mucosa. *Nature* **205**, 799–800.

Kirthikar KR & Basu BD (1935): *Indian Medicinal Plants*, ed. Lalithmohan Basu, pp. 168, 2149. Allahabad:

Lowry OH, Rosebrough NJ, Farr AL & Randall RJ (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Patwardhan MV & Sastry LVL (1957): Effect of asafoetida on the intestinal enzyme systems *in vitro*. *Food Sci.* **6**, 27–28.

Ramachandran K & Ambasta SP, eds (1986): *The useful plants of India*. New Delhi: Publication and Information Directorate, CSIR

Sambaiah K & Srinivasan K (1991): Secretion and composition of bile in rats fed diets containing spices. *J. Food Sci. Technol.* **28**, 35–38.

Sambaiah K, Satyanarayana MN & Rao MVL (1978): Effect of red pepper and capsaicin on fat absorption and liver fat in rats. *Nutr. Rep. Int.* **18**, 521–528.

Snedecor GW & Cochran WG, eds (1976): *Statistical Methods*, 6th edn, p. 298. Ames, USA: Iowa State University Press.

Sreenivasamurthy V & Krishnamurthy K (1959): Place of spices and aromatics in Indian dietary. *Food Sci.* **8**, 284–88.

Thimmayamma BVS & Rao P & Radhaiah G (1983): Use of spices and condiments in the dietaries of urban and rural families. *Indian J. Nutr. Diet.* **20**, 153–162.