ORIGINAL RESEARCH PAPER

Ayurveda

KEY WORDS: Curd,Alpha Amylase Inhibition ,rules of eating curds.

STUDY OF ALPHA AMYLASE INHIBITION ACTIVITY OF COW CURD AND ITS COMBINATIONS AS PER CHARAK SAMHITA

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Introduction

Food is a very basic requirement of every cell.Each living cell needs food or energy for day to day activities and for survival. Ayurveda as a science of life ,have a definite focused approach on food for healthy life.

A complete balanced food can make life healthier and can increase longevity. Food can act as medicine. As per Taitariya Upanishad, man or life is originated from food.

Formation of 5 elements and food

[1]Vayu is originated from akash,Teja element is originated from vayu,water is originated from teja,earth is originated from water, plants are originated from earth, food is originated from plants. And ultimately human is originated from food.

If the origin of human is food, then existence is certainly depends upon quality of food. For healthy and long survival, food should be balanced and nutritious It should not cause any harm to body tissues, but it should nourish them.

[2]Food replenishes and grows the body entities. Food of opposite qualities is responsible to bring back overgrown body entities within physiological limit3.Food is best for healthy status of leaving being.

[4]Food is life of human being. Food offers colour, luster, speech, life,innovative skills,happiness,contentment,nourishment,strength and intelligence.

[5]one should not eat food greedily and unknowingly.[6]Wholesome diet has tremendous capacity of offering healthy longevity. A man disciplined to remain on healthy diet ,lives for 36000 nights or hundred years.

Deprivation of food causes many symptoms and diseased conditions. [7]If an individual fails to eat food when he is hungry, it causes weight loss,weakness,loss of original colour,body aches,hallucinations.He has to eat light and unctuous food to overcome these manifestations [8]When individual is extremely hungry, liveliness of that person get hampered, complexion get shadowed, eyesight get hampered, symptoms like depression, emaciation,draining out ,pain in cardiac region, fatigue appears.this shows that normal life get hampered without food.

[9]There is no medicine like food.It is possible to cure diseases with correct and wholesome food regeimn.human remain healthy with consumption of appropriate food.If a patient is on medication, he can't leave without eating food. Hence physicians call food as Mahaushadhi or the great medicine.

As we know,food is very popular food globally.Some rules and regulations are provided in Ayurvedic compendia about consumption of curd.According to that,[10]curd should not be consumed daily. Inappropriate curd consumption is a cause of many diseases like anemia, bleeding disorders,jaundice,fever ,diabetes and so many. There are some food items with which curd should be eaten.

[11]curd should not be eaten in plain form.It should be eaten with adding one of these things sugar,amalaki ,honey.ghee,green gram curry.Curd should not be eaten after heating it.

[12]With ghee,curd become vata smoothening and kapha facilitating.It does not increase pitta but helps for digestion. When mixed with sugar, it alleviates thirst and burning sensation.Mixed with soup of green gram it pacifies the rakta dhatu and vata dosha.When mixed with honey ,it becomes tasty and onwanted effects are overcome.When mixed with amalaki it pacifies dosha.When heated it vitiates pitta dosha and rakta dhatu.

[13]Curd should be avoided if anyone does not get above mentioned food items.

This was the main stimulating concept behind the research process.

[14] curd is one of the cause of diabetes .The study depend up on laboratory analysis to revalidate the statements of Ayurvedic compendium.

- Material and methods

Materials:

Samples: Cow's milk curd samples (100gm each), khadisakhar, amalaki churna, cow's ghee (clarified butter), *Mudgayush* (Curd mixed with cooked green gram dal) ,madhu.

Raw materials were procured from local authorized shop and the samples were prepared at laboratory scale.

Control sample: pure cow's milk curd
(Curd was prepared as per the standard method described in Dairy Products' Technology Handbook of class XII, CBSC, Delhi)

Preparation of curd samples with ingredients directed by Charaka - Samhita:

Sample1. Curd + honey
Curd (100 gm) + honey (100 gm)

Madhu (honey)is procured from National honey bee institute ,pune.

Sample2 Curd + khandasharkara
Curd (100 gm) + khandasharkara (100 gm)
Khandsharkaa is grinded finely and mixed with curd in equal proportion.

Sample3 Curd + cow ghee
Curd (100 gm) + cow ghee (100 gm)
(Cow's ghee was prepared as per standard procedure mentioned in Dairy Products' Technology Handbook of class XII, CBSE, Delhi.)

Sample4 Curd + mudgayusha
Curd (100 gm) + mudgayusha (100 gm)
(*Akruta Mudgayusha* was prepared as per classical Ayurvedic texts.)

20 gm green gram +360 ml water is is boiled to cook the green

gram. After cooking upper watery portion is filtered. Then it is allowed to cool and then mixed with curd.

Sample5 Curd + amalaki churna
Curd (100 gm) + amalaki churna (100 gm)
(amalaki churna procured from Green Pharmacy ,Pune and was be standardized as per API (Ayurvedic Pharmacopeia of India))

Sample 6: heated curd
Methods:
All the samples were analyzed for nutritional, chemical, microbial and organoleptic parameters on the zero day i.e. on the day of sample preparation.

Parameters tested were:
1.*In vitro analysis* of samples for studying the effect on intestinal amylase activity.

Method for alpha amylase inhibition activity tests
α-Amylase is a protein enzyme EC 3.2.1.1 that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. It is the major form of amylase found in humans and other mammals. It is also present in seeds containing starch as a food reserve, and is secreted by many fungi.

Although found in many tissues, amylase is most prominent in pancreatic juice and saliva, each of which has its own isoform of human **α**-amylase. They behave differently on isoelectric focusing, and can also be separated in testing by using specific monoclonal antibodies. In humans, all amylase isoforms link to chromosome 1p21 (see AMY1A).

SALIVARY AMYLASE (PTYALIN)
Amylase is found in saliva and breaks starch into maltose and dextrin. This form of amylase is also called "ptyalin" / ta l n/ It will break large, insoluble starch molecules into soluble starches (amylodextrin, erythrodextrin, and achrodextrin) producing successively smaller starches and ultimately maltose. Ptyalin acts on linear **α** (1,4) glycosidic linkages, but compound hydrolysis requires an enzyme that acts on branched products. Salivary amylase is inactivated in the stomach by gastric acid. In gastric juice adjusted to pH 3.3, ptyalin was totally inactivated in 20 minutes at 37 °C. In contrast, 50% of amylase activity remained after 150 minutes of exposure to gastric juice at pH 4.3. Both starch, the substrate for ptyalin, and the product (short chains of glucose) are able to partially protect it against inactivation by gastric acid. Ptyalin added to buffer at pH 3.0 underwent complete inactivation in 120 minutes; however, addition of starch at a 0.1% level resulted in 10% of the activity remaining, and similar addition of starch to a 1.0% level resulted in about 40% of the activity remaining at 120 minutes.

Pancreatic amylase
Pancreatic **α**-amylase randomly cleaves the **α** (1-4) glycosidic linkages of amylose to yield dextrin, maltose, or maltotriose. It adopts a double displacement mechanism with retention of anomeric configuration.

Alpha amylase inhibition activity taste
Method for calculation of **α**-amylase inhibitory activity –
The **α**-amylase inhibitory activity was calculated by using the formula: The **α**-amylase inhibitory activity = (Ac+) – (Ac-)- (As-Ab) / (Ac+)- (Ac-) x 100 where, Ac+, Ac-, As, Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme) respectively .

To 600 µl of sample, 1.2 ml of starch in phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride was added. The reaction was initiated by adding 600 µl porcine pancreatic amylase and incubated at 37şc. From the above mixture 600 µl was taken and 300 µl of DNSA (1g of DNSA, 30g of sodium potassium tartarate and 20 mL of 2N sodium hydroxide was added and made up to a final volume of 100 mL with distilled water) and kept it in a boiling water bath for 15 minutes. The reaction mixture diluted with 2.7 ml of water and absorbance was read at 540 nm.

For each concentration, blank tubes were prepared by replacing the enzyme solution with 600 µL in distilled water. Positive Control, representing 100% enzyme activity was prepared in a similar manner, without sample. Negative Control, representing 0% enzyme activity was prepared without sample and enzyme. The experiments were repeated thrice using the same protocol.

Observation and results
Alpha amylase inhibition activity

Table 18

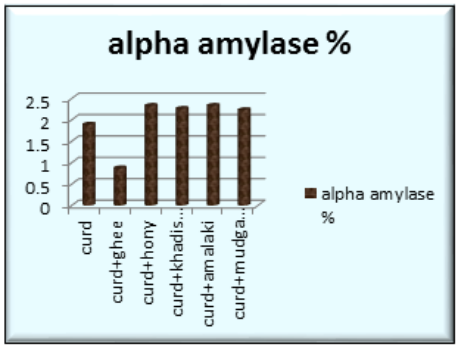
Sr. No.	Sample	% inhibition of alpha amylase
1	Curd	1.888%
2	Curd + Sharkara	2.262%
3	Curd + Yusha	2.234%
4	Curd + Madhu	2.328%
5	Curd + Amalaki	2.065%
6	Curd + Ghruta	0.87%

Formula:
(Ac+)-(Ac-)- [As-Ab]/ (Ac+)-(Ac-) x 100
Ac+ is absorbance of 100% enzyme activity (only solvent with enzyme)
Ac- is absorbance of 0% enzyme activity (only solvent without enzyme)
As is absorbance of test sample (with enzyme)
Ab is absorbance of blank (test sample without enzyme)

Readings:
Table 19

Samples		Reading
Ac+		0.849
Ac-		0.054
Curd	As	0.409
	Ab	1.115
Curd + Sharkara	As	0.613
	Ab	1.616
Curd + Mudga Yusha	As	0.482
	Ab	1.463
Curd + Madhu	As	0.932
	Ab	1.988
Curd + Amalaki kwath	As	0.578
	Ab	1.425
Curd + Ghruta	As	0.25
	Ab	0.147

Sample	Mean Absorbance
Control (A_0)	0.75
Curd	0.95
curd+ Sharkara	0.567
Curd + Mudga Yusha	0.676
Curd + Madhu	0.627
Curd + Amalaki	0.365
Curd + Ghrut	1.247



Discussion-among all the sampls,curd and ghrut has lowest alpha amylase inhibition and curd mixed with honey has highest inhibition activity.the sequence of the samples from lower to higher level is

1. Curd + Ghruta
2. Curd
3. Curd + Amalaki
4. Curd + Yusha
5. Curd + Sharkara
6. Curd + Madhu

[14]If we observe the properties of honey,it is sweet and astringent in taste.It is dry in nature though it feels soft in touch.It has cold potency.with all these properties ,it helps to increase or regulate digestive capacity,. It makes voice soft. It is light in digestion and cause scratching effect on fat. Hence it is useful for weight loss.It has wound healing capacity.It causes reduction in kapha dosha.It is good for eyes and gives luster to the skin.It can reach to the minute channels in the body.It has capacity to pacify all the three doshas.

These properties are though opposite to the properties of curd,the combination does not have vitiating effect on It do not cause any harm to body entities.Hence it proves useful.

Alpha amylase is a enzyme which causes digestion of carbohydrates.Inhibition of this enzyme causes reduction in blood sugar level and glycemic index get reduced.[15]In treatment of diabetis,one therapeutic approach is prevention of carbohydrate absorption after food intake,which is facilated by inhibition of enteric enzyme alpha amylase present in brush boarder of intestine.[16] The **inhibition** of **alpha**-glucosidase and **alpha-amylase,** enzymes involved in the digestion of carbohydrates, can significantly reduce the post-prandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type 2 **diabetic** and borderline patients.

Curd mixed with amalaki shows better results for alpha amylase inhibition than curd.Amalaki fruit is sour and astringent in taste.It is cold in potency.It removes bad or vitiated doshas.It is good for hair. It is aphrodaisiac.It improves vision.It reduces perspiration, removes excess fat in the body.It is useful remedy for skin diseases,anaemia,heart diseases,diabetis,ascitis,irritable bowel syndrome,upper and lower respiratory tract diseases.

Combination of Curd and mudga yusha has also shown good results for alpha amylase inhibition than curd.Mudga yusha is prepared by boiling green gram with 16 fold more water.Mudga has astringent and sweet taste.It has cold potency and exhibits spicy taste after digestion(katu vipak)It is light for in digestion,It is beneficial in decreasing fat,kapha dosha aggrevation,and blood impurities.

If we observe the properties of curd and all these substances ,we can say that they do not harmful to body tissues but definitely reduces untoward properties of curd

conclusion

Laboratory analysis supports the theory in Ayurvedic texts, that curd should not be consumed in plain form but it should be mixed with some useful ingredients which increases its properties.Hence we can conclude on the basis of the testes that, curd should be eaten with honey,amalaki,mudga yusha in the type 2 diabetes patients.Otherwise it should be avoided.

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