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INFLUENCE OF TYPE OF INORGANIC ACID ON YIELD OF PECTIN FROM DIFFERENT FRUIT PEELS AND EFFECT ON SENSORY ATTRIBUTE OF PUDDING MADE WITH EXTRACTED PECTIN

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ABSTRACT

The extraction of pectin was performed after using three type of inorganic acid (Hydrochloric, sulphuric and nitric acid) and two types of boiling methods (Bunsen burner and microwave heating) on different fruit peel like mango, banana, sapodilla, apple and orange. As apple and orange are used to study comparative effects the best yield was obtained from banana peel (5.8%) using nitric acid and microwave heating to extract pectin. later the impact of the different concentration of pectin on the sensory attributes of pudding were investigate. The pudding made from mango and banana peel pectin were failed totally while sapodilla showed promising results. Sensory evaluation of the colour and texture were performed by 20 panellist. The result showed that the puddings made from extracted and standard pectin have almost same physical attributes (texture, colour). It can be suggested from the study that sapodilla pectin does not influence the textural property of pudding and can be used effectively in the formulation of functional foods.

KEY WORDS: inorganic acid, Pudding, pectin, sensory evaluation, texture.

INTRODUCTION

The pectic substances were first discovered by the French Chemist Louis Vauquelin in 1790, while its properties were first reported by Henri Braconnot another French chemist in

1825.[1] Pectin has a key action in the maintenance of the structural integrity of plant by providing firmness to the plant by composing a hydrated cross linked three dimensional network.[2] Pectin not only has a role in ripening and texture building of fresh fruits in plants but it is an important agent in processing industry for its characteristics property of jelling and thickening agent in canned and processed food products.[3] Kertesz in 1951 suggested that the extraction of pectin is a complex physico-chemical process which may involve solubilization, extraction and DE polymerization of pectin complex molecules from plant tissues.[4] In lab-scale extraction, hot water extraction is used for high esterified pectin. While commercially, pectin is extracted through hot dilute mineral acid pH around 2 with varying length of time. The time depends upon the type of pectin required and from one manufacturer to another.[5] Various factors can affect the extraction of pectin. The extracting conditions may affect the probable structure of pectin. Studies on, the use of different pH levels, reveals that it has a profound effect on both the extractability and in the breakdown of pectin molecule.[6]

Functional foods has gained tremendous popularity over the past few years. It provides numerous health benefits and fulfil primary need of nutrient which as a result also helps in controlling health related illnesses and conditions. Pectin being a natural dietary fibre is among the various functional ingredients which are included in this category and are also studied to design new food and pharmaceutical formulations to manage proper health and life style.[7] The products which have been used to enrich fibre capacity includes supplementation in frozen foods, cereals, beverages and milk based products.[8] Due to prominent property of fiber, these are able to alter the many attributes of a food products which includes the consistency, texture, rheological behavior and sensory characteristics. The investigation of new sources to obtain fibers and their use in food has opened new vista for food industry to modify and improve their products in terms of stability, acceptability and cost. The advanced functional products are manufactured by changing traditional food methods and after replacing known ingredients with the newly or under investigation products. Pectin are among water soluble fibers which belong to complex group of polysaccharides containing D-galacturonic acid as the backbone unit. In plants pectin provide structural integrity to the plant cell wall by acting as a cementing agent. Due to its affinity towards water which makes it water soluble, and thus is completely metabolized effectively by colonic bacterial flora of colon.[8]

Pudding is a sweet desert, usually containing flour or cereal products that has been boiled, steamed or baked and the gel network of a pudding is essential for its desirability.[9] The use of pectin in the formulation of pudding is considered as an effective substitute of starch or carrageenan as it does not co-precipitate with casein especially at low pH which actually positively affect the food product by increasing its shelf life.[10] However the stability of the pudding also depends on other ingredients used in its manufacturing, such as milk stabilizers, milk components, sucrose, flavors and colorants etc.[11] Although the amount of hydrocolloids present in pudding is less than 1% of its weight but it has a huge impact on the textural and sensory properties of the final product.[12-13] Among the basic characteristics, a milk pudding should possess a reasonable softness, which is measured in terms of deformation on little resistance.[14] Therefore, the objective of this experiment was to determine the differences in the texture, mainly softness, of milk puddings produced using different concentration of sapodilla pectin and food grade pectin.

MATERIALS AND METHODS

Extraction of pectin from five different fruits

The extraction of pectin from five different fruits (mango, sapodilla, banana, apple and orange) was performed after using different types of inorganic acid i.e. hydrochloric, sulphuric and nitric acid. Peels of the selected fruits were taken weighed to desired weight cut into smaller pieces and were finally grounded using mechanical blender to form a loose slurry. For each fruit peel 25ml of water was taken in blender and the pH was adjusted using 1N HCl, H_2SO_4 and HNO_3. The slurry was then boiled using two boiling methods (Bunsen burner and Microwave oven) and cooled to room temperature. The boiled mixture was then filtered using a Buchner funnel and the filtrate was then precipitated with 95% ethanol. The pectin precipitated was separated from the solution after centrifugation and was dried using freeze dryer to a constant weight. The finally dried pectin was finely grounded and kept in an air tight container for future use.

Three factor factorial completely randomized design (CRD) was applied and mean comparison was done by using Tukey HSD[15] at 5% level of significance. Statistical analysis was performed using SPSS13 and Minitab13.1.

Formulation and sensory evaluation of pudding from selected sapodilla pectin

The study was conducted in faulty of Pharmacy, University of Karachi. Sample puddings with three different concentration of pectin were prepared. Two pudding samples were also made as a reference from food grade pectin which has the same concentration as two among the three sample puddings while one of the pudding has less quantity of pectin than the reference pudding.

Samples of milk pudding was prepared by using different concentration of extracted and a standard concentration of food grade pectin. The pudding was prepared after mixing required amount of extracted pectin with 100 gram of sugar, 1 egg and approximately 990 ml of milk (Table- 1) in a saucepan. The mixture was stirred to dissolve sugar and pectin into the mixture, after 10 min of stirring the mixture was heated till boil. After the required time of boiling the mixture was kept aside from fire and then poured into the desired containers for cooling. The cooled pudding mixtures were then kept in refrigerator for 10 hrs till further analysis. Sensory evaluation of puddings were performed by the 20 panellist specified for the job.

The sensory evaluation of pudding was performed in by 20 panellist .The testing procedure chosen for the study was Duo-trio test.[16] Three milk pudding samples were provided to the panellist among which one was the reference pudding sample while among the remaining two one of the sample pudding was same in concentration as reference pudding in terms of pectin. Among the remaining two samples one of the sample was same as reference sample while the other was different. The pudding made from standard pectin was used as reference for the first 10 panellist while pudding made with extracted pectin in the same quantity as reference for the remaining 10 panellist. To avoid any biasness the samples were coded. An evaluation form was given to each participant to rate the sensory attributes. The example of evaluation form is given in Figure 1. Same procedure was followed for the remaining pudding samples.

Table 1: showing different pectin concentration used for each selected extracted fruit pectin

Material	P1	P2	P3	Ps_1	Ps_2
Pectin (gram)	5	10	15	10	15
Sugar (gram)	100	100	100	100	100
Milk (ml)	990 (approx.)	990 (approx.)	990 (approx.)	990 (approx.)	990 (approx.)
Vanilla custard (grams)	10	10	10	10	10

P1, P2 and P3 are sapodilla pectin pudding samples while Ps_1 and Ps_2 and reference samples

RESULT AND DISCUSSION

The difference in yield of pectin indicated the influence of type of inorganic acid used in the process of extraction as showed in Table 2. The different yield of pectin obtained point towards the best and least effective source of pectin in terms of yield of pectin among the three under investigation sources (mango, sapodilla and banana) and two known sources of pectin (apple and orange). The fruits apple and orange are used for comparative purpose and to show that the selected medium is capable of extracting pectin from fruit peels. The best source of pectin among the three fruits was banana (5.8%) using nitric acid and microwave heating to extract pectin at pH 3. While mango gave 5.05% and sapodilla 5.00% after using hydrochloric acid and microwave heating for the process. The yields of banana and mango are similar as previous studies, [17] but with different organic acid while there is no previous study found on extraction of pectin from sapodilla peel.

The study revealed the effect of inorganic acid on the yield of pectin from different types of peels without any pretreatment of the peels. The study clearly defines the effect a particular acid has on the yield at particular pH and boiling method. Microwave proved to be more effective than conventional heating method (Bunsen burner) by giving more yield from all the type of peels used in the study. Usually the commercial extraction of pectin is performed at pH 1 till 4.5 hence the pH in the current study was kept at pH 3 in order to integrate similar medium. Similar effects were seen on the yield of pectin from orange peel when sulphuric acid was used along with other organic and inorganic acids.[18] Although literature contains the amount of pectin that can be extracted from apple pomace (10 to 15%)[19] but it was not comparable with the present study because to synchronize the study, apple peels, instead of pomace was used. The extracted pectin yield from orange peels was also comparable with previously extracted pectin.[18]

Table: 2 Yield of pectin from five different fruits using different types of inorganic acids.

Fruit	Mechanical procedure	pH	Acid	% yield	
				B	M
Mango	Homogenizing	3	1N HCL	4.4	5.05
			1N H_2SO_4	1.05	1.35
			1N HNO_3	0.05	0.1
Sapodilla			1N HCL	4.3	5
			1N H_2SO_4	0.8	2.6
			1N HNO_3	2.35	0.15

Banana			1N HCL	0.15	0.1
			1N H_2SO_4	2	3.55
			1N HNO_3	0.1	5.8
Apple			1N HCL	0.1	3.8
			1N H_2SO_4	5.05	5
			1N HNO_3	0.25	4.9
Orange			1N HCL	8.15	8.6
			1N H_2SO_4	9.05	12.55
			1N HNO_3	3.5	4.2

Statistical evaluation of yield from five different fruits using different types of inorganic acid

Analysis of variance to show the effect of boiling method and acid type is shown in Table 3. It shows that boiling method has significant effect (P<0.01) on the yield of three fruits banana apple and orange while it has non-significant effect (P>0.05) on the yield of mango and sapodilla. While the acid type has highly significant effect (P<0.01) on yield of pectin from all five fruits.

The mean comparison of yields also showed variable responses for different fruit types. The effect of hydrochloric acid was most significant on yield of mango (Table 4) and sapodilla (Table 5) as compared to the other two acids while the boiling methods had no significant difference and were equally effective to extract pectin from mango and sapodilla peels. The interactive effect of type of acid with boiling method on the yield of mango peel showed in Table 4 revealed the maximum yield (5.05±0.13) with hydrochloric acid while Table 5 showed (5.00±0.06) maximum yield observed from sapodilla fruit peel after using same inorganic acid.

Table 3: Analysis of variance (mean squares) of yield for different fruits.

Source of variation	Degree of freedom	Mean squares				
		Yield (Mango)	Yield (Sapodilla)	Yield (Banana)	Yield (Apple)	Yield (Orange)
Boiling method (BM)	1	0.056^{NS}	0.045^{NS}	25.920**	34.445**	10.811**
Acid	2	37.136**	20.465**	15.034**	15.849**	74.659**
BM x Acid	2	0.392*	6.405**	13.211**	9.264**	4.301**
Error	12	0.073	0.011	0.013	0.035	0.120
Total	17					

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 4: Means comparison on yield of pectin from mango fruit peel.

Boiling method	Acid type									Mean		
	1N HCL			1N H_2SO_4			1N HNO_3					
B	4.40	±	0.13a	1.05	±	0.03b	0.05	±	0.00c	1.83	±	0.66A
M	5.05	±	0.13a	0.68	±	0.34bc	0.10	±	0.01c	1.94	±	0.79A
Mean	4.73	±	0.17A	0.87	±	0.17B	0.08	±	0.01C			

B = Bunsen burner, M= Microwave. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5: Means comparison on yield of pectin from sapodilla fruit peel.

Boiling method	Acid type									Mean		
	1N HCL			1N H_2SO_4			1N HNO_3					
B	4.30	±	0.09b	0.80	±	0.01d	2.35	±	0.07c	2.48	±	0.51A
M	5.00	±	0.06a	2.60	±	0.08c	0.15	±	0.01e	2.58	±	0.70A
Mean	4.65	±	0.16A	1.70	±	0.40B	1.25	±	0.49C			

B = Bunsen burner, M= Microwave. B = Bunsen burner, M= Microwave. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 6: Means comparison on yield of pectin from banana fruit peel.

Boiling method	Acid									Mean		
	1N HCL			1N H_2SO_4			1N HNO_3					
B	0.15	±	0.01d	2.00	±	0.05c	0.10	±	0.01d	0.75	±	0.31B
M	0.10	±	0.01d	3.55	±	0.08b	5.80	±	0.13a	3.15	±	0.83A
Mean	0.13	±	0.01B	2.78	±	0.35A	2.95	±	1.28A			

B = Bunsen burner, M= Microwave. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 7: Means comparison on yield of pectin from apple fruit peel.

Boiling method	Acid									Mean		
	1N HCL			1N H_2SO_4			1N HNO_3					
B	0.10	±	0.01c	5.05	±	0.13a	0.25	±	0.02c	1.80	±	0.81B
M	3.80	±	0.16b	5.00	±	0.13a	4.90	±	0.11a	4.57	±	0.20A
Mean	1.95	±	0.83C	5.03	±	0.08A	2.58	±	1.04B			

B = Bunsen burner, M= Microwave. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 8: Means comparison on yield of pectin from orange fruit peel.

Boiling method	Acid									Mean		
	1N HCL			1N H_2SO_4			1N HNO_3					
B	8.15	±	0.08b	9.05	±	0.30b	3.50	±	0.07c	6.90	±	0.86B
M	8.60	±	0.24b	12.55	±	0.27a	4.20	±	0.08c	8.45	±	1.21A
Mean	8.38	±	0.15B	10.80	±	0.80A	3.85	±	0.16C			

B = Bunsen burner, M= Microwave. Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

The effect of nitric acid was most significant on yield of banana (Table 6) as compared to the other two acids while the microwave showed significant effect on yield of pectin. The interactive effect of type of acid with boiling method on the yield of banana peel showed in Table 6 described the maximum yield (5.80 ±0.13) with nitric acid. While the effect of sulphuric acid was most significant on yield of apple (Table 7) and orange (Table 8) as compared to the other two acids while the microwave heating significant effect on the yield of pectin from apple and orange peels. The interactive effect of type of acid with boiling method on the yield of apple peel showed in Table 7 revealed the maximum yield (5.05±0.13) with sulphuric acid while Table 8 showed (12.55±0.27) maximum yield observed from orange fruit peel after using same inorganic acid.

Sensory evaluation of pudding from different concentration of sapodilla pectin

The result of the sensory evaluation of pudding made from extracted pectin from three fruits (mango, banana and sapodilla) showed different results. The pudding made from mango and banana pectin failed to show any promising results and were omitted for statistical analysis. Only the pudding made from sapodilla peel pectin showed comparable results with food grade pectin and was studied accordingly. The results for sapodilla pectin (10 gram) through duo trio test indicated among the 20, 9 correctly identified the difference in sample while 10 failed to identify any difference. To constitute significant differentiation among the samples 15 correct judgement among 20 were required. The result showed that the panelist as a whole did not found any significant difference (P > 0.05) between the pudding made from standard and extracted pectin. While the pudding made from 15 gram pectin for both reference and sample pudding also showed the same result.

The failure of the participant to identify any difference in the texture of the pudding made from two sample concentration of sapodilla pectin indicated that the pudding made from these extracted pectin has similar physical attribute as that of pudding made from the standard or food grade pectin. Among the many properties of pectin its role in milk products is studied during the formulation of pudding from the extracted pectin. Pectin in milk products help in avoiding the agglomeration of protein which in turn helps in prevention of phase separation in milk products. Pectin is also used to create a specific mouthfeel which is a result of its thickening property.[20] According to some details in the Cargill foods for acid milk drinks HM pectins are very good stabilizers and probably the reason for passing the test of the two puddings made from sapodilla pectin. It is explained further on the site that HM pectin tends to form a coat on the casein particles.[21]

The present study provides a preliminary insight of the consumer response on the change of the one of the ingredient of pudding after its sensory evaluation. The process is called preference mapping and it provides useful information about the basic product characteristics and its effect on the consumer response.[22] Pectin is a useful functional ingredient which can be used in the preparation of pudding. However problems may arise with the replacement of any functional ingredient with the known or traditionally used component hence it is better to learn the effects on the physical attributes of a food product first before any further investigation of the food product.

The prime reason to conduct this study was to learn the effect of pectin in this new system other than gel formulation in which it is reported to contribute in color stability of the product. Pectin is already in use as a functional ingredient in many formulation like jam, jellies and spreads, the use in the formulation of this new product can be beneficial nutritionally and economically. Moreover, due to the rise of nutritionally dependent illnesses like type II diabetics, cardiovascular and digestive system disorders a need of animal free food also aroused which lead to many new investigations like protein-polysaccharide system studies.[23] (Nunes *et al*., 2003)

DUO TRIO TEST

1. Among the three provided samples of pudding R is the reference sample while among the remaining two one of the sample is same as the reference sample and the other is different.
2. Evaluate the attributes of the sample given below from left to right. Please spit the sample into the spitter and rinse your mouth before taking the next sample.
3. Tick the box of the sample which feels same as the reference sample.

Attributes	Sample 1	Sample 2
Softness (texture)		
Colour		

Thank you

Figure-1: Showing the due trio test sample used.

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