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Evaluation of fruit liquefying ability of pectinolytic enzyme system of *A.niger*

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Techno-economical feasibility up gradation of fruit processing is underlined on the basis of yield and quality of finished food products by involvement of biotechnology. It has been postulated that the most effective enzymatic liquefaction of fruit mash is result of synergistic action governed by combination of pectinase quotients. The desired yield of fruit juice coupled with clarity of juice, reduced waste index and easiness in separation of seeds from pulp of unconventional fruits such as custard apple (*Annona squamosa*), and ramphal (*Annona reticulata* L.) against conventional method. Enzymatic liquefaction exhibited higher per cent yield (74% transmittance T.) of sparkling clear juice *i.e.* up to and overall improvement in quality. Increase in per cent yield of juice by 25 per cent over that of conventional method is an outstanding achievement of enzyme processing technology.

Key Words : Pectinase, *A.niger*, Un-conventional fruits, Enzyme assay, Liquefication

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INTRODUCTION

Presently enzymes are becoming indispensable part of most of the industrial applications. According to conventional technology fruit juice is extracted by crushing and pressing of fleshy and edible mass of fruits. It doesn't ensure higher yield, quality and adequacy in hygiene and to provide safety of finished products. The quality of finished fruit products depends up on quality of fruit pulp and extracted juice. The monitoring of natural quality parameters of extracted fruit juice is far away from conventional processing methods (Bhat, 2000). The process ability of fruit is justified on the basis of per cent yield of extracted juice and sustainability of nutritional and sensorial quality parameters. The biotechnological involvement in processing of fruits and vegetables revolutionized the processing scenario feasible for expected value addition (Busto, 2006). The food grade enzymes and their applications in processing progressively entered in satisfying innovative demands of consumers during post GAT scenario. The role of food grade enzymes for maceration induced liquefaction, pulping, homogenization, clarification, cloud stabilization and texturization become indispensable for monitoring quality parameters of fruit processed products (Pawar, 2000).

Food grade pectinases from *A. niger*, are predominantly used as processing aids in industries. The application of these enzymes improves overall quality and the efficacy of the fruit juice processing technology, irrespective of *in vivo* enzyme system present in the fruit (Blandino, 2001). Processing of raw fruit coupled with pectinases resulted in reduction in the viscosity of the pectin-rich juice is referred as pulp enzyming (Mutenda *et al.,* 2002). It significantly improves the process-ability of fruit mass and yield of the juice (Sangeeta, 2009).

The fruit juices production is facilitated through maceration induced liquefaction of the fruit tissues. In this process the fruit tissues are disintegrated by using a broad spectrum of polysaccharide degrading enzymes, such as pectin, hemicellulase and cellulose degrading enzymes (Bhat, 2000). The liquefaction technique is relatively simple and economical to stimulate higher yields of clear or cloudy juices. This

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technology is applicable for pulpy fruits (mango, guava and banana) from which tissue entrapped juice can not be obtained by pressing (Pawar, 2000 patented process). Liquefaction also helps in monitoring the losses of nutrients occurring during mechanical pressing, for example manufacture of carrot (multicomponent fleshy mass) juice (Demir, 2001).

METHODOLOGY

Production of crude enzyme (CE):

The *A. niger* Van Tiegh (a protected organism) fed over a notified substrate in the form of standard media and controlled fermentation conditions specified in the production technology (Pawar, 2000).

Crude enzyme extract (CE) was obtained by the process of submerged fermentation in Biostat B^+ fermenter. The 90 ml innoculum of spore suspension (ca. $1x10^6$ spores/ml) of *A .niger* inoculated in 4 lit synthetic media contained in a 5 lit capacity vessel of automatically operated fermentor. The fermentation process was carried out at controlled conditions of pH (5.2), temperature (28 ± 2^0 C) and incubation period of 5 days. At the end of incubation period, the contents were filtered through Whatman filter paper No. 541. The Culture Filtrates (CF) were used as a source of crude pectinolytic enzyme.

Analysis of liquefying ability of crude enzyme:

It was performed by using 'Brookfield digital viscometer, model Lv-E (USA). The procedure is outlined for making a viscosity measurement in the recommended 600 ml low form Griffin beaker. To make a viscosity measurement, spindle was rotated with speed 100 rpm at different temperatures of test samples *i.e.* 5^0C, 30^0C, 60^0C.

Enzymatic liquefaction of unconventional fruits:

Uniformly ripened fruits were cleaned with wet cloth to remove adhering particles. Around 3 kg of ripened fruits were taken as experimental material. The fruits (custard apple and ramphal) were manually cut into two halves. The fruits were steam blanched (82^0C at 0.55 kg/cm^2 pressure) and processable edible mass of was subjected to liquefaction.

Enzymatic liquefaction:

A known quantity (200 g) pre processed fruits were taken in a 500 ml capacity conical flask. To preserve the natural quality parameters preservative (KMS 350 ppm) was added. The crude enzyme extract in varying proportions was added in flashy mass of fruit to facilitate liquefaction process. The phase transfer phenomenon associated with transformation from semi-solid to liquid was assessed by visual observations and evaluating with respect to its viscosity/consistency. In case of custard apple and ramphal liquefaction index is assessed by easy separation of seed from pulp and its free flowing nature. Liquefaction process is standardized under controlled conditions like incubation time, temperature and enzyme dose.

Extraction of the juice:

The liquefied pulp in the form of homogeneous puree was then subjected to centrifugation at 4080 g for 10 to 15 min. The centrifuged juice was filtered through 2 layers of cheese cloth. Sparkling clear juice obtained was assessed on the basis of per cent transmittance (T) at 660 nm and recovery with respect to weight of fruit and puree. Clarified juice was pasteurized at 70^0C for 10 min, filled in glass bottles and processed in boiling water for 15 min. Bottles were stored at temperature $<10^0$C.

Chemical compositions of raw and processed products:

Raw pulp, processed puree and clarified juice of fruits were analytically assessed with respect to total soluble solids (TSS), titratable acidity, alcohol insoluble solids (AIS), reducing sugars, total sugars, pectin as calcium pectate and pH by using standard procedures (Ranganna, 1986).

OBSERVATIONS AND ASSESSMENT

The results of the present study have been discussed and presented under the following heads:

Enzymatic liquefaction of unconventional fruits:

Enzymatic processing for extraction of juice from unconventional fruits appeared to be a most effective means of juice extraction as against the conventional methods, as all the fruits gave substantially improved quality and per cent yield of juice (Table 1). Highest per cent increase was observed in the case of guava (150.84%), followed by custard apple (64.21%), ber (55.89%), pomegranate (43.46) and ramphal (18.28%) over corresponding values by conventional method. In the case of ramphal lower per cent yield was attributed to fine pulp where grit pulp (35.83%) was comparatively higher than that of custard apple. Data also indicated that per cent pomace was drastically reduced because of enzymatic maceration of the fruit tissues followed by liquefaction. Highest reduction in per cent pomace was noticed for guava (67.57%) followed by pomegranate (60.50%), ber (50.59%) and custard apple (34.30%).

Chemical composition of enzyme liquefied unconventional fruits:

In order to investigate the effect of enzymatic liquefaction on the chemical composition of unconventional fruits and processed products the samples from different stages of sequential processing were collected and analyzed for different parameters. Data on chemical composition of fruits and its intermediate processed products are presented in Table 2. From the observations, it is appeared that TSS decreased during blanching treatment followed by slight increase during

Table 1. Enzyme liquefaction for extraction of juice from some unconventional fruits

Fruit	Method	Initial wt. (kg)	Waste index (%)	Pulp yield (%)	Juice yield (%)		Pomace (%)		Clarity (% T)
					Puree	Fruit	Puree	Fruit	
Custard apple	Conventional	3	51.75	48.25	46.40	22.38	53.59	25.86	9.7
	Enzymatic liquefaction	3	46.25	53.75	68.39	36.75	31.61	16.99	73.6
Ramphal	Conventional	2.7	Because of tallow consistency of fruit pulp it was difficult to extract the juice without liquefaction						
	Enzymatic liquefaction	2.7	27.17	32.48	56.17	18.24	43.83	14.24	61.2
Pomegranate	Hydraulic pressing	2.5	56.23	75.15	58.22	43.74	41.78	31.40	65.4
	Enzymatic liquefaction	2.5	37.25	75.15	83.50	62.75	16.51	12.40	85.6
Ber	Conventional	2.5	4.93	95.07	47.50	45.16	52.50	49.91	27.4
	Enzymatic liquefaction	2.5	4.93	95.07	74.06	70.40	25.94	24.66	74.2 (G:94.0)
Guava	Conventional	2.5	4.00	96.00	30.93	29.70	69.06	66.30	14.5
	Enzymatic liquefaction	2.5	4.00	96.00	77.60	74.55	22.39	21.59	54.4

G: Gelatin clarification

Table 2. Chemical composition of enzyme liquefied unconventional fruits

Fruit	Processing	TSS ^{0}Bx	Acidity (% citric acid)	pH	Reducing sugars (%)	Total sugars (%)	Pectin as Calcium pectate	Alcohol in soluble ash
Custard apple	Fresh pulp	26.0	0.12	5.18	13.69	14.07	0.83	6.61
	Blanched pulp	19.0	0.14	5.19	10.52	10.91	0.80	5.49
	Liquefied pulp	19.5	0.24	4.22	11.97	12.45	0.15	2.79
	Clarified juice	18.0	0.26	4.24	12.08	12.45	0.13	0.39
Ramphal	Fresh pulp	20.0	0.14	5.58	12.50	12.82	ND	12.03
	Blanched pulp	18.0	0.18	5.23	10.53	11.98	ND	11.27
	Liquefied pulp	19.0	0.24	4.35	11.50	11.28	ND	4.25
	Clarified juice	17.5	0.26	4.15	11.92	11.95	ND	2.10
Pomegranate	Hydraulic press expressed juice	14.0	0.32	3.50	7.85	8.44	ND	ND
	Enzyme liquefied juice	14.0	0.36	3.20	8.20	9.09	ND	ND
Ber	Fresh puree	10.0	0.28	5.01	2.94	8.66	0.26	3.78
	Blanched puree	10.0	0.30	4.90	2.49	7.83	0.27	3.62
	Liquefied puree	10.5	0.35	4.75	2.61	7.98	0.08	1.89
	Clarified juice	10.5	0.37	4.63	2.38	6.99	0.06	0.24
	Gelatin clarified juice	10.0	0.37	4.62	2.38	6.89	0.00	0.02
Guava	Fresh puree	10.5	0.39	4.73	4.33	6.24	1.69	12.00
	Blanched puree	9.5	0.40	3.98	4.22	5.98	1.68	10.85
	Liquefied puree	9.5	0.40	3.95	4.29	6.20	0.28	6.62
	Clarified juice	9.5	0.42	3.70	4.72	6.29	0.20	2.60

ND: Non determined

Table 3. Effect of enzyme concentration on viscosity of liquefied pulp

Temperature (^{0}C)	Enzyme concentration (%)			
	0.5	0.75	1.0	Control
	Relative viscosity units (cP)			
5	6752	6651	6680	6821
30	6220	6136	6230	6480
60	6225	6120	6232	6432
Mean	6397	6302	6330	6577
S.E.	2.276	1.950	8.740	9.860
C.D. (P=0.05)	7.269	6.230	2.791	3.149

enzymatic liquefaction. Highest reduction in TSS (26.92%) during blanching was observed in the case of custard apple followed by ramphal and guava (10.00%). Increase in acidity during liquefaction of fruit pulp was more pronounced as compared to other processing steps like blanching, pureeing etc. custard apple and ramphal showed substantial increase (116.66 and 64.28%) in acidity during liquefaction followed by pomegranate and guava, which reported a meagre increase in acidity (12.50 and 7.69). unlike other constituents, reducing sugars and total sugars exhibited similar pattern of reduction during blanching followed by an increase during enzymatic liquefaction and in clarified juice. Alcohol Insoluble Solids (AIS) of fruits varied from fruit to fruit. AIS content was highest in ramphal (12.03%) followed by custard apple (6.61%) remarkable decrease in AIS during enzymatic liquefaction was apparent in all the fruits.

Pectin content of custard apple and ber were found to be 0.82 and 0.26 per cent, respectively. As anticipated enzyme liquefaction exhibits drastic reduction in pectin content of fruit pulp. Maximum reduction (83.43%) was noticed in the case of guava followed by custard apple (81.92%) and ber (69.23%). Clarity of the juice expressed as per cent transmittance was highest for pomegranate (85.6%) and lowest for guava (54.4%). Custard apple and ramphal showed 73.6 and 61.2 per cent T, respectively. In the case of ber clarity of enzyme treated juice was enhanced by gelatin (0.1%) treatment from 74.2 to 94.0 per cent T.

Liquefying ability of crude enzyme:

Statistically analyzed data on viscosity of liquefied ber pulp in Table 3 used to realize the liquefying ability of pectinases preparation. The reduction in viscosity of ber fruit pulp at 30^{0}C temperature using 0.75 per cent CE concentration was found technologically feasible to liquefy the fruit mash on commercial scale.

It is also surprising to note that 60^{0}C temperature recorded similar viscosity reduction profile as that of 30^{0}C. Two fold increase in temperature (30^0 to 60^{0}C) also remained ineffective to reduce viscosity bellow 6302cP. This clearly indicates that most effective temperature accessible to highest activity of pectinase is 30^{0}C. Processing parameter limitations of ber fruit does allow liquefaction process at 60^{0}C temperature because of apparent stimulation of nonenzymic browning responsible for brown colour discoloration. Use of higher concentration of crude pectinase extract may increase cost of liquefaction process and hence not economical.

Conclusion:

The fundamental constraint of conventional technology is inability for summary application to all types of morphologically diversified fruits (custard apple, ramphal, pomegranate etc). This aspiratory achievement could be positively restructured by introducing modern processing technologies like enzymatic processing of pulpy fruits, juice and pulp concentration with aroma recovery, diffusion extraction of fruit juice, ultra filtration, reverse osmosis and nano processing etc. It has been observed that the enzymatic liquefaction of fruit appeared to be most effective with respect to increased yield of fruit juice, improved juice clarity, reduced waste index and predominant ease in separation of seeds from pulp (ramphal) against conventional method. Enzymatic liquefaction exhibited higher per cent yield of sparkling clear juice and overall improvement in quality. Increase in per cent yield of juice by 24.60 over that of conventional method is an outstanding achievement of enzymatic processing technology.

The pre dominant chemical parameters notifying quality of raw pulp, processed puree and clarified juice of ramphal and apple were analytically assessed with respect to Total Soluble Solids (TSS), titratable acidity, Alcohol Insoluble Solids (AIS), reducing sugars, total sugars, pectin as calcium pectate and pH. Summary application of processing unit operations for both the types of fruits appeared that there was decrease in TSS during blanching (18%) followed by slight increase in enzyme liquefied juice (19%). The chemical composition of ber flesh and its processed counter parts remained unaltered except Alcohol Insoluble Solids content (AIS). AIS content of clarified juice decreased from 3.78 to 0.24. It might be associated with the increase in content of soluble sugars as a result of liquefaction.

It has been observed that reduction in viscosity of ber fruit pulp at 30^{0}C temperature using 0.75 per cent CE concentration found technologically feasible to liquefy the fruit mash on commercial scale.

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