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Development and evaluation of fiber enhanced extrudates with vegetable waste powders

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Abstract
The life style changes are increasing the demand for junk foods that lack the fibers but are rich sources of energy. The cauliflower trimmings powder was added at 5, 10, 15 and 20% w/w to extrudates and the best accepted was for 5% addition. Beetroot pomace powder was added at 7.5, 10, 12.5 and 15% w/w to extrudates and the best accepted was for 7.5% addition. The incorporation of powders to extrudates improved the sensory properties.
The dietary fiber content increased by 3.0 to 4.0 times in the value added extrudates in comparison to rice bran extrudate. The vegetable waste powders decreased the requirement of α – glucosidase enzyme by formation of resistant starch and unavailability of carbohydrates for digestion. The change in the inhibition percentage due to the addition of vegetable waste powders that carbohydrate availability remained more or less the same to α-amylase enzyme was minimum.

Keywords: α-amylase enzyme, α – glucosidase enzyme, dietary fiber, rice bran incorporated extrudates, cauliflower trimmings and beetroot pomace

Introduction
The changing life style increased the demand for ready to eat junk foods. These junk foods are rich in starch, fat and energy but depleted in fiber and studies showed that the diets lacking in fiber can cause gastrointestinal and cardiovascular diseases (Grewal, 2007) [8]. India ranks second in the world in the production of vegetables and third in production of fruit (Chauhan and Intelli, 2015) [5]. Vegetable wastes occur throughout the supply chain and vary widely depending on its processing. Globally, more than 30 % of the food wastes are produced by a variety of sources from agricultural operations to household consumption with 38% from food processing (Singh *et al.*, 2012) [17].
The food processing industry produces large quantities of waste products. Over one million tonnes of vegetable trimmings from the vegetable processing industry are produced every year which can be used for value addition. They are inexpensively available in large quantities characterized by a high dietary fiber content resulting with high water binding capacity and relatively low enzyme digestible organic matter (Serena and Knudsen, 2007) [15]. Among vegetables, cauliflower (*Brassica oleracea*) is the most popular Cole vegetable grown extensively in India. It is rich in nutrients but has highest waste index. The leaves and trimmings which are generally thrown away as waste are also rich source of iron and β-carotene.
Many by-products may be useful as source of nutrients and potential functional ingredients to obtain added value products potential for future use (Dominguez-Perles *et al*., 2010) [7]. Researchers have found that fruit and vegetable pomace powders are good sources of beneficial dietary fiber and antioxidants that can be utilized to develop commercially viable food supplements (Gorinstein *et al.,* 2001) [9].
Extrusion is a versatile and continuous process combining processing steps like mixing of different components, degassing, thermal and mechanical heating, forming and expanding. Cold extrusion takes place at temperatures above glass transition but below starch melting temperatures, while the melting temperature of starch are exceeded in hot extrusion (Steiger *et al.,* 2014) [18].

Materials and methods
Preparation of rice bran extrudates: The powdered MTU 1001 rice variety and refined wheat flour in 2:3 ratio was added with stabilized rice bran at 15% and extrudates were standardized.

Preparation of vegetable waste powders

Preparation of beetroot *(Beta vulgaris* L.) pomace powder (BPP): Beetroot *(Beta vulgaris* L.) was washed, juice extracted and the pomace was collected for powder preparation. The pomace was spread on the trays and dried at temperature of 60 °C in a tray dryer for 5 hr so that the moisture content of the sample was reduced to 5±1%. The dried pomace was ground, sieved and stored in air tight container till further use (Kumar *et al.*, 2010) [11].

Preparation of cauliflower (*Brassica oleracea*) trimmings powder (CTP): The cauliflower trimmings were separated from flower and leaves, washed under running water and blanched. After blanching, the trimmings were cut into pieces and kept at 4 °C for 16 hr. Then they were dried at 80 °C for 10 hr in a tray drier. The dried sample was powdered and sieved using 0.5 mm mesh screen, packed, sealed in air tight container and stored at room temperature until use (Stojceska *et al*., 2008) [19].

Value addition with CTP or BPP to rice bran extrudates: The rice bran extrudates were incorporated with cauliflower trimmings powder (CTP) at 4 different levels i.e. 5, 10, 15 and 20 % levels and beet root pomace powder (BPP) at 4 different levels i.e. 7.5, 10, 12.5 and 15 % by the process of folding and passing through rollers of pasta presto making machine several times. Sheeted dough was extruded through a suitable die (width, 2.0 mm), cut to have desired size of extrudates and shade dried. Now these standardized cold extrudates were steamed, spread over trays and dried for 1 hr at 60 °C. The dried products were boiled for 6 min and the sensory evaluation was conducted using 9 point hedonic scale by 15 semi trained panelists.

Estimation of fibers in extrudates: The crude fiber content of samples was determined by boiling with 1.25% dilute H_2SO_4, washed with water, further boiled with 1.25% dilute NaOH and the remaining residue after digestion was taken as crude fiber (AOAC, 1990) [2].

The dietary fiber content of samples was determined by sequential enzymatic digestion of dried and defatted samples (in case of samples containing <10 % fat) by heat stable α-amylase, pepsin and pancreatin enzymes to remove starch and protein present in the samples. Ethanol is added to the digest to precipitate soluble dietary fiber. The TDF is the residue left after subsequent washing of the insoluble residue and the precipitate with alcohol and acetone, dried, weighed and corrected for protein and ash content (AOAC, 2000) [3].

Estimation of *in vitro* starch carbohydrate digestibility: The ability of the samples to inhibit α – amylase and α - glucosidase enzyme activity was determined with the method described by Singh & Jambunathan, (1982) [16] and Vishnu & Murugesan, (2013) [21] respectively.

Results and discussion

Sensory evaluation of beetroot pomace powder (BPP) incorporated rice bran extrudates: BPP was added at four levels viz. 7.5, 10.0, 12.5 and 15.0% to the rice bran incorporated extrudate. The results of sensory evaluation of extrudates prepared with beetroot pomace powder were given in the Table 1. The results showed that colour varied from 7.0 to 7.60. The best colour rating was for BPP at 7.5% incorporation and the least was for 12.5% BPP incorporation. The sensory scores for texture ranged from 6.93 to 8.00, taste 6.60 to 7.67, flavour 6.47 to 8.00 and overall acceptability from 6.47 to 7.60. The texture of extrudates after incorporation of BPP was comparatively lower than the control sample. There was no significant difference in the texture of the extrudates as the BPP percentage increased. The results of taste, flavour and over all acceptability showed no significance difference (p<0.05) amongst the extrudates. The extrudate with 7.5% BPP incorporation showed the best score for taste, flavour and overall acceptability in comparison to other extrudates.

Table 1: Mean sensory scores of BPP incorporated stabilized rice bran extrudates

Extrudate	Colour	Texture	Taste	Flavor	Overall acceptability
CRB	7.47^{a}±0.64	8.00^{a}±0.93	7.67^{a}±0.49	7.93^{a}±0.70	7.47^{a}±0.52
BPP_1	7.60^{a}±0.51	7.13^{b}±0.99	7.73^{a}±0.49	8.00^{a}±0.65	7.60^{a}±0.62
BPP_2	7.33^{a}±0.49	7.47^{a}±0.57	7.20^{b}±0.68	6.47^{b}±0.51	6.67^{b}±0.72
BPP_3	7.00^{b}±0.85	7.33^{a}±1.17	6.80^{bc}±0.78	6.47^{b}±0.52	6.47^{b}±0.52
BPP_4	7.13^{ab}±0.83	6.93^{b}±1.03	6.60^{c}±0.63	6.47^{b}±0.52	6.47^{b}±0.52
Mean	7.31	7.37	7.20	7.07	6.93
SE	0.247	0.366	0.215	0.168	0.192
CD	0.495	0.733	0.431	0.337	0.384
CV (%)	9.26	13.585	8.189	6.514	7.580

Note: Values are expressed as mean ± standard deviation of three determinations.

Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$

CRB: Control with 15% stabilized rice bran incorporated extrudate

BPP_1: 7.5% BPP incorporated stabilized rice bran extrudate

BPP_2: 10% BPP incorporated stabilized rice bran extrudate

BPP_3: 12.5% BPP incorporated stabilized rice bran extrudate

BPP_4: 15% BPP incorporated stabilized rice bran and extrudate

Sensory evaluation of cauliflower trimmings powder (CTP) incorporated rice bran extrudates: CTP was added between 5 to 20% to the selected rice bran incorporated extrudates. The sensory evaluation results are given in Table 2. The sensory evaluation of CTP incorporated extrudates for colour, texture, taste, flavour and overall acceptability were in the range from 6.93 to 7.53, 7.00 to 8.00, 7.00 to 7.80, 7.07 to 7.93 and 6.93 to 7.67 respectively. The highest rating of colour was for extrudate with 5% CTP and least was for 15% and 20% CTP incorporation, texture for extrudate was highest with no CTP incorporation and least for 20% CTP incorporation, taste of extrudates was highest for 5% CTP incorporation and least for 15 and 20% incorporation, flavor was highest for extrudate with no CTP addition and least for 15% addition. Finally the overall acceptability was highest for 5% added CTP but least for 15% added CTP.

Hence as the results showed that CTP at 5% addition had significantly highest rating for colour, texture, taste, flavour and overall acceptability on the 9 point hedonic scale, this extrudate was selected for further analysis.

Table 2: Mean sensory scores of CTP incorporated rice bran extrudates

Extrudate	Colour	Texture	Taste	Flavor	Overall acceptability
CRB	$7.47^{a}\pm0.64$	$8.00^{a}\pm0.93$	$7.67^{a}\pm0.49$	$7.93^{a}\pm0.70$	$7.47^{ab}\pm0.16$
CTP_1	$7.53^{a}\pm0.52$	$7.80^{a}\pm0.56$	$7.80^{a}\pm0.94$	$7.73^{ab}\pm0.79$	$7.67^{a}\pm0.62$
CTP_2	$7.13^{ab}\pm0.64$	$7.47^{ab}\pm0.52$	$7.33^{ab}\pm0.72$	$7.33^{ab}\pm1.04$	$7.13^{ab}\pm0.91$
CTP_3	$6.93^{b}\pm0.96$	$7.33^{b}\pm1.06$	$7.00^{b}\pm1.00$	$7.13^{ab}\pm0.99$	$6.93^{b}\pm0.79$
CTP_4	$6.93^{b}\pm0.79$	$7.00^{b}\pm1.07$	$7.00^{b}\pm1.00$	$7.07^{b}\pm1.10$	$7.00^{ab}\pm0.76$
Mean	7.20	7.48	7.36	7.44	7.24
CD	0.5085	0.6597	0.6134	0.6924	0.5429
SE	0.2539	0.3293	0.3062	0.3457	0.2710
CV (%)	9.66	12.06	11.39	12.72	10.25

Note: Values are expressed as mean ± standard deviation of three determinations.

Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$

CRB: Control with 15% stabilized rice bran incorporated extrudate

CTP_1: 5% CTP incorporated stabilized rice bran extrudate

CTP_2: 10% CTP incorporated stabilized rice bran extrudate

CTP_3: 15% CTP incorporated stabilized rice bran extrudate

CTP_4: 20% CTP incorporated stabilized rice bran extrudate

The rice bran extrudates added with BPP of 7.5% or CTP of 5% were found to be most accepted products from the sensory scores as shown in Figure 1. These best extrudates were further analysed for fibers and *in-vitro* carbohydrate digestibility with α-glucosidase and α-amylase enzymes.

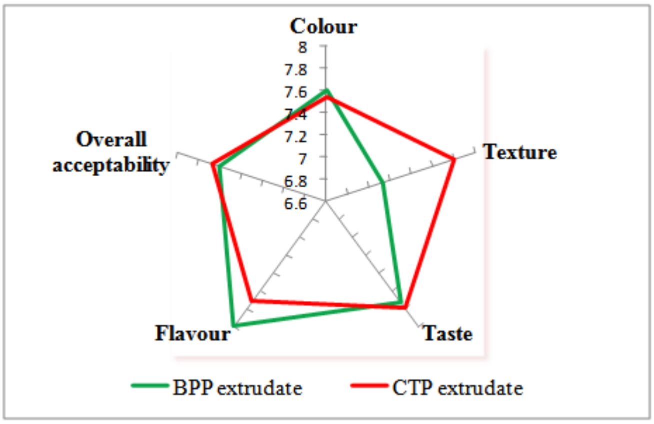

Fig 1: Sensory scores of standardized and selected extrudates

Kaur *et al.*, 2013 [10] who prepared chapatti, buns, cookies and pancake by incorporating 5, 10 and 15% fruit pomace and cauliflower greens. The study concluded that the increase in levels of both food remnants (pomace and cauliflower green) was decreasing acceptability.

Similarly Altan *et al.* (2008a) [1] added tomato and grape pomace to barley based extrudates and investigated the *in vitro* starch digestibility and was concluded that increasing level of both tomato and grape pomace led to reduction *in vitro* starch digestibility.

Stojceska *et al.* (2010) [20] studied the rice flour based extrusion processing of gluten-free products with apple, beetroot, carrot and cranberry pomace. The relationship and interaction between raw ingredients, extrusion processing parameters like pressure, material temperature and torque were monitored and resulting extrudates nutritional and textural properties were investigated. Extrusion technology has the potential to increase the total dietary fibre levels using vegetable and fruit pomace in gluten free rice flour based products up to 30%.

Crude and total dietary fiber:

Crude fiber is the residual part remaining after treatment with acid, alkali and alcohol. Dietary fiber is the portion that is resistant by digestion to enzymes secreted in human body consisting of hemi cellulose, cellulose, lignins, oligosaccharides, pectin, gums and waxes. Crude fiber is a part of dietary fiber (Dhingra *et al.*, 2012) [6].

The analysis of crude and total dietary fiber content in the extrudates was given in Table 3. The crude fiber ranged between 0.31 to 1.23 g/100g in the extrudates. The descending order was as follows CTP extrudate > BPP extrudate > CRB extrudate > control.

The total dietary fiber ranged from 2.18 to 8.64 in the extrudates. The descending order was as follows BPP extrudate > CTP extrudate > CRB extrudate > control. The extrudate with highest crude fiber was CTP extrudate where as total dietary fiber was BPP extrudate. The lowest crude and total dietary fiber was seen for control sample.

The percentage increase in the crude and total dietary fiber content in stabilized rice bran and BPP or CTP added extrudates was shown in Figure 2. As shown in the figure, in comparison with control the crude and total dietary fiber increased by 2.4 and 2.9 times in CRB extrudates, 3.5 and 4.0 times in BPP extrudate and 3.9 and 4.0 times in CTP extrudates. The rice crackers incorporated with apple pomace showed 10% increase in total dietary fiber content. The total fiber content of control crackers (3.01-3.10%) was significantly lower (p<0.05) than the apple pomace incorporate crackers (7.41-7.61%) (Mir *et al.*, 2015) [12].

Table 3: Fiber content of extrudates

Extrudate	Crude fiber	Total dietary fiber
Control	$0.31^{d}\pm0.03$	$2.18^{d}\pm0.09$
CRB	$0.90^{c}\pm0.004$	$5.18^{c}\pm0.09$
BPP	$1.07^{b}\pm0.06$	$8.64^{a}\pm0.01$
CTP	$1.23^{a}\pm0.007$	$8.42^{b}\pm0.06$
Mean	0.88	6.10
CD value	0.0679	0.1178
S.E	0.0276	0.0481
C.V (%)	3.84	0.96

Note: Values are expressed as mean ± standard deviation of three determinations.

Means within the same column followed by a common letter do not significance differ at $p \leq 0.05$.

Units expressed as g/100g.

Control: Rice flour + refined wheat flour extrudate

CRB: Control + stabilized rice bran extrudate

BPP: Beetroot pomace powder incorporated CRB extrudate

CTP: Cauliflower trimmings powder incorporated CRB extrudate

Reddy *et al.* (2014) [13] observed that crude fiber content in extruded RTE snacks was least in the control ranged from

0.70 to 1.4 g/100g. The fiber content in extruded snacks made with rice and chickpea also showed a similar value of 1.38 g/100g. The consumption of diets with high dietary fiber lowered the risk of cardiovascular diseases by reducing the plasma and LDL cholesterol levels but did not alter the concentration of HDL cholesterol or triglycerides (Schneeman, 1999) [14].

The processings like heat treatment and extrusion cooking decrease the dietary fiber content in any food product, the cold extrusion technique may not affect the dietary fiber as heat is not used (Charunuch *et al.*, 2014) [4]. This significant increase improved the nutritional quality of the products as consumption of foods with high dietary fiber lowers the risk of cardiovascular diseases. The use of cold extrusion also did not affect the fiber content and hence significant increase of 2.5 and 4.0 times was observed as shown in figure 2.

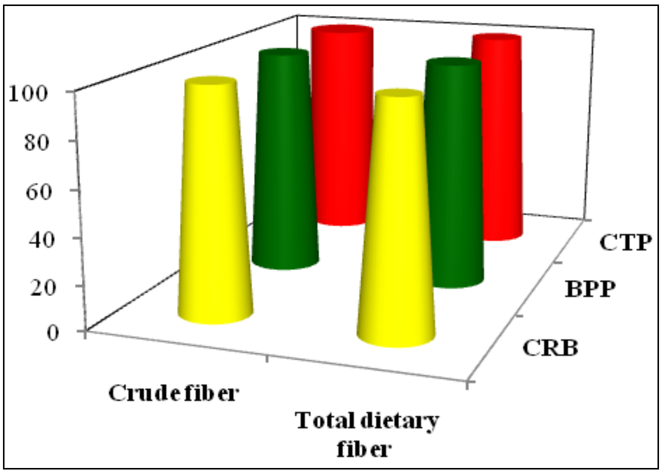

Fig 2: Percentage increase in fiber content of extrudates

In the current study, extracts of cold extrudates showed inhibitory activity against α-glucosidase enzyme activity as a measure of *in vitro* carbohydrate digestibility. The percentage inhibition ranged from 10.0 to 88.0% for various concentrations of extrudates as shown in Figure 3.

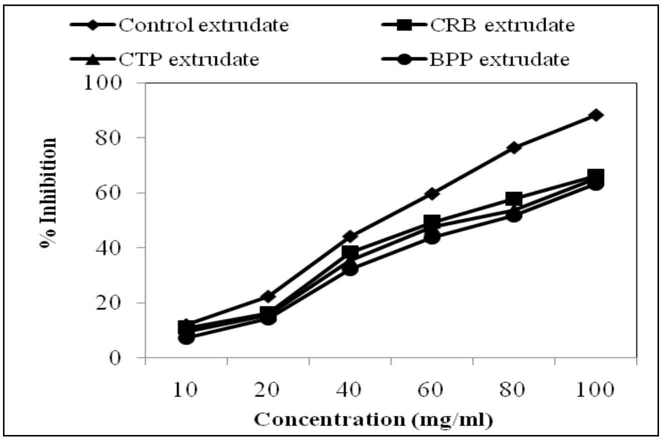

Fig 3: Percentage inhibition of α – glucosidase enzyme activity

The maximum percentage inhibitory activity was recorded for control extrudates with 88.43%. The α-glucosidase inhibitory activity of extrudates was expressed as IC_{50} values. The IC_{50} inhibitory activity for control extrudate was 68.50 mg/ml indicating that starch was readily available for inhibitory activity. The adding of rice bran and vegetable pomace powders decreased the requirement of α – glucosidase enzyme as shown with IC_{50} activity for CRB extrudate of 62.19 mg/ml; CTP extrudate 60.24 mg/ml and BPP extrudate with 45.28 mg/ml in comparison with control. The decrease for α – glucosidase enzyme to inhibit was 10, 34 and 12% for CRB, BPP and CTP extrudates respectively which may mainly be due to the formation of resistant starch and unavailability of starch for digestion.

Table 4: α – glucosidase enzyme activity in extrudates

S. No.	Parameter	Extrudates			
		Control	CRB	BPP	CTP
1.	IC_{50}	68.50±0.06	62.19±0.09	45.28±0.05	60.24±0.01
2.	S.E	0.1238	0.0864	0.1401	0.1849
3.	C.D	0.2759	0.1925	0.3123	0.4121
4.	C.V (%)	0.42	0.28	0.33	0.56

Note: Values are expressed as mean ± standard deviation of three determinations.

Means within the same column followed by a common letter do not significance differ at $p \leq 0.05$.

Units expressed as mg/ml

Control: Rice flour + refined wheat flour extrudate

CRB: Control +stabilized rice bran extrudate

BPP: Beetroot pomace powder incorporated CRB extrudate

CTP: Cauliflower trimmings powder incorporated CRB extrudate

In this study, the ability of the extrudate extracts to inhibit the *in vitro* carbohydrate digestibility was compared on the basis of percentage inhibition and expressed as IC_{50} values shown in Figure 4. The various samples showed a dose dependent inhibition of α-amylase enzyme activity. The maximum inhibition of 94.73% was recorded for control extrudate.

The IC_{50} values of samples calculated for control, CRB, CTP and BPP extrudates were 40.03, 40.4, 40.97 and 41.74 mg/ml respectively. There was not much change in the inhibition percentage due to the addition of stabilized rice bran and vegetable waste powders in comparison with the control extrudate indicating that carbohydrate availability remained more or less the same as shown in Table 5.

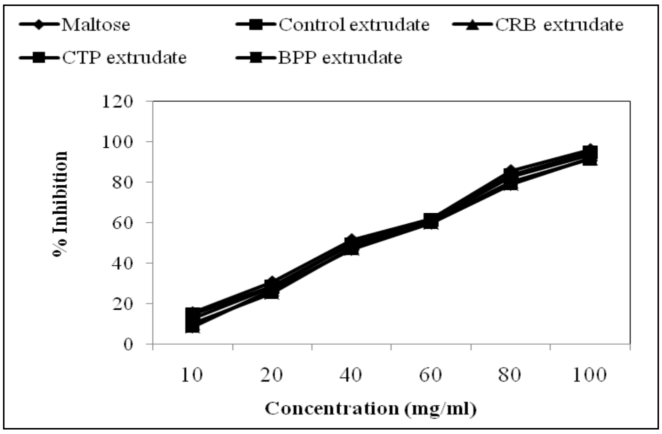

Fig 4: Percentage inhibition of α – amylase enzyme activity

Table 5: Inhibition of α – amylase enzyme activity in extrudates

S. No.	Parameter	Extrudates				Maltose
		Control	CRB	BPP	CTP	
1.	IC_{50}	40.03±0.06	40.4±0.08	41.74±0.01	40.97±0.07	36.25±0.02
2.	S.E	0.1552	0.1824	0.1335	0.1488	0.0342
3.	C.D	0.3460	0.4065	0.3464	0.3315	0.0762
4.	C.V (%)	0.34	0.41	0.35	0.34	0.07

Note: Values are expressed as mean ± standard deviation of three determinations.

Means within the same column followed by a common letter do not significance differ at $p \leq 0.05$.
Units expressed as mg/ml
Control: Rice flour + refined wheat flour extrudate
CRB: Control + stabilized rice bran extrudate
BPP: Beetroot pomace powder incorporated CRB extrudate
CTP: Cauliflower trimmings powder incorporated CRB extrudate

Conclusion

From the present study, it can be concluded that incorporation of rice bran at 15%, cauliflower trimmings powder (5%) or beetroot pomace powder (7.5%) in extrudates with rice flour and refined wheat flour not only improves the texture, taste and overall acceptability but also improves the nutritive value of these extrudates, which are generally thrown away can be utilized in a better way thus reducing wastage.

Also these vegetable wastes improved the dietary fiber in the extrudates. The *in-vitro* carbohydrate digestibility with α – amylase enzyme showed no change among the various extrudates but α – glucosidase enzyme showed its effects on the extrudates. BPP extrudate had carbohydrates that are cannot be digested by α – glucosidase enzyme followed by CTP extrudate and CRB extrudate in comparison to control extrudate.

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