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Optimization of process for the preparation of antioxidant rich ginger candy by response surface methodology

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Abstract

Ginger is well known for its medicinal properties and health benefits through ages in almost all system of medicines against many diseases and infections but have moderate antioxidant activity. Pomegranate peels are employed in traditional medicine because of their strong phytochemicals profile. An attempt was made to optimize the process for production of antioxidant rich ginger candy by response surface methodology. The experimental variables considered were dried pomegranate peel powder (1-5% of total syrup) and blanching time (5-15 minutes) using response surface methodology i.e. central composite design (CCD). Ginger slices were blanched as per the design followed by dipping in 75°B sugar syrup having different concentrations of pomegranate peel as per design and 2.0% citric acid for 24 hours at room temperature and dried at 50°C for 1 h. The candies so prepared were evaluated for different physico-chemical and sensory attributes. The optimum product qualities; total soluble solids (TSS) (67.24oB), titratable acidity (1.11%), antioxidant activity (66.70%), TSS: acid ratio (60.61), total phenolics (18.03 mg/100 g), total proteins (7.16 mg/100 g), overall acceptability (7.18) and acceptability index (79.74%) with 0.894 desirability were obtained using 5% peel concentration and 15 minutes blanching time. It was further observed that the loss of antioxidant activity due to blanching has been recovered with the addition of pomegranate peel powder. In nutshell, it is concluded that in the processing of antioxidant rich ginger candy, pomegranate peel had a great influence on antioxidant activity and total phenolics content of antioxidant rich ginger candy which is desirable from health point of view, whereas, blanching time had influence on the TSS, titratable acidity, TSS: acid ratio which is desirable from the sensory point of view.

Introduction

Ginger *(Zingiber officinale)* belongs to the family zingiberaceae consumed worldwide as a spice or condiment for over 2000 years (Bartely and Jacobs, 2000). The rhizomes of ginger plants are typically consumed as cooking spice or as a fresh ginger paste, dried powder, preserved in syrup or for flavouring tea (Bhattarai *et al*., 2001; Badreldin *et al.*, 2008). Ginger is widely used as a medicinal plant in Asian, Indian and Arabic herbal traditions since ancient times (Altman *et al*., 2001). The medicinal properties of ginger is due to the presence of polyphenols, terpenoids, isoterpenoids compounds i.e. gingerol and its derivatives which are responsible for therapeutic, anti-oxidative properties, anti-bacterial property (Langner *et al*., 1998; Habsah *et al.*, 2000).

In food and beverages, ginger is used as an additive for its aroma and pungency. The aroma or pungent taste of ginger is due to the presence of the key components such as volatile essential oils and non-volatile oleoresin (Famurewa *et al*., 2011) which includes zingeberene, curcumene (Govindarajan, 1982), gingerols and shagoals (Wohlmuth *et al*., 2005). These compounds contains bioactive constituents which help against many pharmacological action such as common cold, high cholesterol, ulcers, arthrosclerosis, sore throats, indigestion, diabetes and cardiovascular disease (Tapsell *et al*., 2006; Shukla and Singh, 2007; Badreldin *et al*., 2008). Ginger has intense effect in curing many diseases, but the pungent taste of raw ginger limits its use. Ginger candy is a traditionally ready to eat product have great demand in confectionery due to the acceptable sensorial characteristics. Till date, limited numbers of the valued added products from ginger are available in market (Nath *et al*., 2013) like ginger candy, tonic, syrup etc. and of these, candy is one of the most important product. For the preparation of ginger candy, blanching is an important pre-treatment

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but it results in leaching of the phytochemicals and ultimately a decrease in the antioxidant potential. Till date, no documentation has been reported regarding increase in phytochemical potential of the ginger candy. So, there is a strong need to develop the alternative or modified methods to increase the phytochemical as well as antioxidant potential of the ginger candy.

Nowadays, there has been increasing interest in applying the fruit processing waste in the manufacturing of high value added products because of presence of high amount of dietary fibre as well as phenolic compounds (Balasundram *et al*., 2006) which have considerable economic benefits to food processors. Pomegranate peel is an inedible part of pomegranate which was obtained during the processing of the juice. Pomegranate peel is a rich source of the bioactive compounds which is often discarded as waste (Fawole *et al*., 2012). Pomegranate peel are relatively high in polyphenolic class (Li *et al.*, 2006) which possesses a wide range of action such as antimicrobial, antioxidant, anti-inflammatory and therapeutic properties (Rajan *et al.*, 2011). The peel can used to increase their shelf life as well as their functional properties due to the presence of antioxidant and antimutagenic properties which can be exploited as bio-preservatives in various food applications and nutraceuticals (Negi *et al.*, 2003; Knatt *et al.,* 2010).

The current study was carried out to optimize the process parameters by response surface methodology for the production of antioxidant rich ginger candy. The results so obtained are discussed here.

Material and Methods

Preparation of pomegranate peel powder

Pomegranate peel was collected from local market juice shop of Jalandhar (Punjab, India) and was washed with distilled water to remove the undesirable dirt particle or to reduce the microbial load. The peel was dried in a tray drier at 50°C. The dried peel was pulverised and sieved to obtain fine powder.

Preparation of antioxidant rich ginger candy

Fresh uniform size ginger rhizomes (73.8±3% moisture content) were collected from the local market of Jalandhar (Punjab, India) and washed thoroughly with water to get rid of dirt or additionally to reduce the microbial load. After washing, rhizomes of ginger were air dried at room temperature (27±2°C) for 1-2 h for removal of surface moisture followed by manual peeling and slicing (Nath *et al.*, 2013). The slices so prepared were used for preparation of antioxidant rich ginger candy by blanching them in boiling water having 2% citric acid for 5-15 minutes according to the design and then steeped in 75°Brix sugar syrup having the different concentrations of pomegranate peel (1-5% of total syrup) with 2% citric acid for 24 hrs at room temperature followed by draining and drying at 50ºC for 1 hour (28.3±2% final moisture content), cooling and packing and used for further analysis. Response surface methodology (RSM) was used to predict optimal parameters such as pomegranate peel powder concentration (X_1) and blanching time (X_2). Table 1 shows the maximum and minimum levels of variables (peel concentration and blanching time) chosen for trials (runs) in the CCD. Second-order experimental design, i.e., CCD with two factors at five levels was employed to investigate the first and higher-order main effects of each factor and interactions among them. The design involved 5 center design points with α value being ± 1.41. The five coded levels investigated in the current study were -2, -1, 0, +1 and +2.

Statistical analysis

A second-order polynomial equation was established based on analysis of variance and the optimum ratio of the medium component was found using the Design-Expert 7.1 software optimization toolbox. Standard deviation, r^2 values were also determined. The mathematical model generated during RSM implementation was validated by conducting check point studies.

Analytical methods

The Total soluble solids (TSS) and titratable acidity of the candy were analysed as per the standard methods (AOAC, 1980). Antioxidant activity (DPPH free radical scavenging activity) of candy was measured as per the standard method of Brand-Williams *et al*. (1995). The TSS:acid ratio was calculated by dividing the total soluble solids by per cent acid as described by Grewal *et al*. (2000). The total phenols in dried pomegranate peel were determined by using Folin-Ciocalteu reagent using gallic acid as standard (Sadasivam and Manickam, 1991). Total proteins of candy were determined by Lowry's method as described by Sadasivam and Manickam (1991). In order to study the effect of multiple replicate analysis on the physico-chemical characteristics of antioxidant rich ginger candy, each analysis was performed three times at the same conditions. The overall acceptability of candy was evaluated by panel of 10 judges in respect of colour, texture, flavour and taste using the standard method as suggested by Amerine *et al*. (1965).

Result and Discussion

Effect of process parameters

Antioxidant rich ginger candy was prepared using two variables i.e. pomegranate peel concentration (1-5% of total syrup) and blanching time (5-15 min). The values of all the responses at different experimental combination for antioxidant rich ginger candy are presented in Table 1. The models conducted for responses functions were examined for their adequacy and fitness by using regression analysis and ANOVA technique. F-value for lack of fit was found to be non-significant which indicates the validity of models. Evaluation of experimental values for responses shows that coefficient of determination (R^2) and coefficient of variation (CV) values were adequate and satisfactory (Table 2). As a result the models had been valid for further analysis.

Due to the effect of blanching time and peel concentration, the TSS of the antioxidant ginger candy varied from 65 to 70^{o}B (Table1). Blanching time is the most critical parameter affecting the TSS ($p<0.05$) at linear and quadratic terms (Table 2). However, pomegranate peel concentration has no significant affect on TSS. Highest TSS of antioxidant ginger candy was observed at blanching time 5-10 minutes whereas minimum was observed at 10-15 minutes blanching treatments. Figure 1(a) shows that the TSS of antioxidant rich ginger candy increased with increase in blanching time till 10 minutes blanching but after that it decreased with further increase in blanching time. This might be due to increase in blanching time up to a certain limit caused an increase in TSS through softening of the tissues and permitting faster penetration of sugar through osmosis, whereas, a further increase in blanching time may affects the semi-permeability of the cell walls and reduces the rate of osmosis. Azian *et al.* (2004) reported that longer blanching time will increase the permeability of cell wall and solid gain will increase with immersion time, blanching time and syrup temperature. Similar results were reported by Alam *et al*. (2010) for aonla slices and Nath *et al.* (2013) for ginger candy.

Titratable acidity of the antioxidant ginger candy varied from 1.10 to 1.33% (Table1). Pomegranate peel concentration was the most significant parameter affecting the titratable acidity ($p<0.05$) at linear terms, whereas, in at quadratic terms it was pomegranate peel concentration and blanching time (Table 2). However, there was no significant contribution at blanching time linear and interaction terms. However, there was no significant contribution at blanching time linear and interaction terms. Highest titratable acidity of antioxidant ginger candy was observed at 3% peel concentration and 10 minutes blanching time (1.33%). The result observed from Figure 1(b) revealed that peel concentration and blanching time have the indirect effect on the titratable acidity of antioxidant rich ginger candy. Increase in blanching time caused increase in titratable acidity; this probably due to longer heat treatment and in addition due to extended exposure of slices for more penetration of acid into the product. Similar influence was accounted by Alam *et al.* (2010) for anola slices.

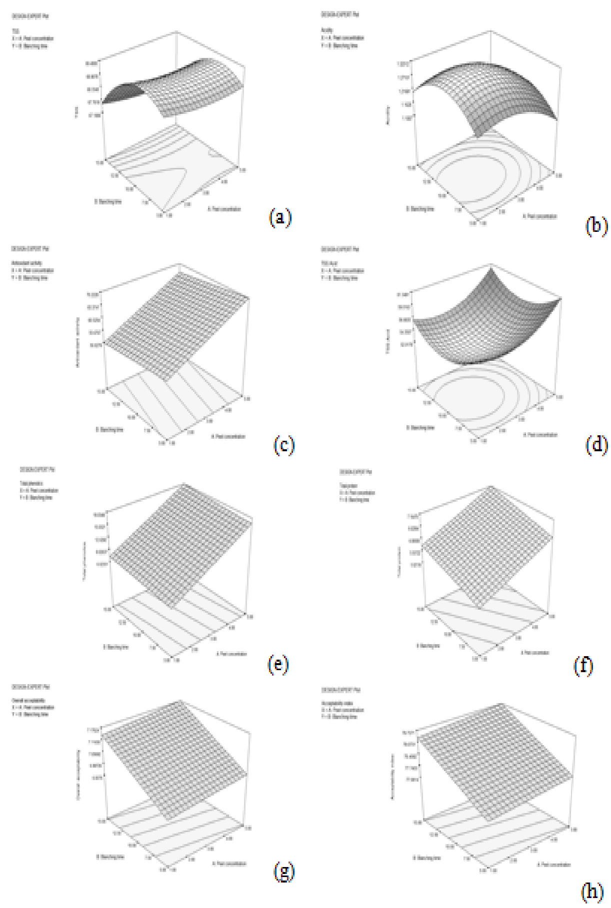

Figure 1. Response surfaces plots for effect of pomegranate peel concentration (%) and blanching time (min) on different quality parameters viz., TSS (a), titratable acidity (b), antioxidant activity (c), TSS:acid ratio (d), total phenolics content (e), total protein content (f), overall acceptability (g) and acceptability index (h)

Antioxidant activity of the dried pomegranate peel and fresh ginger extract was 79.08% and 71.54% respectively. The antioxidant activity of the antioxidant rich ginger candy varied from 44.45 to 71.65% (Table 1) due to the effect of blanching time and peel concentration. Pomegranate peel concentration is the most significant parameter affecting the antioxidant activity ($p<0.05$) at linear

Table 1. Experimental data for antioxidant rich ginger candy under the different treatment of pomegranate peel concentration (%) and blanching time (min)

Run	Process variables (Coded terms)		Responses							
	Peel concentration (%)	Blanching time (minutes)	TSS (°B)	Titratable acidity (%)	Antioxidant activity (%)	TSS:acid	Total phenolics (mg/100g)	Total protein (mg/100 g)	Overall acceptability	Acceptability index (%)
1	1.00	1.00	68.00	1.13	71.65	60.18	13.39	7.22	7.15	79.44
2	0.00	0.00	69.00	1.32	59.59	52.27	9.48	5.79	7.12	79.11
3	0.00	0.00	69.00	1.31	58.76	52.67	9.47	5.76	7.10	78.89
4	0.00	0.00	69.00	1.32	58.91	52.27	9.48	5.79	7.12	79.11
5	0.00	1.41	65.00	1.10	54.16	59.09	15.10	6.95	7.28	80.89
6	1.00	-1.00	68.00	1.13	71.51	60.18	13.27	7.16	7.15	79.44
7	-1.00	1.00	68.00	1.22	58.07	55.74	10.09	7.10	6.90	76.67
8	0.00	0.00	69.00	1.30	58.41	53.08	9.54	5.61	7.12	79.11
9	-1.00	-1.00	68.00	1.22	57.87	55.74	10.05	6.90	6.80	75.55
10	1.41	0.00	69.00	1.11	70.09	62.16	30.56	6.91	6.90	76.67
11	-1.41	0.00	70.00	1.25	44.45	56.00	4.87	3.25	7.22	80.22
12	0.00	-1.41	69.00	1.13	64.37	61.17	11.54	5.00	6.78	75.33
13	0.00	0.00	69.00	1.33	58.99	51.88	9.54	5.77	7.10	78.89

TSS: total soluble solids

level (Table 2). However, there was no significant contribution of blanching time. Highest antioxidant activity of antioxidant ginger candy was observed at high peel concentration (5%) and high blanching time (15 minutes) (71.65%), this might be due to pomegranate peel which was characterized by substantial amounts of phenolic compounds including flavonoids, anthocyanin and hydrolysable tannins (Afaq *et al.*, 2005; Zahin *et al.*, 2010). Figure 1(c) show that pomegranate peel concentration has direct effect on the antioxidant activity of antioxidant rich ginger candy, this is due to the higher content of polyphenols and tannin of pomegranate peel (Ashoush *et al.*, 2013) which allow them to act as hydrogen donor, singlet oxygen quenchers and also may have a metallic chelating potential (Ozkal and Dinc, 1994; Adedapo *et al.*, 2008). Chidamabaramurthy *et al.* (2002) reported that the presence of gallic acid and ellagic acids in pomegranate peel are responsible for antioxidant activity.

Due to the effect of blanching time and peel concentration, TSS:acid ratio varied from 51.88 to 62.16 (Table 1). The data presented in Table 2 indicates that pomegranate peel concentration and blanching time are the most significant parameter affecting the titratable acidity ($p<0.05$) at quadratic terms. However, there was no significant contribution at blanching time linear and interaction terms. Highest TSS:acid ratio (62.16) of antioxidant ginger candy was observed at 5.83% peel concentration and 10 minutes blanching time. Figure 1(d) indicates that peel concentration and blanching time have the direct effect on the TSS:acid ratio of antioxidant rich ginger candy due to the acidity of pomegranate peel and also due to the higher blanching time, which increases the sugar level in the product which gradually increases the TSS:acid ratio. The results of the current study were in line with the findings of Potter and Hotchkiss, (1995) who reported that the higher the Brix the greater the sugar concentration in the juice; the higher the Brix to acid ratio the sweeter and less tart is the juice.

The total phenolics of the antioxidant ginger candy varied from 4.87 to 30.56 mg/100 g (Table 1). Pomegranate peel concentration is the most significant parameter affecting the total phenolics ($p<0.05$) at linear level (Table 2). However, there was no significant effect of blanching time at linear terms. Highest total phenolics of antioxidant ginger candy were observed at high peel concentration (5.83%) irrespective to blanching time (30.56 mg/100 g). Total phenolics content was observed at high pomegranate peel concentration; this might be due to the presence of ellagic acid, ellagitannins and gallic acid (Nasr *et al.*, 1996). Figure 1(e) revealed that pomegranate peel concentration have direct effect on the total phenolics of antioxidant rich ginger candy. Ashoush *et al.* (2013) reported that pomegranate peel powder are a good source of total phenolics content and had a great free radical scavenging activity. Several studies have revealed that total phenolics content in plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents (Adedapo *et al.*, 2008).

Total proteins of the antioxidant ginger candy varied from 3.25 to 7.16 mg/100 g (Table 1). Close perusing of data given in Table 2 indicates that there was no significant contribution of peel concentration

Table 2. ANOVA and regression coefficients of the second-order polynomial model for the response variable

Variables	DF	Estimated coefficients								F-values							
		TSS	TA	AA	TSS:acid	Total phenolics	Total protein	OA	AI	TSS	TA	AA	TSS:acid	Total phenolics	Total protein	OA	AI
Model	5	69.00	1.32	60.53	52.43	12.03	6.09	7.06	78.41	4.11	58.48	17.91	31.49	5.43	2.41	1.94	1.95
X_1	1	-0.18	-0.047	7.94	2.20	5.36	0.69	0.018	0.20	0.40	54.40*	34.14*	36.94*	10.70*	3.71	0.13	0.13
X_2	1	-0.71	-5.303E-003	-1.76	-0.37	0.65	0.38	0.10	1.12	6.40*	0.69	1.68	1.03	0.16	1.10	3.75	3.77
X_1^2	1	0.19	-0.061		2.91					0.39	79.17*		56.32*				
X_2^2	1	-1.06	-0.094		3.44					12.57*	185.75*		78.46*				
$X_1 X_2$	1	-.00	0.000		0.00					0.00	0.00		0.00				
Lack of fit											4.56	132.47	10.39	29291.81	298.88	300.90	360.70
R^2		0.8461	0.9766	0.7818	0.9574	0.8207	0.8248	0.8793	0.8802								
Adj R^2		0.5647	0.9599	0.7381	0.9270	0.4248	0.1897	0.1352	0.1362								
CV%		1.15	1.48	6.35	1.82	38.49	16.73	2.09	2.09								

TSS: total soluble solids, TA: titratable acidity, AA: antioxidant activity, OA: overall acceptability, AI: acceptability index, X_1: pomegranate peel powder concentration, X_2: blanching time
* Significant at $p<0.05$

and blanching time at linear terms. Highest total proteins of antioxidant ginger candy was observed at high peel concentration (5%) at low blanching time (5 minutes) (7.16 mg/100 g). Figure 1(f) for the effect of pomegranate peel concentration and blanching time indicates that peel concentration have direct effect on the total proteins of antioxidant rich ginger candy irrespective to the blanching time although the effect was non-significant (Table 2). Increase in peel concentration results in increase in total protein content of antioxidant rich ginger candy; this is due to the total protein content of the pomegranate peel (Bandal *et al.*, 2014). On contrary, increase in blanching time, decreases the total protein content due to the denaturation of total protein caused by effect of heat during blanching. Adejumo *et al.* (2013) reported that total protein content will decrease irrespective of blanching time and temperature.

The overall acceptability score of the antioxidant ginger candy varied from 6.78 to 7.28 (Table 1) due to the effect of blanching time and peel concentration. The evaluated data given in Table 2 indicates that there was no significant contribution of peel concentration and blanching time at linear terms. Highest overall acceptability score of antioxidant ginger candy was observed at moderate peel concentration (3%) at high blanching time (17.07 minutes). Figure 1(g) shows for the effect of pomegranate peel concentration and blanching time of antioxidant rich ginger candy indicates that blanching time have direct effect on the overall acceptability score of antioxidant rich ginger candy irrespective to the peel concentration. This might be due to longer immersion times and higher temperature increased the migration of solute which consequently resulted in improvement of colour, sweetness and overall acceptability of ginger candy (Rastogi *et al.,* 2002).

The acceptability index of the antioxidant ginger candy varied from 75.33 to 80.89 (Table 1) due to the effect of blanching time and peel concentration. The evaluated data presented in Table 2 indicate that there was no significant contribution of peel concentration and blanching time in linear terms. Highest acceptability index of antioxidant ginger candy was observed at moderate peel concentration (3%) at high blanching time (17.07 minutes) (80.89). Blanching time has direct effect on the acceptability index score of antioxidant rich ginger candy irrespective to the peel concentration (Figure 1h). This might be due to increase in blanching time caused an increase in TSS through softening of the tissues which permitted the faster penetration of sugar through osmosis up to some extent and resulting in improvement of its sensory indexes. From this it can be concluded that higher sugar concentration gives the higher acceptability for antioxidant rich ginger candy as the taste is somewhat influenced by sweetness (Bhuiyan *et al*., 2012).

Optimization

As per desired goals, each factors and responses was chosen to optimize the process parameters for the production of antioxidant rich ginger candy (Table 3). The desirability function was obtained by running central composite design. The software generated two optimum condition for the production of antioxidant rich ginger candy. The solution no.1 having extreme desirability (0.894) with 5% pomegranate peel concentration and 15 minutes blanching time having 67.24°B TSS, 1.11% titratable acidity, 66.70% antioxidant activity, 60.61 TSS: acid ratio, 18.03 mg/100 g total phenolics, 7.16

Table 3. The optimum conditions of independent variables with the predicted values of response for the production of antioxidant rich ginger candy

Sr. No.	Process variables		Responses								Desirability
	Peel concentration	Blanching time	TSS (°B)	Titratable acidity (%)	Antioxidant activity (%)	TSS:acid ratio	Total phenolics (mg/100g)	Total protein (mg/100 g)	Overall acceptability	Acceptability Index (%)	
1	5.00	15.00	67.24	1.11	66.70	60.61	18.03	7.16	7.18	79.74	0.894
2	5.00	14.78	67.36	1.12	66.77	60.34	18.00	7.14	7.17	79.68	0.887

TSS: total soluble solids

mg/100 g total proteins, 7.18 overall acceptability and 79.74% acceptability index were predicted on those conditions and the desirability graph (Figure 3) was chosen as the ideal procedure condition for production of antioxidant rich ginger candy.

Conclusion

In the processing of antioxidant rich ginger candy, pomegranate peel had greater influence on antioxidant activity and total phenolics, whereas, blanching time had influence on the TSS, titratable acidity and TSS: acid ratio. Antioxidant rich ginger candy having functional and nutritional properties of both ginger and pomegranate peel, can be developed by blanching of ginger slices for 15 minutes and steeping in sugar syrup having 5% pomegranate peel powder (0.894 desirability) to achieve optimum product characteristics. This led to the enhancement of the functional properties of ginger candy which is available traditionally in the market and at the same time cost effective management of pomegranate processing industry waste.

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