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Functional hard-boiled candy formulation employing Plackett Burman design

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Plackett-Burman experimental design was employed as a screening experiment with five variables for preparation of hard boiled candy. The effect of varying the levels of hard-boiled candy ingredients such as ratio of liquid glucose (30-40%): sugar (60-70%) addition of nutraceuticals namely green tea extract (0.5-1%) and *Amla* powder (0.5-1%) along with salt (0.1 to 0.2%) on the physicochemical responses such as hardness, colour, total polyphenol, antioxidant radical scavenging, vitamin C and overall quality were studied. Results indicated that a formulation of: sugar 70%, liquid glucose 30%, *amla* powder and green tea extract 1% and salt 0.1% was considered as the optimum for obtaining a hard-boiled candy (HBC) with highest overall quality. The present study showed that the hard-boiled candy with added green tea extract and *Amla* powder were natural sources of polyphenols, vitamin C and antioxidants.

Keywords: *Amla* powder, Green tea extract, Hardboiled candy, Plackett Burman design, Vitamin-C

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One of the oldest industrialized sugar confectionery is hard boiled candy[1], it is a super-cooled, non-crystalline liquid, which is below the melting or softening points[2]. It is prepared from sugar along with invert sugar, glucose syrup, citric acid, colour and flavours.

Sugar on heating melts into an amorphous mass that can be pulled and rolled as needed. On cooling this mass becomes thicker and starts to hold shape which can be fashioned into various shapes, when cooled completely the crystals remain merged and form solid hard boiled candy[3].

The texture, taste and appearance characteristic of hard boiled candies are mainly due to the raw material combinations of sugar and liquid glucose. Undesirable changes increase hygroscopicity and these changes are caused due to hydrolysis of sugars, which are responsible for decreasing the cooling rate during moulding which in turn affects sugars[3]. In order to prevent the excess sucrose from recrystallizing when solution becomes supersaturated, interfering agents like corn syrup rich in glucose, fats, acids and proteins are added. These *agents* can prevent or slow the crystal growth.

Attempts have been made to enhance the nutritional benefits of hard-boiled candy by addition of nutraceuticals. Hard-boiled confections are empty calories without nutrition; to fortify this popular product and provide nutrients to the consumers especially children natural ingredients like green tea and *amla* can be added. Anand, *et al.* (2007)[4] have shown that micronutrient fortified candies were effective in improving the haemoglobin level and decreasing anaemia prevalence in children. They concluded that it serves as a suitable vehicle for micronutrient supplementation in children and other target groups. Green tea is rich in phenolic compounds and is a potential source of natural antioxidants, the health benefits of green tea or its extract is well documented[5]. Damaging effects caused by reactive oxygen species in cells are protected by antioxidants. Green tea boosts mental alertness and thinking, epidemiologic studies have reported about the protective effect of green tea[6]. Green tea consumption is known to lower incidences of diseases, such as cancer, diabetes, chronic fatigue, dental caries, kidney stones, and skin damage and obesity. It is also aids in weight loss and stomach disorders[7]. The major green tea ingredients, including catechin and (-)-epigallocatechin 3-gallate (EGCG), are responsible for the biological activity of green tea. Novel formulations can be formulated with green tea so as to increase the general antioxidant content for nutritional or technological purposes[8,9].

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Amla, (*Emblicaofficinalis* Gaertn) belongs to the family *Euphorbiaceae* and is an important medicinal plant in Ayurveda system of medicine. It is a rich source of vitamin C (478.56 mg/100 mL) higher than those in oranges, tangerines, or lemons[10]. *Amla* is known to be cardioprotective, gastroprotective, anti anaemic, antihypercholesterolemic, antidiarrhealetc. Furthermore, experimental studies have reported that *amla* and its phytochemicals also exhibit anticarcinogenic, chemopreventive, free radical scavenging, antioxidant, anti-inflammatory, antimutagenic and immunomodulatory activities[11].

The Phenolics in green tea and *amla* exhibit antioxidant activity by providing additional protection against oxidative reactions and reactive species. These antioxidants also lower oxygen concentration which in turn avoids the initiation of free radical scavenging. Formation of reactive oxygen species, generated and which cause damage to DNA, lipids, proteins and other biomolecules are prevented by antioxidants. Free radicals have been implicated in aging and other diseases as they are known to alter lipids, proteins and DNA. These radicals are also a part of the ROS that cause oxidative damage in biological and food systems[12].

In the present work, green tea extract (GTE) and *amla* powder (AP) were incorporated into hard-boiled candy and the effect of their addition on Physico-chemical properties of the candy was studied.

The objective of the present work was to optimize the formulation to obtain a nutraceutical enriched candy using Plackett–Burman experimental design technique to screen independent variables (Liquid glucose (LG), Sugar, Salt, Green Tea Extract (GTE) and *Amla* powder (AP)) these were taken as independent variables. Effect of these variables on the quality attributes such as texture, colour, vitamin-c and total polyphenols of the hard-boiled candy was determined.

Materials

Fresh *amla*, sugar, liquid glucose and common salt were procured from the local market, Mysuru, Karnataka, India. Green Tea Extract (GTE) was procured from Pellagic Food Ingredients Private Limited, Bangalore, Karnataka, India. The chemicals and reagents used for analysis were of analytical reagent grade.

Methods

Preparation of *Amla* powder

Fresh *amla* was grated using a prototype electrically operated grating machine, and the resultant gratings were spread as thin layers in each tray of a tray drier (Cross-Flow Tray Drier). The samples were subjected to tray drying at a temperature of 55-60°C for 8 h dried samples were ground and sieved through a 60 mesh sieve. The final moisture content of the *amla* powder was found to be 5to 7%.

Preparation of hard-boiled candy

Sugar, liquid glucose and water were heated to 145-150°C, GTE and salt were added after the final temperature was reached. Citric acid and *amla* powder were added at the last so as to retain the natural vitamins; this mass was cooled and then shaped using a lab-scale, hand-operated candy roller, to obtain small bits. Sugar: liquid glucose ratio which is a critical factor in hard-boiled candy was maintained, *amla* powder, green tea extract and salt were added according to the Plackett - Burman Design. 5 factors were altered with 12 base runs (Table 1). In all the runs, the amount of citric acid (1%), water (40-45%) and other processing

Table 1 — Physiochemical analysis of HBC showing experimental values of the response functions

Expt. no	Sugar	LG	GTE	AP	Salt	Hardness (N)	Color (L*)	TPP mg/g	RSA IC 50 Mg/ml	OQ*	Vit C mg/100g
	Sugar: liquid glucose										
1	70 (1)	30 (-1)	1.0 (1)	0.5 (-1)	0.1 (-1)	319.35	12.52	5.94	1.51	11.5	1.29
2	60 (-1)	30 (-1)	0.5 (-1)	0.5 (-1)	0.1 (-1)	277.79	17.73	3.09	2.26	9.8	1.87
3	60 (-1)	30 (-1)	1.0 (1)	1.0 (1)	0.2 (1)	245.27	14.01	7.54	1.15	9.5	2.75
4	60 (-1)	30 (-1)	0.5 (-1)	1.0 (1)	0.2 (1)	242.36	17.23	4.12	1.88	9.8	2.68
5	60 (-1)	40 (1)	0.5 (-1)	0.5 (-1)	0.1 (-1)	220.56	16.85	3.13	1.43	8.9	1.32
6	70 (1)	30 (-1)	1.0 (1)	1.0 (1)	0.1 (-1)	340.65	12.35	7.65	0.62	12.0	2.56
7	60 (-1)	40 (1)	1.0 (1)	1.0 (1)	0.1 (-1)	246.56	16.79	7.61	0.72	10.1	2.61
8	70 (1)	40 (1)	0.5 (-1)	1.0 (1)	0.1 (-1)	381.55	12.72	4.30	0.89	9.5	2.51
9	60 (-1)	40 (1)	1.0 (1)	0.5 (-1)	0.2 (1)	269.86	15.18	5.89	0.74	9.8	1.37
10	70 (1)	30 (-1)	0.5 (-1)	0.5 (-1)	0.2 (1)	350.56	13.1	3.19	1.53	12.1	1.32
11	70 (1)	40 (1)	1.0 (1)	0.5 (-1)	0.2 (1)	369.87	9.42	5.99	0.97	10.0	1.26
12	70 (1)	40 (1)	0.5 (-1)	1.0 (1)	0.2 (1)	365.65	14.68	4.30	1.01	9.5	2.58

conditions (temperature, cooling etc.) were kept constant. 500 g batches were prepared for the experimental runs. The prepared candy was packed in polythene pouches of 150 gauges and stored at 20±2°C till further analyses. Natural coffee flavour (100 µL) was added for improving sensory acceptability to the optimised sample and compared with control which had no added flavour.

Plackett–Burman experimental design for hard-boiled candy independent variables

Plackett–Burman theory[13], was adopted in the experimental design (Table 1) to determine the eﬀect of a total of 5 independent variables each at two levels on six response functions. The variables included sugar (%), liquid glucose (%), green tea extract, *amla* powder (%) and salt (%). The response functions were Hardness (N), Color L*, Total polyphenol content (mg/g), Radical scavenging activity IC 50 (mg/mL), Vitamin-C (mg/100 g and overall quality).

Quality attributes of hard-boiled candy

Texture analysis

The texture of hard-boiled candy was analyzed using texture analyser (LLOYD-LR-5K, Ametek, United Kingdom). A calibrated 5 kg load cell was used and set to zero automatically lowering the plate until the plate just touches the table where the hard boiled candy is placed. All the candy samples were subjected to a single bite compression test using 50% compression at the rate of 50 mm/min average of eight replicates were reported.

Color measurement (L*)

Colour measurement Instrument (Konica Minolta, CM5, Japan), was standardized using standard tiles. The L* values (10° view angle) were measured[8,14] and an average of five values reported.

Estimation of vitamin C

0.2 mg ascorbic acid mL^{-1} was prepared and metaphosphoric acid (3% w/v) added to increase the stability of ascorbic acid. This was titrated with 2, 6 di-chlorophenol indophenol solution (0.5 mg mL^{-1}concentration) till a pink colour appeared. Ascorbic acid as mg of sample was calculated equivalent to di-chlorophenolindophenol[15]. All experiments were carried out in triplicates.

Estimation of total polyphenols (TPP)

Different concentrations of *Amla* powder, Green tea extract and hard-boiled candy extracts (70% methanol extract) were mixed with 0.5 mL of 1:1 diluted FC reagent and saturated sodium carbonate solution. Allowed it to rest for 30 min at 30°C and absorbance measured using a UV-visible spectrophotometer (model UV 160, Shimadzu, Japan) at 760 nm[5]. All experiments were carried out in triplicates. A standard Gallic acid curve was used to calculate the total phenolics and results expressed as mg Gallic acid equivalent/g of sample.

Radical scavenging activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical assay was used to analyze the individual scavenging activities and expressed as a percentage of DPPH inhibition. The concentration at which 50% radical scavenging activity occurs (IC 50 value) was also calculated.

Different concentrations of *amla* powder, green tea extract and hard-boiled candy extracts (70% methanol extract) were mixed with 2.5 mL of DPPH. And allowed to stand for 20 min at 30°C in dark, the Absorbance was measured at 517 nm using a UV-visible spectrophotometer (model UV 160, Shimadzu, Japan) respectively according to the method of kodama *et al.* (2010)[5]. Radical scavenging activity of samples was compared with standard antioxidant BHA.

Sensory evaluation

Sensory evaluations of hard-boiled candies were carried out for colour, appearance, texture, taste, flavour and overall quality by a panel of 10 panellists. Representative samples of hard-boiled candy (coded sample) were given to the panellists for listing the sensory attributes of the product. A linear rating scale of 15-points ranging from 0 (No perceivable intensity) to 15 (Highest intensity)[16], was used to mark the attributes by the panellists.

Statistical analysis

Coded variables related to responses (Hardness, Colour, L*, Total polyphenol content, Radical scavenging activity IC 50, Vitamin-C and overall quality) for the various experimental matrix (xi, i = 1, 2, 3, 4, 5 and 6) were given by a first order multiple regression Eq. (1).

$$y = bo + \sum_{i=1}^{k} bixi + \in \quad \dots (1)$$

The polynomial coefficients are representedbyb_0 (constant term), b_1,b_2,b_k (linear effects) and ε(random error).Gauss–Jordan method[17] was used to obtain

Table 2 — Coefficients of the first order multiple regression equations for response functions in coded level of variables

Coefficient	H	C	TPP	IC50	OQ	Vit-C
b_o	-426.43*	43.66*	-1.10*	6.27*	6.85*	2.00*
b_1	10.42*	-0.38*	-0.00	-0.03	0.11**	-0.02
b_2	1.30	-0.02	-0.01	-0.05**	-0.12**	-0.01
b_3	-15.63	-4.01**	6.16*	-1.09**	1.10	-0.15
b_4	4.68	0.99	2.76*	-0.72	-0.57	2.42*
b_5	95.18	-8.90	-1.15*	-0.25	-1.83	-0.33
R^2	0.90	0.86	0.99	0.84	0.73	0.97

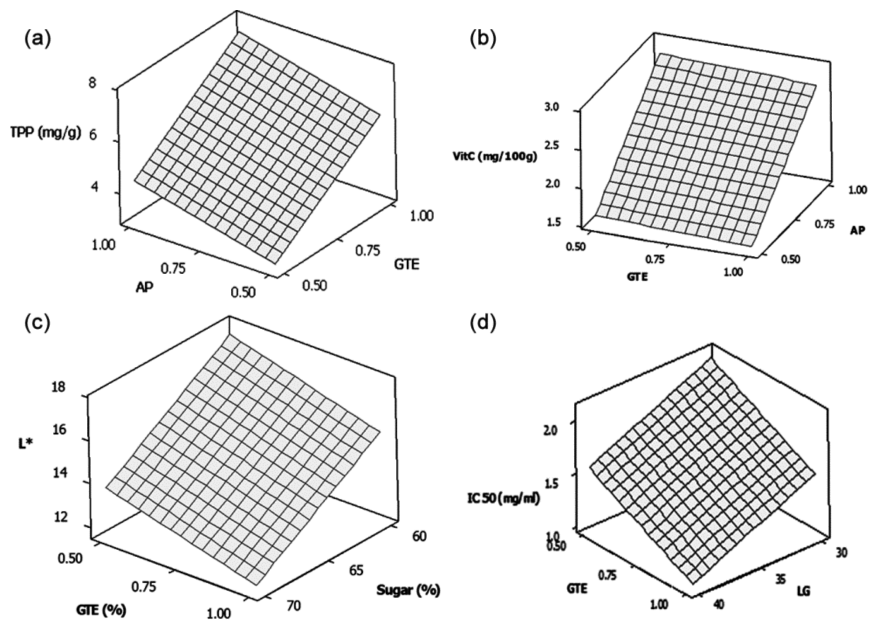

Fig. 1 — (1a) Response surface plot for the TPP (mg/g) and (1b) Vit-C as a function of Amla powder and Green tea extract by keeping other independent variables constant. (1c) Response surface plot for the L* values as a function of sugar and Green tea extract by keeping other independent variables constant. (1d) Response surface plot for the IC-50 (mg/mL) as a function of Liquid glucose and Green tea extract by keeping other independent variables constant.

coefficients of the polynomialand correlation coefficient (r) was determined at a probability (p) level of 0.01. The regression coefficients have been used to generate three-dimensional plots from the first order multiple regression model.

Results and Discussion

Six responses in the experiments namely, hardness (Y_1), Color L* (Y_2), total polyphenol content (Y_3), radical scavenging activity IC 50 (Y_4), vitamin C (Y_5) and overall quality (Y_6) were measured. The experimental design (coded and uncoded) and results for all the dependent response variables (Table 1). Table 2 shows coefficients of the first order multiple regression equations (in the coded level of variables). Three-dimensional plots based on effects of important variables (in actual level of variables) are shown in Figure 1 to aid visualisation effects. In general, the results of the responses indicate a complex interaction between the variables.

The magnitude of the multiple correlation coefficients of the first order equations (Eq. 1) shows that the equations predict effectively the dependent response variables in relation to the independent variables. Based on the sign (+or-) of the regression coefficients in coded variables (Table 2), the effect of the individual variables are considered as a positive or negative effect on response variable. Further, the magnitude of these coefficients indicates the relative effects of the variables on the dependent response variables. Independent variables with strong effects, as judged by comparing the magnitudes and identified for presence in the second stage of the experiment; thus, the number of variables can be reduced[8]. According to these results Plackett–Burman design can be suitably employed for screening of variables of larger numbers and to reduce the number of experiments.

Effect of green tea extract and amla powder on TPP and Vitamin C

Among the individual variables, the presence of *amla* powder markedly increased the total polyphenol and vitamin C. On the other hand, the total polyphenol content increases with GTE, but sugar, liquid glucose and salt had an opposite effect. The response surface (Fig. 1a and Fig. 1b) for TPP and vitamin C were drawn as a function of green tea extract and *amla* powder while keeping remaining variables constant at a coded level of zero. At the lowest and highest level of green tea extract (0.5%, coded value -1), the total polyphenol content and vitamin C values were found to increase with an increase in *amla* powder (Fig. 1a and Fig. 1b). The nutritional and antioxidant activity of a hard boiled Candy with added sacred figs (*Ficusreligiosa*) was studied by Verma & Guptha (2015)[18], their results indicated that the antioxidant activity was found to be good. Vitamin C undergoes degradation during drying and ascorbic acid is oxidised to dehydroascorbic acid, which is then hydrolysed to 2, 3-diketogulonic acid. This is further oxidised and polymerised to form a wide range of other nutritionally inactive products[19]. Loss of vitamin C is mainly due to high temperature and chemical degradation during preparation or processing. Significant loss occurs by leaching in freshly cut fruit due to the high solubility of vitamin C in aqueous solutions. Loss can also occur during storage and handling[19,20].

Effect of green tea extract and sugar on L* values

The L* values of hard-boiled candy were in the range of 9.42 to 17.73 with an average value of 14.38 (Table 1). At the highest level of sugar content (70%, coded value 1) the L* values of hard-boiled candy were found to decrease with increase in green tea extract. At the lowest level of sugar content (60%, coded value -1) the L* values of hard-boiled candy were found to marginally decrease with increase in green tea extract. The coefficients of the first order multiple regression equation and other statistics are presented in Table 2. For all levels of green tea extract, L* values of the hard-boiled candy were found to decrease with an increase in sugar content (Fig. 1c). This may be due to the fact that increases in sugar content leading to caramelization. Sugar syrup begins to caramelize above 140°C creating an intense flavour and rich colour, from light and clear to dark brown.

Effect of green tea extract and sugar on IC_{50} values

The IC 50 values of hard-boiled candy were found to less than 2.1 mg/mL with an average value of 1.23 mg/mL. At the highest level of liquid glucose (40%, coded value 1) the IC 50 values of hard-boiled candy were found to decrease with increase in green tea extract and the similar effect was observed at the lowest level of liquid glucose (30%, coded value -1) (Fig. 1d). At the highest level of liquid glucose (40%, coded value 1), the IC_{50} values of hard-boiled candy were found to decrease with increase in green tea extract and the similar effect was observed at the lowest level of liquid glucose (30%, coded value -1) (Fig. 1d). The IC_{50} values were noted to decrease when liquid glucose increase from 30% to 40% at the highest and lowest level of green tea extracts content.

Optimization of conditions for the hardboiled candy

The operational parameters were optimized using the factorial design (Plackett–Burman theory)[8] in this study. In order to determine the workable optimum conditions for the hardboiled candy, a response optimizer was used. The main criterion for optimization was maximum possible total polyphenol content, Vitamin C content and overall quality, keeping other responses within the acceptable range. The numerical optimization of process variables were optimized as, sugar (70%), liquid glucose (30%), green tea extract (1%), *amla* powder (1%) and salt (0.1%) (Table 3).

Sensory evaluation of optimized formulation of HBC

Results of the sensory analysis showed that candy had a dark brown colour mainly due to the presence of GTE (Fig. 2). The gloss was quite high which is a desirable attribute in candy mainly due to the liquid glucose and the optimum quantity of GTE contributes

Table 3 — Optimum conditions and predicted and experimental value of response at optimum conditions

Optimum condition	Coded	Actual
Sugar	1.0	70
LG	-1.0	30
GTE	1.0	1.0
AP	1.0	1.0
Salt	-1.0	0.1
Responses	**Predicted value**	**Experimental value**
Hardness	340.6	339.5
Color (L*)	12.26	12.35
TPP	7.54	7.65
IC 50	0.84	0.62
Vit-C	2.57	2.56
OQ	11.56	12.00

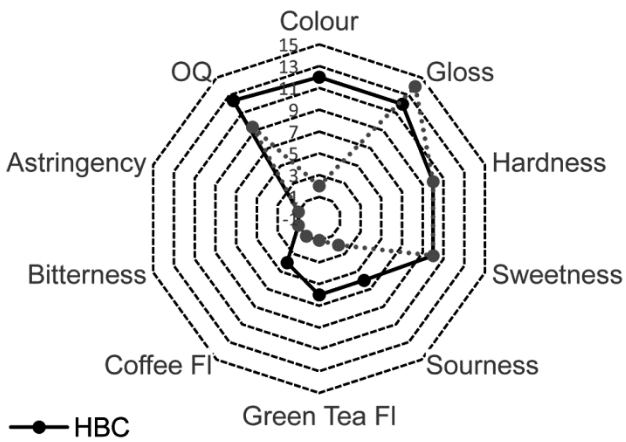

Fig. 2 — Sensory evaluation of optimized formulation of HBC

to the glossiness. Hardness and sweetness were well within the desirable range for a candy. Sourness was slightly lower as the amount of AP added was limited. As the sourness needed could not be achieved with *amla* powder alone, citric acid was added in all the runs during preparation. Higher amounts of AP increased crystallinity and the product became powdery. Silva *et al*. (2016)[21] studied the effect of adding spray dried acai fruit powder to chewy candy and study its effect on the sensory and textural qualities. Their results indicated that the no-added sucrose açai chewy candy was acceptable for all the attributes evaluated.

GTE flavour was well within the acceptable range, which can be seen by the low astringency and bitterness scores. As such GTE had no flavour of its own. Added coffee flavour to the optimised product enhanced the overall acceptability scores and this was widely accepted as the panellists felt that some flavour was necessary. The overall acceptability score was above 12 which indicated a good consumer response for the functional hard-boiled candy rich in TPP and natural Vitamin C.

Conclusion

Preparation and standardization of a hard-boiled candy containing green tea extract and *Amla* powder were optimised using Placket Burman Design. The optimized formulation of functional hard-boiled candy is sugar 70 parts, liquid glucose 30 parts, AP and GTE 1 part and salt 0.1. Citric acid was kept constant at 1% level. Complete replacement could not be carried out due to the astringency in *amla* powder. Responses corresponding to optimised conditions were total polyphenol (TPP), Vitamin C and L* (Colour) value keeping hardness of candy within acceptable ranges. These experimental conditions were validated and compared with predicted values. Results showed that the experimental values agreed with the predicted values (< 5% variation between predicted and experimental values). This study has shown that HBC can be a good carrier for fortification with various nutraceuticals especially Vitamin C from a natural source, besides being cost-effective sweetness from sugar helps to mask the bitter principle of GTE and AP.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Authors' Contribution

All the authors have same contribution in this research (Collected data, analyses, and formatted the manuscript).

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