Biochemical changes in chocolate due to addition of *Digera muricata* (Kondhra) leaves powder

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Abstract

Digera muricata (Kondhra) green leaves, various biochemical characteristics were estimated Sonal and Dr Nutan (2022). Leaves were converted to powder by standard method and supplemented to chocolate to further study the biochemical changes in the chocolate. The experiment was plan with four combinations namely control, 2.5g, 5g, 7.5g in chocolate and the analysis was carried out with sensory evaluation on a 9 point-hedonic scale and necessary and sufficient statistical analysis was done. The correlation analysis was carried out for different attributes of chocolate containing *Digera muricata* (Kondhra) leaves powder and presented as a correlation matrix. The analysis was carried out for selected parameters of chocolate viz., protein, fat, iron. The supplementation of 2.5g powder was most suitable and accordingly Shelf life was estimated to be 65 days.

Keywords: Chocolate, hedonic scale, correlation, ANOVA, shelf life

1. Introduction

Chocolate is one of the most well-known and widespread confectionery food products in the world, manufactured from cocoa by combining cocoa powder, sugar, and milk powder in a continuous fat phase to create cocoa butter. The market for chocolate is growing fast, and among all confectionery foods, chocolate is the most popular in India (Jyothi, 2003).

It can be utilised as a flavour component in various meals as a liquid, paste, or block (Shahkhalili *et al*., 2000). Spain was the only country where chocolate was introduced to Europe in the 16th and 17th centuries. Beginning in the early 20th century, chocolate production began to become industrialised, but even at that time, it was still

considered a luxury item for adults, only appropriate for festivals, special occasions, or sensitive moments between friends (Jyothi, 2003). A quality bar of chocolate should break smoothly, be lustrous brown in colour, and be free of lumps, tiny burst bubbles, and white flecks. It melts like butter on the tongue, smells more like chocolate than cocoa powder, and is neither greasy nor sticky. The three primary varieties of chocolates are Dark, White, and Milk chocolate, depending on the amount of various ingredients used in their production. The chemistry of the product and the typical compositions of the materials used in chocolate production influence the flavour of the chocolate to some extent. While perceived texture is a result of how the substance melts and breaks apart in the tongue, taste is dependent on the release of flavour compounds to the mouth and nose. The preferred chocolate flavour differs from nation to nation (Kulozik *et al*., 2003). Chocolate has numerous positive and therapeutic effects on the body in addition to having a tasty combination of fat and sugar. Flavonoids and polyphenols, which have significant antioxidant activity, are abundant in chocolate (Pimsentel *et al*., 2010; Schinella *et al*., 2010; Vanzani *et al*., 2011). A recent study has revealed a potential and unexpected role for cacao in "promoting health" of the consumer and preventing from many diseases, despite the fact that theobroma cacao has long been used as a medicine with little advancement in this area (Ding, 2006; Grassi, 2006). Numerous studies have demonstrated the positive effects of chocolate on endothelial function, insulin secretion, and coronary vasculature (Allen *et al*., 2008; Taubert *et al*., 2007).

A number of different ailments have been successfully treated with natural products, and chocolate is one of them. Chocolate has numerous positive and therapeutic effects on the body in addition to having a tasty combination of fat and sugar. *Digera muricata (Kondhra)* leaves powder chocolate has been prepared and wrapped in embossed chocolate wrapping plain foil paper of size 12*8 cm and chocolate weighs about 10g per 100g.

2. Materials and Methods

2.1 Raw material

Digera muricata (Kondhra) fresh leaves were collected from the agricultural fields of BPSMV University, Khanpur kalan, Sonipat (Haryana) and also from different sources including vegetable markets as per the requirements and used in powdered form dried by standard method.

2.2 Chemicals

For the experiment the researcher approached for necessary analytical findings for testing in a laboratory of National and International repute to get best possible results on sample analysis and other experimental framework.

All chemicals used during investigation were of AR (Analytical Reagent) grade and obtained from standard suppliers. The testing of product was carried out at Referral laboratory of ICAR-National Dairy Research Institute, NDRI, Karnal.

2.2 Equipment's

All the equipment's used for the testing of the developed food product are of high quality and standards.

2.4 Development and preparation of *Digera muricata* added food products

Wild edible green leafy vegetable under study was selected for perforation of traditional routine foods on the basis of its easy availability and suitable for the particular recipe. The experiment was initiated to prepare *Digera muricata (Kondhra)* chocolate with the use of other necessary ingredients like cocoa butter, cocoa powder and powdered sugar as a product and finally tested for its acceptability and other necessary quality characteristics.

The food products under study were developed by incorporating *Digera muricata (Kondhra)* fresh leaves powder by using various cooking methods by giving different treatments at different levels. The detailed method of preparation of product under study and the various proportions of materials and powder used for the development of traditional product are as follows:

Table 1: *Digera muricata* (Kondhra) leaves powder chocolate

Ingredients	I	II	III	IV
Cocoa powder	45g	42.5g	40g	37.5g
Melted cocoa butter	45g	45g	45g	45g
Powdered sugar	55g	55g	55g	55g
Kondhra powder	---	2.5g	5g	7.5g

Process: Weight of all the ingredients was according to the requirement. Sieve the cocoa powder and powdered sugar in a bowl and then mix all the ingredients to make liquid consistency. Take a silicon mold of any shape and pour the prepared chocolate mixture into the mold. Then keep the mold in the freezer for 1 hour cooling. Take out chocolate from the mold. Fresh and healthy *Digera muricata* (Kondhra) chocolates are ready to eat. This procedure was followed with all the four treatments as given in the above Table 1. The developed product has been shown in Plate 2.

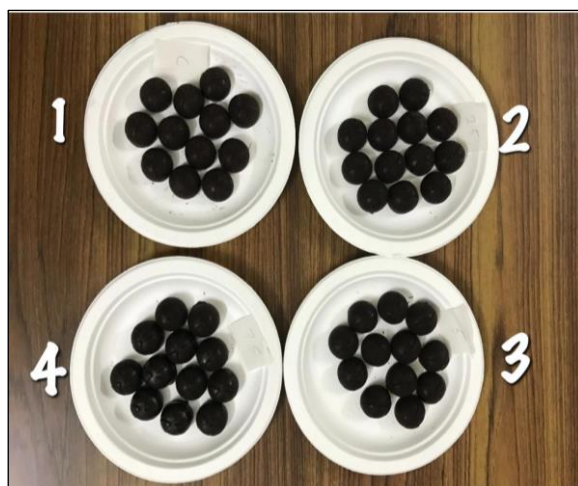**Plate 1:** *Digera muricata* (Kondhra) leaves plant

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Plate 2: *Digera muricata* (Kondhra) leaves powder chocolate

Type I: Control 45g (Cocoa powder).
Type II: 42.5g Cocoa powder + 2.5g *Kondhra* powder.
Type III: 40g Cocoa powder + 5g *Kondhra* powder.
Type IV: 37.5g Cocoa powder + 7.5g *Kondhra* powder.

2.5 Sensory evaluation

The perception of food product is a combination of different senses that contribute to the sensory qualities. These include characteristics like appearance, colour, texture, taste, aroma and overall acceptability of the product.

2.6 Development of score card

A 9-point Hedonic scale score card was developed to assess the organoleptic characteristics mainly based on the appearance and colour, taste, texture, aroma and overall acceptability for this food product developed.

2.7 Selection of sensory panel

Ten judge's experts in quality testing, possessing good health and interested in sensory evaluation were selected from Department of Foods and Nutrition, B.P.S. Institute of Higher learning, BPSMV, Sonipat and to be evaluated individually. All the panel judges were females.

2.8 Sample presentation for sensory evaluation by judges

Before the presentation of the coded product, each product was placed in a sensory evaluation chamber with saline water to rinse the mouth. The selected panels were made to sit comfortably in the chamber and requested to score for different sensory characters according to their importance in evaluating the acceptability of the product on the score card (9-point hedonic scale). The evaluation scores for different characteristics were subjected to further statistical analysis.

2.9 Shelf life

A food product's shelf-life is the amount of time during which it will continue to have a respectable flavour, texture, aroma and appearance. Microbiological safety, eutectics, bloom, migration (of fat or moisture), loss of texture, and rancidity are important aspects of shelf life for the confectioner (Marlene Stauffer., 2007). With a complex nutritional composition that includes fat (either cocoa butter or, if it is a confectionary coating vegetable fat), carbs, and protein, chocolate is a high-energy food. It does offer certain vitamins and minerals. It is an intimate based fat-and solid-suspended particle. It melts at body temperature and releases flavour quickly. Tocopherols found in cocoa solids, are a type of natural antioxidant and short-chain fatty acids, which are produced when cocoa butter is broken down. Because of this, the majority of chocolate products fall into the category of medium-or long-life products. Shelf-life elements can be changed when adding inclusions and using chocolate to make sweets.

They can be divided into two groups:

- Those which are inherent in the product and cannot be avoided by packing alone,
- Those which are reliant on the environment and are controllable.

2.9 Statistical Analysis

The data generated was statistically analysed for analysis of variance (ANOVA), Karl Pearson's coefficient of correlation, Kendall's coefficient of concordance and Physicochemical properties to determine significant difference. Data has been analyzed using the IBM SPSS software, version 25 at the 5% level of significance (α = 0.05).

The physicochemical properties of bar prepared corresponding to 4 treatment combinations namely control, 2.5g, 5g, 7.5g addition of *Digera muricata* (Kondhra) leaves powder were subjected to laboratory analysis as per methodology adopted above in 4 replicates for each parameter. Thus, the statistical analysis corresponding to data generated in a table with four replicates and four treatments was carried out according to Two-way analysis of variance (ANOVA). The statistical model was: (George W. Snecdecor., 1989).

$$y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

Where;

y_{ij} = Value of observation corresponding i^{th} row and j^{th} column

μ = Overall mean.

α_i = Effect of ith replicate, i = 1 to 4.

β_j = Effect of jth replicate, j = 1 to 4.

ϵ_{ij} = Random error.

3. Results and Discussions

The experiment was initiated to prepare *Digera muricata (Kondhra)* chocolate with the use of other necessary ingredients like cocoa butter, cocoa powder and powdered sugar as a product and finally tested for its acceptability and other necessary quality characteristics.

3.1 Sensory evaluation

The standard technique was adopted to evaluate the product for its acceptance and a 9-point hedonic scale was used to study various parameters namely: colour, appearance, flavour, texture and taste for all the four products as prepared above. Well qualified, experienced having good knowledge of tasting the product, ten judges were selected for the proper sensory evaluation of the products. It is worth mentioning that all the judges were females from the Institute of higher learning, BPSMV University, Khanpur Kalan, Sonipat. In other words, it could be mentioned that there was a homogenous group of judges involved in the sensory evaluation of the products. The acceptance scores were recorded by the judges on a well-tested score card.

3.2 Assessment of Quality

The scores given by judges on 9-point hedonic scale, the overall acceptability was measured on the basis of total mean scores for all the four products as mentioned below:

Table 2: Overall Sensory Scores of the Chocolate

	Digera muricata (Kondhra) powder			
Judges	**Control**	**2.5g**	**5g**	**7.5g**
1	9	9	8.8	8.8
2	7	7.2	7.2	7.2
3	7.6	8	8	8
4	9	9	8.8	8.6
5	8.8	9	8.8	8.6
6	9	9	8.6	8.4
7	8	8.4	7.6	7
8	8	7.6	7.4	7.4
9	8.4	8.6	8.2	8.2
10	8.4	8.4	8.8	8.2
Mean Scores	**8.32**	**8.42**	**8.22**	**8.04**

As per methodology adopted *Digera muricata* (Kondhra) Chocolates were prepared with the supplementation of (0, 2.5g, 5g and 7.5g) of *Digera muricata* (Kondhra) leaves powder and subjected to sensory evaluation with a panel of 10 expert judges and variable under study were color, appearance, flavor, texture and taste measured on a 9-point hedonic scale. The results so obtained were further subjected to correlation analysis and the results are summarized below in the form of the correlation matrix.

Table 3A: Correlation analysis of Chocolate

3(A)Chocolate Control	Colour	Appearance	Flavour	Texture	Taste
Colour	1				
Appearance	**0.701***	1			
Flavour	**0.709***	**0.763***	1		
Texture	**0.788****	**0.655***	**0.858****	1	
Taste	0.619	**0.836****	**0.923****	**0.793****	1
Mean Scores	**8.3**	**8.6**	**8.2**	**8.2**	**8.3**

Table 3: (B, C, D) Correlation analysis of 2.5g, 5g, 7.5g Digera muricata (Kondhra) leaves powder incorporated Chocolates

3.(B)Chocolate 2.5g	Colour	Appearance	Flavour	Texture	Taste
Colour	1				
Appearance	**0.800****	1			
Flavour	**0.733***	0.574			
Texture	**0.733***	0.574	**1.000****	1	
Taste	**0.898****	**0.912****	**0.668***	**0.668***	1
Mean Scores	**8.4**	**8.6**	**8.3**	**8.3**	**8.5**

3.(C) Chocolate 5g	Colour	Appearance	Flavour	Texture	Taste
Colour	1				
Appearance	**0.836****	1			
Flavour	**0.646***	0.352	1		
Texture	**0.676***	**0.676***	0.622	1	
Taste	**0.725***	**0.725***	0.114	0.19	1
Mean Scores	**8.3**	**8.3**	**8.2**	**8.1**	**8.2**

3(D) Chocolate 7.5g	Colour	Appearance	Flavour	Texture	Taste
Colour	1				
Appearance	**0.653***	1			
Flavour	**0.718***	**0.657***	1		
Texture	**0.761***	**0.715***	0.544	1	
Taste	**0.693***	**0.671***	0.596	0.293	1
Mean Scores	**8.1**	**8.4**	**7.8**	**8**	**7.9**

$*p<0.05$ $**p<0.01$.

Chocolate control: From the Table 3. (A), it very clear that correlation coefficient with colour and taste was not found to be significantly related and a set of all other nine correlations are found to be highly significant clearly showing a good interrelationship among all possible combinations of quality indicators.

2.5g *Digera muricata* **(Kondhra) leaves powder incorporated Chocolate:** The Table 3. (B), The correlation matrix for the evaluated product sensory evaluated by panel suggested strong evidence of relationship between colour and appearance, colour and flavour, colour and texture, colour and taste followed by appearance and taste, flavour and taste and so also texture and taste. ($p<0.01$), it is interesting to note that a correlation between flavour and texture is perfect positive correlation (1.000).

5g *Digera muricata* **(Kondhra) leaves powder incorporated Chocolate:** The Table 3.(C), The correlation analysis suggested no significant relationship among appearance and flavour, flavour and texture, flavour and taste and texture and taste. On the other hand, rest all combinations of variables were highly correlated ($p<0.05$).

7.5g *Digera muricata* (Kondhra) leaves powder incorporated Chocolate: The Table 3. (D), depicts that the product with *Digera muricata (Kondhra)* Chocolate with its supplementation of 7.5g submitted for sensory evaluation suggested non-significant relationship between flavour and texture, flavour and taste and texture and taste. It indicates that with increase (or decrease) is not affecting in one variable with the other. However, rest all combinations of variables were found to be satisfactorily interrelated ($p<0.05$).

The sensory evaluation has suggested that *Digera muricata* chocolate with supplementation of 2.5g were most acceptable and further analysis is given below to test the agreement among all judges with the use of Kendall's coefficient of Concordance W where W was estimated to be 0.432 (p<0.01) Chi square ($\chi^{2)} = 12.06$, with 3 degree of freedom.

Analysis of variance ANOVA

For Chocolate, as per methodology adopted chocolate was taken as a product and through sensory evaluation parameters 2.5g of *Digera muricata (Kondhra)* powder supplementation was the most acceptable level. On the other hand, the protein content was also reported to be comparatively higher for this level. The ANOVA in Table 4. Suggested a highly significant difference between all the four levels studied ($p<0.05$).

Table 4: Analysis of variance ANOVA of selected parameters of Chocolate

Anova	For	Protein			Fat			Iron			
Source of Variation	df	MS	F	CD (5%)	MS	F	CD (5%)	MS	F	CD (5%)	F crit
Rows	3	0.00467	9.46E+13	NG	0.00787	9.96E+12	NG	0.00204	81.66667	0.025	3.86255
Columns	3	0.85067	1.72E+16		16.5572	2.1E+16		24.2182	9.69E+05		
Error	9	4.9E-17			7.9E-16			2.5E-05			
Total	15										

CD = *$p<0.05$.

The Table 4. Shows that the estimated fat in chocolate was like protein content and so also the analysis of variance. As reported earlier, iron is one of the most important factors associated with the products under study. In the case of chocolate, no doubt the iron content was estimated to be significantly higher for 7.5g compared to 2.5g supplementation but due to certain sensory evaluation indicators has been found to be having better acceptability than others. The analysis of variance carried out suggested a highly significant difference among all four levels of *Digera muricata* (Kondhra) supplementation ($p<0.05$).

3.2 Shelf-life study of the *Digera muricata* (Kondhra) Chocolate

According to FFA and PV levels the expected shelf-life has been estimated with the help of Linear two-dimensional growth (decay) curve. The figure 1, shows that the growth rate in Free fatty acid (FFA) and Peroxide value (PV) values was found to be 0.002 and 0.015 units per day giving an expected shelf - life of 65 days for Free fatty

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acid (FFA) and 64 days for Peroxide value (PV) values. Further, it is pointed out that the melting point of chocolate was at 34 °C. It is recommended that 34 °C the chocolate maintains good taste and other quality characteristics and may be considered as optimum temperature.
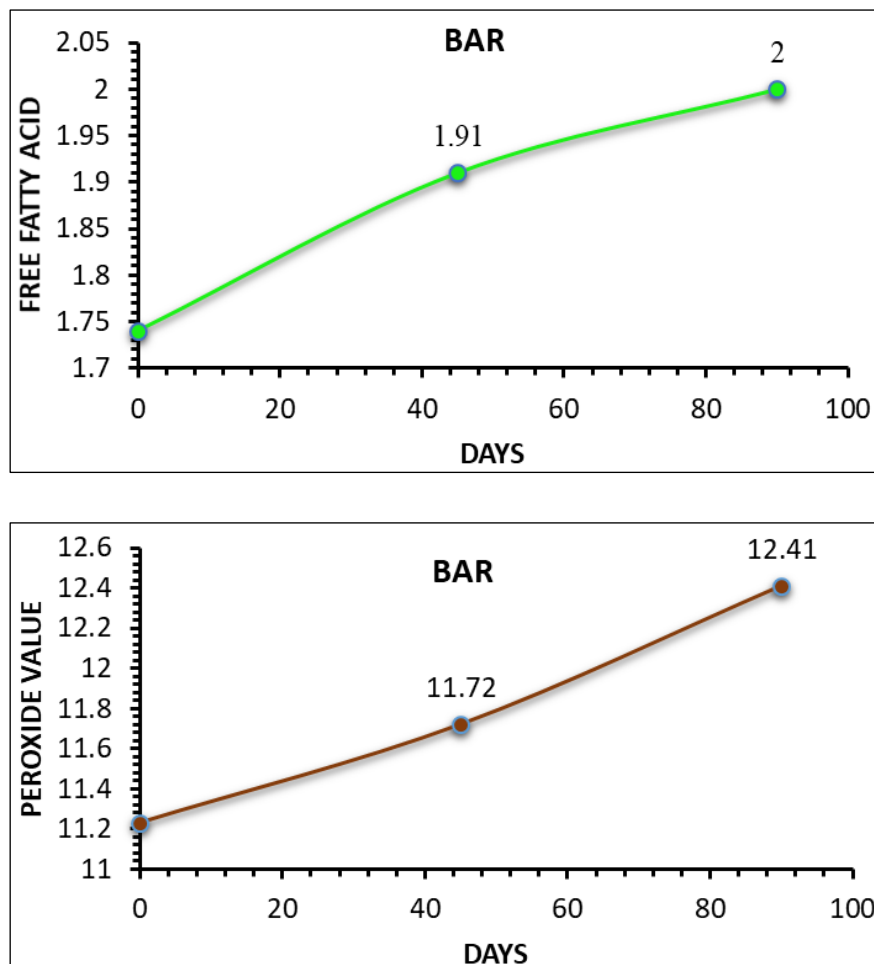

Fig 1: Graphs showing FFA and PV of Bar under Shelf-Life study (0-90 days)

Conclusion

It was concluded, that in control the correlation between colour and taste was found to be non-significant. Similarly, in 2.5g flavour and appearance; flavour and taste in 5g and texture and taste are found to be non-significant. Individually, in control and 2.5g highest mean scores was observed to be 8.6 in appearance while 8.3 for colour and appearance in 5g and 8.4 in appearance for 7.5g as per panel of judges.

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