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Analysis of Physicochemical parameters of *Annona squamosa Linn* Leaves and Development of Value-added Products for Diabetic patients

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Abstract

Prevalence of diabetes mellitus is increasing rapidly. Diabetes effect 6.25% of global estimation. Immediate and rapid intervention need to be executed to deal with increasing trend of diabetes. Use of traditional methods and plant for the treatment of various diseases is very common worldwide as it is not only a cost-effective method but also has fewer side effects. One such plant is *Annona squamosa* or Custard apple. Custard apple leaves have been claimed to possess anti-diabetic property. The present study aimed to 1) Assess the Physicochemical parameters; 2) Assess the anti-diabetic property; 3)Develop the Value-added products and 4) Calculate the Cost of Value-added products. It was found that dried leaf powder had 3.11% (protein), 3.22% (fats) and 5.65% (carbohydrate). The anti-diabetic property was highest for sample with maximum concentration of leaf extract. The maximum acceptability for product varied for every value-added product. The cost of the products was reported to be highest for Oats Cheela followed by Falafel and was least for custard apple flavoured yoghurt.

Keywords

Custard Apple, Leaf Powder, Antidiabetic Properties, Flavoured Yoghurt, Oats *Cheela,* Falafel

Introduction

Prevalence and incidence of type 2 diabetes continues to grow at a rapid pace. According to Khan et al. (2019), 462 million individuals are affected by type 2 diabetes around the globe that is 6.26% of the world population (Khan et al., 2019). The incidence of diabetes has increased from 11.3 million (1990) to 22.9 million (2017), which shows an increase of 102.9%. There is an estimation that the prevalence of diabetes will increase up to 570.9 million and incidence of diabetes will increase up to 26.6 million respectively by the year 2025, if effective interventions are not planned (Lin et al., 2020). Besides having various drugs available having anti-diabetic effect, much focus is directed towards use of natural products to deal with diabetes, as they are less toxic and have very few side effects (Jung et al., 2006; Modak et al., 2007). According to the World Health Organisation (WHO),

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herbal medicines are being used by around 80% of the world population as a primary health care treatment (Mazid et al., 2012). However, only 6% of the total estimated 250,000-400,000 plant species, have been studied for biological activity (Balabdrin et al., 1985). Plants hold various chemicals and contents that have potential therapeutic and nutraceutical values.

Custard apple

Custard apple or *Annona squamosa* is one such plant having immense therapeutic properties. One such property is the anti-diabetic effect (Singh et al., 2019). The goal of this study was to develop value-added products from custard apple leaves and fruits to utilise their anti-diabetic properties for reducing blood glucose level of diabetic patients.

A. squamosa Leaves (ASL) extract consists of many phytochemicals such as proteins, carbohydrates, saponins, alkaloids, phenolics, glycoside and flavonoids (Lakshmi et al., 2019). Another study represented the presence of alkaloids, oils, flavonoids, tannins and phenols in both methanolic and aqueous extract of seeds and leaves of custard apple (Nguyen et al., 2012).

The aqueous as well as methanolic extract of ASL were found to be rich in protein and amino acids (Sampathkumar, 2012). The ASLs consist of various minerals such as magnesium (65.65 mg/100 g), phosphorus (43.1 mg/100 g), zinc (0.455 mg/100 g), copper (0.312 mg/100 g), and selenium (in trace amounts). They are also rich in many vitamins like vitamin B1 (0.065 mg/100 g), B2 (0.155 mg/100 g), B3 (1.105 mg/100 g), C (34.4 mg/100 g), and A and E (in traces) (Varadharaj et al., 2014).

Leaf oil majorly consist of Sesquiterpene hydrocarbons (76.0%), β-caryophyllene (23.0%), germacrene D (21.3%), oxygenated sesquiterpenes (7.1%), β-elemene(7.8%), and bicycloger-macrene (8.5%), monoterpene hydrocarbons (2.5%) (Shenoy et al., 2009). Malik et al. (2018) isolated a compound namely, quercetin-3-rhamnosyl glucoside from the ethanolic extracts of ASLs which is a flavonoid compound. The ethanolic extract of the leaves contains Alpha linolenic acid, Eicosopentaenoic acid (EPA) and Docosohexaenoic acid (DHA) which are the Omega-3-Fatty acids (Varadharaj et al., 2014).

Besides having numerous chemical constituents, Custard Apple Leaves also possess various health benefits. Kalidindi et al. (2015)reported high free radical scavenging property of chloroform extract, moderate activity of the methanol extract and very less

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activity of aqueous extract of ASL, suggesting presence of antioxidant activity in the ASL extracts. Same study also reported high antifungal activity of chloroform extract of ASL against 3 fungus species (*A. alternata, C. albicans,* and *F. solani*). Al-nemari et al. (2020) reported strong cytotoxic and anti-proliferative activity of ASL extract against breast cancer cell at the concentration of 100 µg/mL.In another study, hepatoprotective activity of alcoholic extract of A. squamosa leaves was evaluated on swiss-albino mice. The results showed presence of hepatoprotective activity of ASL (Raj et al., 2009).Tiangda et al. (2000) investigated the anti-hyperlipidemic activity of aqueous extract of custard apple on the reduction of blood glucose in rats. The polyherbal formula led to a significant reduction of blood glucose, increase in insulin levels and significant decrease in tissue lipids.

To assess the anti-diabetic property of ASL extract, Panda and kar (2008) isolated a compound namely, quercetin 3-O-glucoside (Q3G) from the ASL extract and demonstrated its anti-diabetic effect in the alloxan-induced diabetic rats. It was found that Q3G improved the insulin level and reduce the blood sugar levels. In another study, Mujeeb et al. (2009) reported that oral administration of 350 mg/kg body weight of ethanolic leaves extract reduced blood glucose level in normal rats, alloxan-induced diabetic rabbits and streptozotocin (STZ)-induced diabetic rats significantly. Further, a study conducted on streptozotocin-induced diabetic rats to evaluate the anti-diabetic effect of ASL, claimed that 100 mg/kg/d dosage of ASL extract to the STZ induced diabetic rats for 30 days led to restoration of blood glucose and an increase in insulin levels. (Basha & Subramaniam, 2011).Kumar et al. (2019) in another study, evaluated the anti-diabetic effect of methanol leaves extract of 30 genotypes of custard apple plant by determining their α-amylase inhibition activity. The result claimed that the genotype Aml-11, Aml-12, Aml-4 showed the highest PPA inhibition and hence highest anti-diabetic activity.

Objectives

- To assess proximate composition of the custard apple leaves powder
- To assess antidiabetic effect of custard apple leaves powder
- To develop value-added products (Flavoured Yoghurt, Oats *Cheela* and Falafel) by incorporating custard apple leaves powder
- To do the sensory evaluation of the developed value-added products
- To do the cost analysis of the developed products

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Research Methodology

The study was carried out in following steps -

1. Procurement of Raw Materials

Custard apple leaves were procured from the kitchen garden of ICG College, SFS Mansarovar, Jaipur. Custard apple fruits were procured from Thadi market sabji mandi, Mansarovar, Jaipur.Saras Yoghurt was procured from local market of SFS, Mansarovar, Jaipur.Quaker oats, chickpea, and other raw materials were procured from the local market of SFS, Mansarovar, Jaipur.

2. Processing of Raw materials

Custard apple leaves

The leaves were washed and shade dried at room temperature for 1 to 2 weeks. After shade drying, the leaves were put in a Hot air oven for oven drying to remove any extra moisture present in leaves for 1-2 days. The leaves were then kept in desiccator for few hours. After desiccation, the dried leaves were grinded in grinder to form a powder texture. This powder was then sieved to remove any thicker particles. The obtained thin powder was stored in an air-tight jar.

Custard apple fruit

The fruit pulp was in a mixer to form a milky uniform paste. This paste was then kept in refrigerator.

Yoghurt

Hung curd was made from the Yoghurt by hanging it in a muslin cloth for 5-6 hours.

Oats

Oats were grinded into a grinder to obtain a thick powder.

Chickpeas

The chickpeas were kept soaked overnight. The soaked chickpeas were then grinded after being removed from water to form a thick, grainy paste.

3. Proximate Analysis of Custard Apple leaves

Ash content

10g of dried leaf sample was taken in previously weighed crucible. The sample was then kept in muffle furnace at 600℃ for 7-8 hours till a light grey ash was obtained. The sample

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was then cooled down in desiccator and weighed repeatedly until 2 concordant readings were obtained (AOAC, 2017).

Fat content

Fat content was determined using Soxhlet Method. The reagent used was petroleum ether (AOAC, 2017).

Crude Fibre content

The method used for determination was Acid-Alkali Wash Method. The reagents used were 1.25% sulphuric acid solution and 1.25% sodium hydroxide solution (AOAC, 2017).

Protein content

Micro Kjedahl Method was used for determination of Crude Protein Content. The protein content was calculated by using 6.25 as conversion factor from Kjedahl Nitrogen(AOAC, 2017).

Carbohydrate content

The carbohydrate content was calculated using Composite Method (AOAC, 2017).

4. Assessment of Anti-diabetic Property of Custard Apple Leaf powder

Anti-diabetic property of the custard apple dried leaf powder was analysed using % alpha inhibition assay (Kumar et al., 2019).

Methanol extract of the dried leaf powder was prepared and centrifuged at 5000 rpm for 15 min. Standard sample was prepared by addition of starch in distilled water in the concentration of 0.1, 0.2, 0.3 and 0.4 mg/ml to 4 test tubes (namely standard 1, standard 2, standard 3, standard 4 respectively) to make the final concentration 1ml in standard test tubes. These test tubes were incubated at 25℃ for 3 min.1 ml DNS reagent was added to the test tubes. These were then incubated in boiling water bath for 5minutes. Then cooled to room temperature. 10ml distilled water was added. Absorbance was measured at 540nm at 25℃.

Enzyme solution was composed of sodium phosphate buffer (0.02M), porcine pancreatic α-amylase and sample extract in the concentration of 0.1, 0.2, 0.3 and 0.4 mg/ml in 4 test tubes named sample 1, sample 2, sample 3, sample 4 respectively. 5mg starch solution (1%) was added to each test tube and kept for incubation for 15 min. at 37℃. Reaction was terminated by adding 1ml DNS reagent to each test tube and then incubating them in boiling water bath for 5min.The test tubes were cooled down, diluted and absorbance was recorded at 540 nm at 25℃.

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The anti-diabetic activity was measured by calculating % Porcine Pancreatic Inhibition Activity (% PPA Inhibition) (Karthic et al., 2008).

$$\%\ PPA\ Inhibition = \frac{Absorbance\ of\ standard - Absorbance\ of\ sample}{absorbance\ of\ standard} \times 100$$

5. Standardisation of Recipe and Development of Variations

- **Preparation of control**

Custard Apple Flavoured Yoghurt

50 gm pulp paste was mixed with 50gm of hung curd in a mixer to form a thick pasty texture. No sugar was added since the recipe is being prepared for diabetics.

Oats *Cheela*

To 60 gm of Oats powder, 15 gm of onion, 15 gm of tomato, 5 gm of green chilli and 5 gm of coriander leaves were added. Spices like red chilli powder, salt, cumin powder were added as per the taste. The ingredients were mixed property to form a semi dilute paste. A small amount of oil was used to grease the non-stick pan. The semi dilute paste was then spread over the no-stick pan and heated on moderate flame till the *Cheela* turned slight golden-brown in colour.

Falafel

50 gm of grinded Chickpea were added with 30 gm of coriander leaves. To this, 10 gm of ginger, 10gm of garlic were added and mixed together in a mixer. Spices like red chilli powder, salt, cumin powder were added as per the taste and mixed properly. Small balls were prepared from the lot. A small amount of oil was used to grease the non-stick pan. The balls prepared were heated on the non-stick pan on low flame till they turn golden-brown in colour.

- **Preparation of variations**

Custard apple flavoured yoghurt

Custard apple dried leaf powder was added to the standard recipe in 4 different concentration- 3%, 6%, 9%, 12%. These variations were coded as Y112, Y113, Y114, Y115. The control was coded as Y111.

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Oats *Cheela*

Four variations of the Oats *Cheela* were prepared by adding dried custard apple leaf powder in the proportion of 3%, 6%, 9%, 12% and were coded as O112, O113, O114, O115 respectively. The control was coded O111.

Falafel

The dried custard apple leaf powder was added in 4 concentrations: 3%, 6%, 9%, 12% to the standard recipe of falafel and 4 variations coded F112, F113, F114, F115 were formed. F111 code was given to the control.

6. Sensory Evaluation of Value-added Products

Sensory evaluation of each recipe with their 4 variations and a control was done to check the acceptability of the products. The overall acceptability of the Value-added products was evaluated on the bases of 5 parameters namely, Appearance, Colour, Texture, Taste and After taste.

Evaluation was done by a panel of 7 semi-trained members which were selected through sensory threshold test (sugar for yoghurt and salt for *Cheela* and falafel) at IIS (Deemed to be University), Jaipur. The coded samples were served to the panel members separately in different serving cutleries. The test panel evaluated each variation with respect to the control and filled the ratings in the 9-point hedonic rating scale form provided to them. The panel scored each of the sensory parameters namely, Appearance, Colour, Texture, Taste and After taste and the Overall acceptability of all Variations and Control of each product. 9-point hedonic scale was used to assess 5 sensory characteristics of the recipe.

The data was recorded and analysed and the mean scores of all Variations and Control of all products were calculated for each parameter.

7. Cost-analysis of Value-added products

The cost calculation of all 3 Value-added products with 3% concentration of dried leaf powder (Y112, O112, F112) was done as per the latest market price. The food and total cost were calculated. Total cost included 50% Food Cost (FC), 10% Labour Cost (LC), 25% Overhead Cost (OC) and 15% Profit (P).

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Results and Discussion

1. Proximate Analysis of Custard Apple leaves

Parameters	Amount
Moisture	60.14 %
Ash	7.58 %
Fat	3.22 %
Protein	3.11 %
Crude fibre	20.30 %
Carbohydrate	5.65 %

Table 1: Proximate composition of Custard apple leaves

2. Assessment of Anti-diabetic Property of Custard Apple Leaf powder

Porcine Pancreatic α-amylase (PPA) is an enzyme present in our digestive system that catalyses the reaction of starch degradation that is it promotes conversion of maltose to glucose. This process leads to increase in blood glucose levels which is an important aspect for diabetes. The inhibition of the α-amylase will also lead to reduction in starch degradation which correlates to the blood glucose level. The inhibition of α-amylase, hence can be used as a treatment for diabetes. The more the inhibition of the α-amylase, the more is the anti-diabetic activity of the sample (Kumar et al., 2019).

Absorbance of the both standard and sample at all 4 concentrations that is 0.1, 0.2, 0.3 and 0.4 mg/ml was measured at 540 nm at 25℃. The anti-diabetic activity was found to be maximum for the test sample with concentration 0.4 mg/ml that is 43.39%. The anti-diabetic activity for sample 3 (0.3 mg/ml) was found to be 39.58% followed by sample 2 (0.2 mg/ml) with 34.37% and the lowest activity was showed by sample 1 (0.1 mg/ml) with 28.57% activity.

The results shows that the anti-diabetic activity increased with the increase in concentration of the sample extract. Kumar et. al. (2012) studied anti-diabetic effect of methanolic extract of 30 genotypes of custard apple leaves by α-amylase inhibition assay and found that more than 9 genotypes had 30% α-amylase inhibition activity. This proves the presence of anti-diabetic activity of custard apple leaves.

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3. Standardisation of Recipe and Development of Variations

- **Preparation of control**

Custard apple flavoured yoghurt

100 gm yield 1 Bowl of Custard apple flavoured yoghurt.

Figure 1: Standard of Custard Apple Flavoured Yoghurt

Oats *Cheela*

100 gmyield 4 medium sized Oats *Cheelas.*

Figure 2: Standard of Oats *Cheela*

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Falafel

100 gm quantity resulted in preparation of 5-6 Falafels.

Figure 3: Standard of Falafel

- **Preparation of the variations**

Different concentration of dried custard apple leaf powder was added to each recipe to form 4 variations of all the 3 Value-added products.

Figure 4: Control and Variations of Custard apple flavoured yoghurt

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Figure 5: Control and Variations of Oats *Cheela*

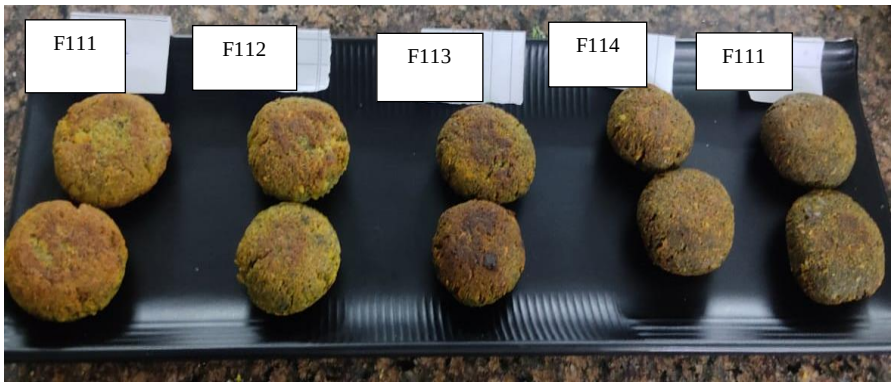

Figure 6: Control and Variations of Falafel

4. Sensory Evaluation of Value-added Products

Custard apple flavoured yoghurt

The Variants Y112 & Y113 were found to be acceptable whereas Y114 & Y115 were found to be less acceptable.

Attributes	Y112	Y113	Y114	Y115
Appearance	7.2 ± 1.16	8.5 ± 2.61	8.4 ± 2.91	8.1 ± 1.84
Colour	7.4 ± 2.60	6.8 ± 2.41	5.4 ± 2.06	5.3 ± 2.25
Texture	8.2 ± 1.16	8.0 ± 1.15	6.5 ± 2.69	5.1 ± 2.79
Taste	7.8 ± 2.05	7.4 ± 2.29	6.3 ± 2.40	3.2 ± 1.36
After taste	7.7 ± 1.88	7.1 ± 2.73	5.2 ± 2.36	3.5 ± 2.29
Overall acceptability	7.7 ± 2.06	7.5 ± 2.35	5.6 ± 2.91	3.3 ± 1.98

Table 2: Mean scores of sensory attributes (Appearance, Colour, Texture, Taste, After taste and Overall acceptability) of the Custard apple flavoured Yoghurt for all variations

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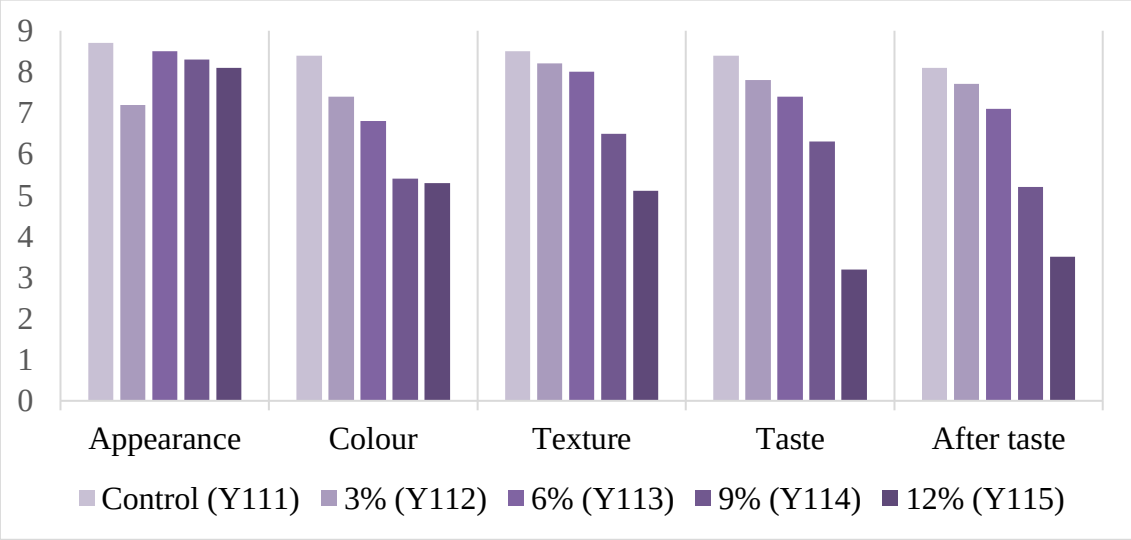

Figure 7: Graphical representation of the mean scores of sensory attributes (Appearance, Colour, Texture,Taste and After taste) of the Custard apple flavoured yoghurt for all variations and control

Oats *Cheela*

The Variants O112, 0113 & O114 were found to be acceptable whereas O115 was found to be unacceptable.

Attributes	O112	O113	O114	O115
Appearance	8.5 ± 0.54	8.1 ± 0.75	7.6 ± 1.03	7.0 ± 1.09
Colour	8.6 ± 0.51	8.1 ± 0.75	7.5 ± 0.83	7.0 ± 1.03
Texture	8.3 ± 0.51	8.1 ± 0.40	7.6 ± 0.81	7.0 ± 0.89
Taste	8.5 ± 0.54	8.1 ± 0.75	7.5 ± 0.81	7.0 ± 0.89
After taste	8.5 ± 0.54	8.1 ± 0.51	7.6 ± 0.81	7.1 ± 0.98
Overall acceptability	8.1 ± 0.40	7.6 ± 0.81	7.1 ± 0.75	6.6 ± 0.81

Table 3: Mean scores of sensory attributes (Appearance, Colour, Texture, Taste, After taste and Overall acceptability) of the Oats *Cheela* for all variations

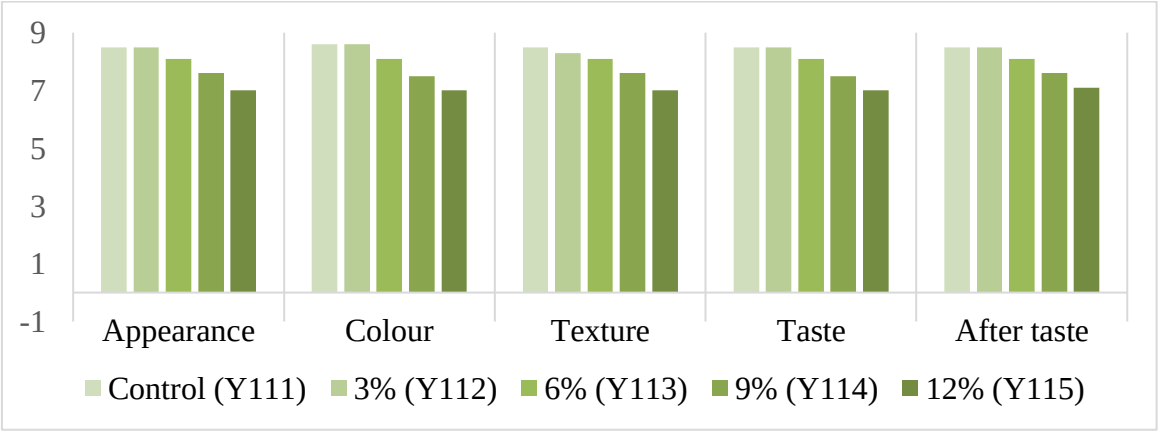

Figure 8: Graphical representation of the mean scores of sensory attributes (Appearance, Colour, Texture, Taste, and After taste) of the Oats *Cheela* for all variations and control

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Falafel

The acceptability was highest for F112 followed by F113 and F114. The acceptability was found to be lowest for F115.

Attributes	F112	F113	F114	F115
Appearance	8.6 ± 0.54	7.5 ± 1.26	7.5 ± 1.37	7.0 ± 1.54
Colour	8.5 ± 0.54	7.6 ± 1.21	7.1 ± 1.04	6.8 ± 1.47
Texture	8.1 ± 0.75	7.8 ± 0.40	6.8 ± 0.75	5.8 ± 0.75
Taste	8.1 ± 0.40	7.1 ± 0.75	6.3 ± 1.03	5.5 ± 1.37
After taste	8.1 ± 0.75	8.3 ± 0.63	7.6 ± 0.98	7.1 ± 0.81
Overall acceptability	8.6 ± 0.54	8.5 ± 1.21	7.5 ± 0.89	6.5 ± 1.03

Table 4: Mean scores of sensory attributes (Appearance, Colour, Texture, Taste, After taste and Overall acceptability) of Falafel for all variations

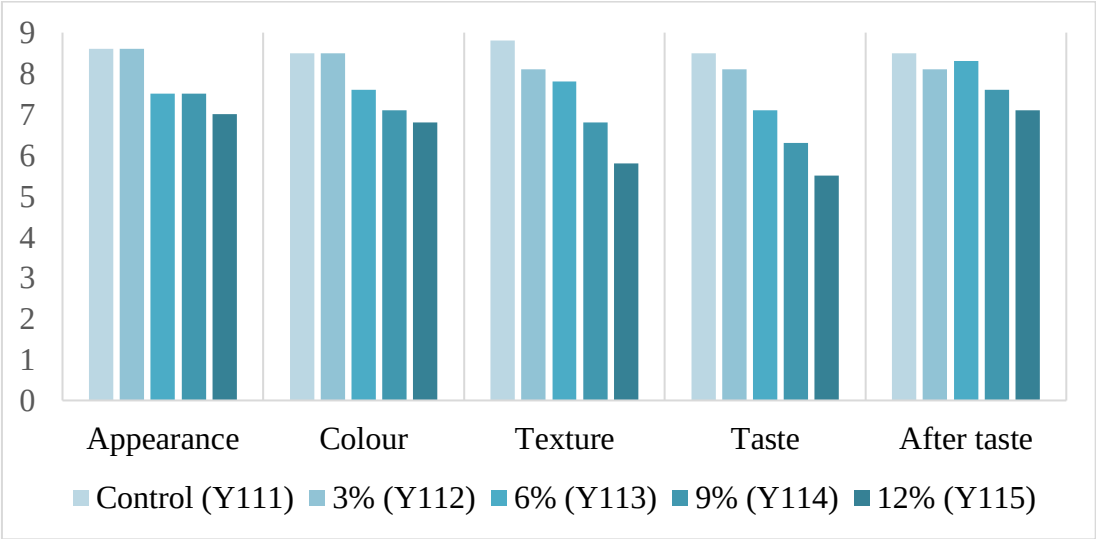

Figure 9: Graphical representation of the mean scores of sensory attributes (Appearance, Colour, Texture, Taste and After taste) of the Falafel for all variations and control

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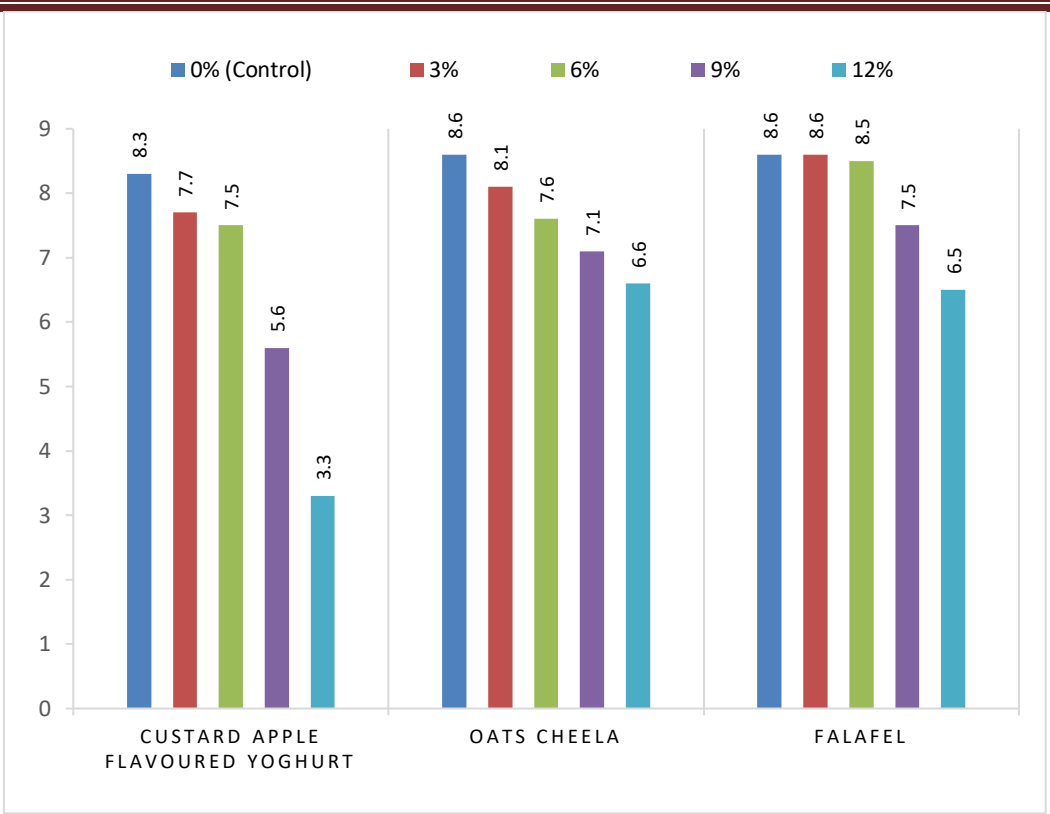

Figure 10: Graphical representation of the mean scores of Overall acceptability custard apple flavoured yoghurt, oats *Cheela* and falafel of all variations and control

5. Cost-analysis of Value-added products

The cost analysis shows that Oats *Cheela* has the maximum market price followed by the Falafel and then the Yoghurt. Although, Seasonal variations may be present.

Cost	Food Cost	Total Cost (FC (50%) + LC (10%) + OC (25%) + P (15%)
Custard apple flavoured yoghurt (Rs. /100g)	7.75	15.5
Oats *cheela* (Rs. /100g)	14.16	28.32
Falafel (Rs. /100g)	10.00	20.00

Table 5: Food Cost and Total Cost of Custard apple flavoured Yoghurt, Oats *Cheela*, Falafel

Conclusion

Prevalence of Diabetes mellitus continues to grow globally. Individuals from all religions, sex, race, country, economic status are vulnerable to this disease. The increased prevalence, incidence and mortality of diabetes is attributed to the metabolism risk and the lifestyle habits. Although, there are various medicines available for diabetes, all of them

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can have side-effects on health of the consumer after prolonged intake. The treatment of diabetes with intake of plant having anti-diabetic effect is also very common practice globally. One such plant possessing anti-diabetic properties is *Annona squamosa* or Custard apple plant.

The Results of proximate analysis showed presence of good amount of moisture, fibre, protein, ash content in the leaves. The carbohydrate was found to be less in content. The anti-diabetic assessment showed maximum α-amylase inhibition activity for the sample with highest concentration of sample. The activity decreased with decrease in concentration of leaf extract. The Custard apple flavoured Yoghurt incorporated with 6% of dried leaf powder was accepted. While for both Falafel and Oats *cheela*, sample having 9% concentration was found to be acceptable. The cost analysis showed maximum cost for the Oats *Cheela* followed by Falafel and lowest for Custard apple flavoured Yoghurt.

The Custard apple leaves, thus, can be used for its anti-diabetic effect and for development of Value-added Products. Various products should be developed using custard apple leaves for its health benefits. Also, further studied can focus on development of Value-added products by the use of Custard apple fruits which is also claimed to have anti-diabetic properties.

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