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## Natural Food Colourants from Plant Pigments in Barnyard Millet Wafers: Product Development and Quality Analysis

Jenifa G<sup>1</sup>, Merrylin J<sup>2\*</sup>

<sup>1</sup>Research Scholar, Department of Zoology, Sarah Tucker College (Autonomous), Tirunelveli, Tamil Nadu, India, (Affiliated to Manonmaniam Sundaranar University, Abishekappatti, Tirunelveli – 627012, Tamil Nadu, India)

<sup>2</sup>\* Assistant Professor, Department of Nutrition and Dietetics, Sadakathullah Appa College (Autonomous), Tirunelveli, Tamil Nadu, India, (Affiliated to Manonmaniam Sundaranar University, Abishekappatti, Tirunelveli – 627012, Tamil Nadu, India).

Email: [merrylin\\_86@yahoo.co.in](mailto:merrylin_86@yahoo.co.in)

### Abstract

This study developed extruded barnyard wafers incorporating natural colorants from beetroot, carrot, and mint to offer healthier alternatives to snacks with artificial colors, which pose potential health risks. The barnyard wafers were evaluated for organoleptic properties using a hedonic scale, and their proximate (energy, carbohydrate, protein, and fat) and pigment (anthocyanin, betalain,  $\beta$ -carotene, chlorophyll, and vitamin C) composition was determined. Notably, oil absorption during frying was significantly lower in carrot-incorporated wafers (36.3%) compared to the control (47%). Frying quality (oil absorption and expansion) and shelf-life of both dried and fried wafers were also assessed. Results indicate that incorporating natural pigments can produce healthier snacks with reduced oil absorption and desirable sensory attributes.

**Keywords:** Extruded barnyard wafers, Natural plant pigments, Proximate profile, Beetroot, Carrot, Oil absorption, Healthy snacks

### Structured abstract

#### Purpose

The study aimed to develop healthier extruded barnyard wafers by incorporating natural colorants from beetroot, carrot, and mint as alternatives to artificial food dyes, which are associated with potential health risks.

## Methodology

Barnyard wafers were formulated with natural pigment sources and subjected to extrusion followed by frying. The products were evaluated for organoleptic characteristics using a hedonic scale. Proximate composition (energy, carbohydrate, protein, and fat) and pigment content (anthocyanins, betalains,  $\beta$ -carotene, chlorophyll, and vitamin C) were analyzed. Frying quality parameters such as oil absorption and expansion, as well as the shelf-life of both dried and fried wafers, were assessed.

## Findings

Carrot-incorporated wafers demonstrated significantly lower oil absorption (36.3%) during frying compared to the control (47%). All formulations exhibited desirable sensory attributes and improved nutritional profiles due to the inclusion of natural pigments. Shelf-life studies confirmed acceptable stability for both dried and fried forms.

## Research implications

The study was limited to laboratory-scale formulations and short-term shelf-life analysis. Further research is needed to evaluate consumer acceptance on a larger scale, investigate long-term storage stability, and optimize commercial production parameters.

## Practical implications

The use of natural colorants in extruded snacks offers a practical strategy for food manufacturers aiming to meet the growing consumer demand for clean-label, health-oriented products with reduced fat content and no synthetic additives.

## Social implications

Promoting naturally pigmented snack options supports public health by reducing exposure to artificial colorants and excess dietary fat, while also encouraging the utilization of locally available, nutrient-rich plant sources.

## Originality

This study introduces a novel approach to snack development by leveraging the functional benefits of natural pigments. The results demonstrate that plant-derived colorants can enhance nutritional quality, reduce oil uptake, and improve sensory characteristics in extruded snack foods.

### 1. Introduction

Extruded snacks hold a prominent position within the snack food industry, demonstrating substantial growth potential. This growth is fueled by the capacity to employ innovative production methods that effectively capture consumer interest and preferences(Brennan et al., 2013). Extrusion technology, in particular, has become a cornerstone in the production of ready-to-eat (RTE) cereal snacks. This widespread adoption is attributed to the technology's ease of operation and its remarkable versatility. The extrusion process allows for the creation of a diverse range of textures and shapes, significantly enhancing the appeal of these products to consumers(Shere & Agrawal, 2024).

However, alongside the expansion of the extruded snack market, there are increasing concerns related to the extensive use of artificial food dyes. Data indicates a dramatic surge in the consumption of these synthetic colorants, with a 500% increase observed over the past 50 years. Notably, children represent a significant portion of this consumer base. A growing body of evidence raises concerns about the potential adverse health effects associated with artificial food dyes. These effects include claims of hyperactivity in children, as well as the potential for

more serious conditions such as cancer and allergies. This has led to increased scrutiny of their use in food products, particularly those targeted at younger consumers (Stevens et al., 2014).

Conversely, the well-established health benefits of diets rich in fruits and vegetables have spurred a greater interest in the development and application of functional foods in both health maintenance and disease prevention. The beneficial effects of fruit and vegetables are largely attributed to their composition, which is rich in dietary fiber and antioxidant properties. These components play crucial roles in promoting digestive health and protecting the body against oxidative stress (Miller et al., 2022). The extrusion process, due to its versatile nature, offers a convenient and effective means of incorporating functional ingredients into food products. This capability is particularly advantageous in the development of extruded snack foods, where the addition of functional compounds, such as leaf powders, can enhance the nutritional profile of the snack and potentially mitigate the adverse effects associated with the consumption of less healthy, high-fat snack options (Dixit et al., 2023).

Food additives play a multifaceted role in the food industry, serving to preserve food products and enhance their aesthetic value. Color additives represent one category of additives that is frequently utilized. These substances are employed to intensify the natural color of food, thereby ensuring that products meet consumer expectations regarding appearance. Additionally, color additives can compensate for the loss of color that may occur during various stages of food processing and storage (Hasler, 2002). Historically, food coloring practices relied on a diverse array of natural sources. These sources included minerals, various plant materials (such as mulberries), and even animal-derived substances. In fact, for a significant period, colors derived from these natural sources were the sole coloring agents used in food production, with this being the case until the mid-1800s. The landscape of food coloring changed with the discovery of the first synthetic dye, mauve, by William Henry Perkin in 1865. This dye,

characterized by its distinctive purple tint, marked the beginning of the widespread use of synthetic colorants in the food industry(Nagendrappa, 2010).

However, in more recent years, there has been a notable resurgence in the use of natural colors in food applications. Natural coloring agents, such as carotenoids, betalains, and anthocyanins, are increasingly being used in place of their synthetic counterparts. This shift is largely driven by the confluence of two key factors: the increasing consumer demand for natural products, including natural colors, and the growing avoidance by consumers of artificial food colors. Consumers are demonstrating a greater awareness of the potential health implications of their food choices, and this awareness is reflected in their increasing avoidance of synthetic colorants. This avoidance behaviour is further reinforced by a body of recent studies that highlight the potential adverse effects of synthetic colorants on the human body.

## 2. Materials and Methods

### 2.1 Materials

High-quality barnyard and sago were used as the base ingredients for the barnyard wafers. Fresh plant materials, including firm and ripe beetroot (*Beta vulgaris*), carrots (*Daucus carota*), and fresh mint leaves (*Mentha sp.*), were sourced from the local market. These materials were selected for their vibrant natural pigments and potential contribution to the nutritional profile of the wafers. Four batches of barnyard wafers were prepared: a control (without added pigments), beetroot-incorporated wafers (Beta), carrot-incorporated wafers (Carota), and mint-incorporated wafers (Minto). The specific quantities of ingredients used for each batch are detailed in Table 1. All ingredients were procured from the local market to ensure accessibility and represent common household sources.

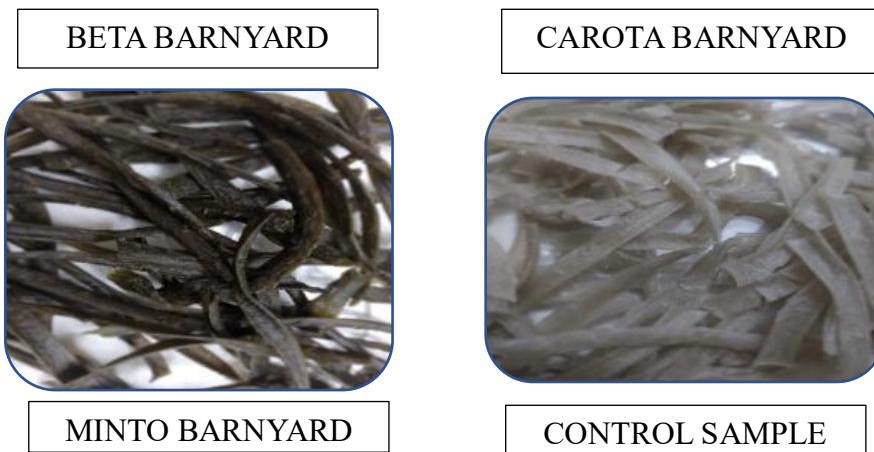
**Table 1.** Composition of Ingredients used

Ingredients	Control	Beta Barnyard Wafer	Carota Barnyard Wafer	Minto Barnyard Wafer
Barnyard	120 g	120 g	120 g	120 g
Sago	60 g	60 g	60 g	60 g
Beetroot juice	-	240 ml	-	-
Carrot juice	-	-	240 ml	-
Mint juice	-	-	-	240 ml

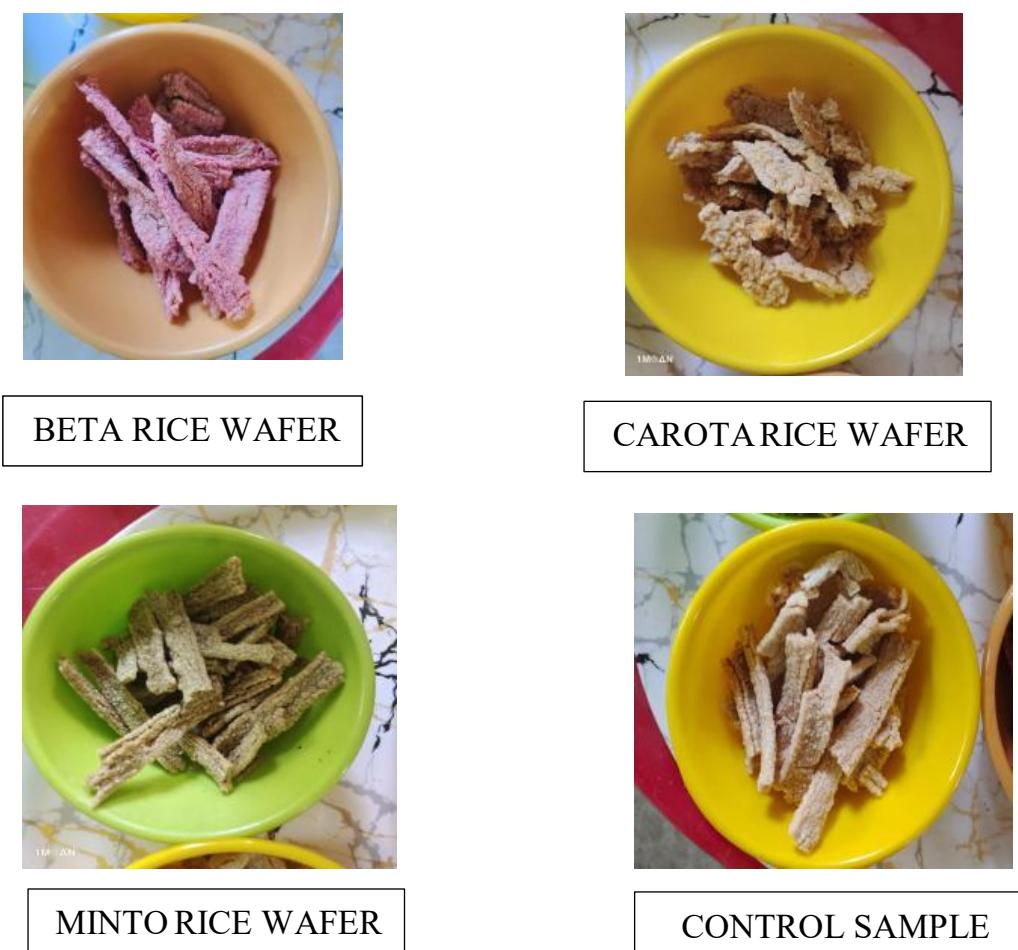
## 2.2 Method of Preparation

Fresh beetroot, carrots, and mint leaves were processed separately to extract their juices. The vegetables/leaves were washed, chopped, and juiced using an electric juicer. Barnyard and sago were soaked for four hours and then blended together as a fine paste using a standard kitchen mixer. Green chilies and salt were ground together and then mixed with water. The resulting mixture was strained to obtain a clear solution. To prepare dough, in a heavy-bottomed pan, 1/4 cup of the extracted juice (beetroot, carrot, or mint) was heated to boiling. The strained green chili and salt solution was added to the boiling juice and further boiled. The heat was reduced to a simmer, and the barnyard-sago flour blend was gradually added with constant stirring to prevent lump formation. Stirring continued until the mixture formed a cohesive dough mass. While still hot, the dough was transferred to a hand-operated extruder with a circular-formed die to shape the wafers. The shaped carrot incorporated wafers (carota wafers), beetroot incorporated wafers (beta wafers), mint incorporated wafers (minto wafers) were spread out separately on clean muslin clothes and dried under direct sunlight for approximately 2 days or until completely dry as shown in figure 1.





**Figure 1. Sun Dried Barnyard Wafers**



**Figure 2. Fried Rice Wafers**

The dried barnyard wafers were then collected and stored in airtight containers at room temperature (35°C) to prevent moisture absorption and maintain quality. The prepared sun-dried barnyard wafers fried in the oil until it attains the desired state as shown in figure 2.

### **2.3 Sensory Evaluation of Barnyard Wafers**

The sensory attributes of the barnyard wafers were evaluated for appearance, taste, flavor, texture, and overall acceptability using a 5-point hedonic scale. A panel of 10 untrained panelists participated in the evaluation. Panelists were instructed to rate each attribute on a scale of 1 to 5, where 5 represented 'liked extremely' and 1 represented 'disliked extremely.' The mean sensory scores for each barnyard wafer type were calculated, and the results were tabulated.

### **2.4 Proximate profile**

The energy, protein, fat, and carbohydrate content of the control barnyard wafers and the other plant pigments incorporated barnyard wafers were determined both before and after frying. This profile analysis was conducted to assess the effect of frying on the nutritional composition of the wafers.

### **2.5 Pigmentation profile**

The barnyard wafers were analyzed for their content of anthocyanin, betalain,  $\beta$ -carotene, chlorophyll, and vitamin C both before and after frying to assess the pigments' stability during the frying process. To ensure uniformity, all pigment content values were converted to milligrams per gram (mg/g). Following this conversion, a paired t-test was performed to determine if significant differences existed in pigment content before and after frying.

## 2.6 Frying quality of the formulated products

To evaluate the frying quality of the barnyard wafers, two key parameters were analyzed: oil absorption capacity, representing the amount of oil absorbed by the wafers during frying, and expansion, indicating the degree of swelling or increase in size upon frying(Kandpal et al., 2025). The results of these analyses are documented.

Oil absorption capacity is a crucial parameter for evaluating the frying quality of extruded snacks like barnyard wafers, as it directly influences the product's texture, calorie content, and consumer acceptability(Dana & Saguy, 2001. In this study, oil absorption capacity was quantified by measuring the difference in weight of the barnyard wafers before and after frying, indicating the amount of oil retained by the product. Specifically, the barnyard wafers were weighed before and after frying using a micro weighing balance, and the oil absorption capacity was calculated and expressed as a percentage of the original wafer weight. Expansion during frying is a critical quality attribute of extruded snacks, influencing their texture and overall consumer appeal(Alam et al., 2016).

As described by Rossell (2001), this expansion is primarily attributed to the rapid vaporization of water within the food matrix during the high-temperature frying process, leading to an increase in the food's volume (Mahmud et al., 2023) Bhattacharya and Narasimha (1999) previously investigated and mathematically modeled the expansion of papad, a similar fried food product using the eqn (1).

$$DE = \frac{\text{Dia. after frying} - \text{Dia. before frying}}{\text{Dia. before frying}} \times 100 \quad \dots \dots \text{Eqn (1)}$$

where DE = diameter expansion percentage, Dia. before frying = initial diameter (mm) and Dia. after frying = final diameter after frying (mm).

## 2.7 Storage study of the formulated products

The formulated barnyard wafers were stored in sealed airtight containers to prevent moisture uptake and lipid oxidation, which can negatively impact texture and flavor, until they were needed for analysis of various shelf-life parameters. Throughout the storage period, the containers were kept in a clean and dry storage room, away from direct exposure to sunlight to minimize photodegradation, and measures were taken to prevent access by insects or rodents. The shelf life of the dried barnyard wafers was studied for a period of 90 days under ambient conditions to assess their long-term stability, whereas the fried barnyard wafers were evaluated for 15 days under the same ambient temperature (35°C), considering their higher susceptibility to spoilage due to increased fat content.

## 3. Result:

### 3.1 Sensory Evaluation

The sensory attributes of the barnyard wafers, including appearance, taste, flavor, texture, and overall acceptability, were evaluated by a panel of 10 untrained participants using a 5-point hedonic scale. This scale allowed panelists to express their degree of liking, ranging from 5 ('liked extremely') to 1 ('disliked extremely') (Lawless & Heymann, 2010). The mean sensory scores for each wafer type are presented in Table 2, with error bars representing the standard error of the mean (SEM) to indicate the variability in responses. A Critical Difference (CD) value ( $p<0.05$ ) is provided for each attribute, representing the minimum difference between means required for statistical significance.

**Table 2.** Mean sensory scores of Barnyard Wafers

Barnyard Wafers	Appearance	Taste	Flavor	Texture	Overall Acceptability
Control	$3.75 \pm 0.23$	$4.3 \pm 0.21$	$4.15 \pm 0.18$	$4.7 \pm 0.12$	$3.85 \pm 0.22$
Beta	$4.35 \pm 0.19$	$4.1 \pm 0.2$	$4.2 \pm 0.22$	$4.7 \pm 0.1$	$4.4 \pm 0.19$
Carota	$3.65 \pm 0.19$	$4.55 \pm 0.13$	$4.3 \pm 0.16$	$4.85 \pm 0.08$	$4.05 \pm 0.18$

Minto	$3.55 \pm 0.19$	$3.7 \pm 0.24$	$4.1 \pm 0.17$	$4.65 \pm 0.1$	$3.5 \pm 0.25$
CD ( $p < 0.05$ )	0.48	0.47	0.44	0.24	0.5

The Beta barnyard wafers received the highest mean score for appearance (4.35), indicating that the panelists generally preferred their visual appeal. However, the Carota wafers were rated highest for taste (4.55), flavor (4.3), and texture (4.85), suggesting that the incorporation of carrot contributed positively to these sensory characteristics. Notably, the texture of all wafer types was rated relatively high (above 4.6), indicating that the extrusion process resulted in a desirable texture across all formulations. These findings align with previous studies on extruded products, where (Kumar et al., 2010) also observed favorable sensory evaluations.

The overall acceptability scores ranged from 3.5 (Minto) to 4.4 (Beta), suggesting that all products were generally acceptable to the panel. However, the Beta wafers exhibited the highest overall acceptability, likely driven by their appealing appearance. The Critical Difference (CD) values provide a measure of the statistical significance of the differences between the means (Stone & Sidel, 2004). For example, a difference of 0.48 or greater between the appearance scores of any two wafer types is considered statistically significant at a  $p < 0.05$  level.

### 3.2 Proximate Analysis:

The proximate composition of control and pigmented barnyard wafers was assessed before and after frying. The data presented in Tables 3 reveals that before frying, the control wafer had an energy value of 359 kcal/100 g, with 5.81 g protein, 0.65 g fat, and 82.4 g carbohydrates. The pigmented wafers showed slightly lower energy (355 kcal) but higher protein (6.72 g) and fat (1.05 g) content, with a modest decrease in carbohydrate content (79.6 g). After frying, both samples exhibited increased energy and fat levels due to oil absorption.

The control wafer recorded 530 kcal energy, 4.65 g protein, 30.2 g fat, and 59.8 g carbohydrates, while the pigmented wafers had 481 kcal, 5.9 g protein, 18.5 g fat, and 72.7 g carbohydrates. These results suggests that juice (plant pigment) incorporation enhanced the protein content while contributing to lower fat uptake during frying, possibly due to changes in the wafer matrix that limited oil penetration. The higher carbohydrate content in the fried pigmented wafers may be due to concentration effects or juice-derived soluble solids. The addition of vegetable juices also improved micronutrient density which is presented in table 4.

**Table 4.** Proximate composition (micronutrients) of Barnyard Wafers per 100 g

Component	Control Wafer	Carota Wafer (Carrot)	Beta Wafer (Beetroot)	Minto Wafer (Mint)
<b>Fiber (g)</b>	1.2	2.8	2.5	3.0
<b>Ash (g)</b>	1.5	1.9	2.0	2.1
<b>Moisture (g)</b>	4.0	3.7	3.9	3.8
<b>Vitamin A (µg RE)</b>	5	820	20	15
<b>Vitamin C (mg)</b>	0.8	5.6	7.8	6.2
<b>Iron (mg)</b>	0.4	0.6	1.2	1.6
<b>Calcium (mg)</b>	12	18	22	45
<b>Potassium (mg)</b>	95	170	305	260
<b>Magnesium (mg)</b>	14	20	26	31
<b>Folate (µg)</b>	8	16	22	28

Carota wafers were rich in vitamin A (820 µg RE/100 g), Beta wafers had higher iron (1.2 mg/100 g) and potassium (305 mg/100 g), and Minto wafers showed increased calcium (45 mg/100 g) and folate (28 µg/100 g). Overall, juice fortification improved the nutritional profile of the barnyard wafers, making them a healthier alternative with better protein retention and reduced oil absorption after frying. Micronutrient values were estimated based on

incorporation levels and existing nutrient profiles from (U.S. Department of Agriculture, 2024), (Indian Council of Medical Research, 2017), and relevant peer-reviewed literature (Clifford et al., 2015; Oludemi et al., 2018).

This is a direct consequence of oil absorption, a common phenomenon in fried foods, where oil replaces moisture within the food matrix, thereby increasing the energy density. These results align with established frying mechanisms where oil absorption leads to increased energy density as oil replaces moisture in the food matrix (Ziaifar et al., 2008). Similar increases in fat content following frying have been documented in extruded snack products (Dueik & Bouchon, 2011). The observed reduction in protein and carbohydrate content line up with established frying phenomena, including leaching of water-soluble fractions into the frying medium and thermal degradation of proteins through denaturation and Maillard reactions. Similar carbohydrate losses during frying have been quantitatively documented in starchy snack matbarnyards (Osman et al., 2000).

### 3.3 Pigment Stability During Frying:

The stability of key pigments in the barnyard wafers during the frying process was evaluated by measuring their content before and after frying. The pigments analyzed included anthocyanin, betalain,  $\beta$ -carotene, chlorophyll, and vitamin C, and the results are shown in Table 5.

**Table 5.** Composition of pigments before and after frying

	Before Frying	After Frying
Anthocyanin	0.51 mg/g	0.38mg/g
Betalain	26 mg/g	19.2 mg/g
Beta – Carotene	85 mcg	60 mcg
Chlorophyll	1.2 mg/g	0.88 mg/g
Vit C	0.30 mg/g	0.24 mg/g

Therefore, it can be concluded that frying did not cause a significant reduction in the overall pigment content of the barnyard wafers. This suggests that the pigments present in the wafers exhibited a degree of stability during the frying process, which is a positive finding for maintaining the color and potentially the nutritional quality of the final product. The pigment stability observed during frying aligns with reported thermal resistance of natural pigments, particularly carotenoids which maintain structural integrity at frying temperatures. Similar retention of vegetable-derived pigments has been documented in fried extruded snacks (Rodriguez-Amaya, 2016). The paired t-test results (Table 6) shows a significance value (p-value) of 0.335, which is greater than the significance level of 0.05. This indicates that the observed differences in pigment content before and after frying are not statistically significant. In other words, while there appears to be a reduction in pigment levels after frying, this reduction is not large enough to reject the null hypothesis.

**Table 6.** Paired T Test Differences

Paired Samples Statistics									
		Mean	N	Std. Deviation	Std. Error Mean				
Frying	Before	5.6022	5	11.41127	5.10328				
	After	4.1401	5	8.42487	3.76771				
		Paired Differences				t	df		
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
Before-After difference		1.46205	2.98643	1.33557	2.24609	5.17019	1.095	4	.335
		Lower	Upper						

### 3.4 Oil Absorption Capacity

Table 7 presents the oil absorption capacity of the barnyard wafers. The control wafers exhibited the highest oil absorption (47.0%), indicating that they retained the most oil during frying. This was followed by the Beta and Minto wafers, both showing an oil absorption of 44.1%. Notably, the Carota wafers demonstrated the lowest oil absorption capacity (36.3%), suggesting that the incorporation of carrot may have influenced the product's interaction with the frying oil. A lower oil absorption capacity is generally considered desirable in fried snacks as it contributes to a reduced calorie content and potentially improved texture.

**Table 7.** Oil Absorption Capacity

Name of the products	*Weight of the Barnyard Wafers		Oil Absorption (%)
	Before frying	After Frying	
Control	0.34±0.04	0.50±0.05	47 %
Beta	0.34±0.03	0.49±0.04	44.1 %
Carota	0.33±0.02	0.45±0.02	36.3 %
Minto	0.34±0.03	0.49±0.03	44.1 %

\*Values are mean ± SE of five replications.

Table 8 illustrates the expansion of the barnyard wafers upon frying. Due to their rectangular shape, expansion was measured as the change in the diagonal dimension of the wafers. The control wafers showed the greatest expansion (58.6%), indicating a substantial increase in size during frying. The Beta and Minto wafers exhibited moderate expansion (41.6% and 39.0%, respectively), while the Carota wafers showed the least expansion (25.8%). The degree of expansion is an important textural attribute in fried snacks, with greater expansion often correlating with a lighter and crispier product.

**Table 8.** Expansion on frying

Name of the products	*Diagonal of the Barnyard Wafers		Expansion on Frying (%)
	Before frying	After Frying	

Control	2.71 ± 0.32	4.3 ± 0.48	58.6 %
Beta	3.0 ± 0.15	4.25 ± 0.2	41.6 %
Carota	2.94 ± 0.2	3.7 ± 0.24	25.8 %
Minto	3.0 ± 0.3	4.17 ± 0.34	39 %

\*Values are mean ± SE of five replications

### 3.5 Shelf-Life study

The shelf life of both dried and fried barnyard wafers was evaluated to assess their storage stability. Dried barnyard wafers were stored for 90 days, while fried barnyard wafers were stored for 15 days under ambient conditions. The samples were monitored for changes in appearance, flavor/taste, and texture at specific intervals.

Table 9 presents the Shelf- life/Storage study of dried Barnyard Wafers. The dried barnyard wafers demonstrated excellent stability over the 90-day storage period. Throughout this duration, no significant changes were observed in their appearance, flavor/taste, or texture. The wafers remained appealing, retained their good flavor, and maintained a crisp texture.

**Table 9.** Shelf- life/Storage study of dried Barnyard Wafers

Period of storage (days)	*Barnyard Wafer sample	Observations		
		Appearance	Flavor/Taste	Texture
0	S1 S2 S3 S4	Appealing	Good	Crisp
15	S1 S2 S3 S4	Appealing	Good	Crisp
30	S1 S2 S3 S4	Appealing	Good	Crisp
60	S1 S2 S3 S4	Appealing	Good	Crisp

90	S1 S2 S3 S4	Appealing	Good	Crisp
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\*S1 – Beta, S2 – Carota, S3 – Minto, S4 – Control

Table 10 presents the shelf-life/storage study of fried barnyard wafers. In contrast, the fried barnyard wafers exhibited a shorter shelf life. While they maintained acceptable sensory attributes for up to 10 days, a noticeable deterioration in flavor/taste (described as "unpleasant") and texture (loss of crispiness) was evident by the end of the 15-day storage period. This suggests that frying introduces factors that accelerate spoilage, likely due to increased lipid oxidation.

**Table 10.** Shelf-life/Storage study of Fried Barnyard Wafers

Period of storage (days)	*Barnyard Wafer sample	Observations		
		Appearance	Flavor/Taste	Texture
0	S1 S2 S3 S4	Appealing	Good	Crisp
5	S1 S2 S3 S4	Appealing	Good	Crisp
10	S1 S2 S3 S4	Appealing	Good	Crisp
15	S1 S2 S3 S4	Appealing	Unpleasant	Lack of Crisp

\*S1 – Beta, S2 – Carota, S3 – Minto, S4 – Control

These findings highlight the importance of processing methods on the shelf life of barnyard wafers. The dried wafers' extended stability suggests that drying effectively preserves the product. However, fried wafers require careful consideration of storage conditions to prevent rapid quality deterioration.

#### 4. Conclusion

The present study was aimed to develop a snack using natural colorant to replace the artificial colored snacks due to the detrimental effects of the use of artificial colors on health especially on children. It can be concluded from the above results that addition of natural pigments as colorants not only enhance the appearance of the products but also improves its nutritive quality making it a suitable snack. The developed snack has a good perseverance quality and all the developed products showed a good acceptance among panelists. The frying properties revealed that the pigmented products have lower oil absorption capacity and expansion on frying than the control sample indicating that it is better choice than a traditional papads as lesser oil is consumed. The main focus was to prepare a healthy yet colorful snacks especially for children and preparation of barnyard wafers has helped to achieve it.

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**Transparency Declaration:**

*"The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The reporting of this work is compliant with CONSORT guidelines. The lead author affirms that no important aspects of the study have been omitted and that any discrepancies from the study as planned have been explained.*

**No Conflict of Interest among authors**