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Alfiya PV
ICAR – Indian Institute of Spices Research, Kozhikode, Kerala, India

Jayashree E
ICAR – Indian Institute of Spices Research, Kozhikode, Kerala, India

Sneha C
ICAR – Indian Institute of Spices Research, Kozhikode, Kerala, India

Corresponding Author:
Alfiya PV
ICAR – Indian Institute of Spices Research, Kozhikode, Kerala, India

Development and shelf-life evaluation of spice flavoured sorghum (*Sorghum bicolor*) cookies: A study on valorisation of millets for food security

Alfiya PV, Jayashree E and Sneha C

Abstract
Food and Agriculture Organization and United Nations has recognised 2023 as the International Year of Millets or IYM2023 for awareness about health and nutritional benefits of millets. This study evaluated the effect of storage period and packaging materials on the quality attributes of spice-flavoured sorghum millet cookies. Quality characteristics such as moisture content, texture, overall acceptability, antioxidant activity, free fatty acid, and peroxide value of gluten-free cookies made from sorghum millet flour were investigated during the storage period under ambient conditions. The result of the study indicated that the moisture content, free fatty acid, and peroxide value of developed cookies increased while hardness decreased with an increase in storage time for cookies packed in both BOPP (biaxially oriented polypropylene) and metallised polyester package. During storage, moisture content increased more in biaxially oriented polypropylene as compared to metallised polyester package. The free fatty acid and peroxide value of cookies increased in both packaging materials during storage whereas, the highest increase was observed in 120 days of storage. The metallised polyester package was found to be the best packaging material than biaxially oriented polypropylene with respect to sensory, textural and shelf stability characteristics of sorghum cookies.

Keywords: Sorghum, cookie, BOPP, metallized polyester, peroxide value

1. Introduction

Cereal-based foods have been consumed as a staple food worldwide for energy and nutrients in various forms such as bakery products, noodles and pasta, snack foods, breakfast cereals and others [1]. The diverse availability of bakery products with lower glycaemic index increased the food choice for individuals with a gluten-free diet. It is estimated that the market of gluten-free products has grown significantly over the past five years. Among these, bread and cookies are considered the most globally consumed cereal-based gluten-free food [2]. Cookies are very popular bakery products among all age groups, especially children, and thus can serve as a vehicle for providing key nutrients [3]. They are characterized by high sugar and fat contents with low moisture and proteins [4].

Millets are gluten-free, therefore being an ideal choice for persons with celiac disease, who are frequently irritated by the gluten content of wheat and other common cereal grains. Phytic acid is one of the antinutrient present in millets. Phytic acid can bind to minerals such as iron, zinc, and calcium, producing complexes that are difficult for the body to absorb. Millets are also rich in dietary fibre, vitamins, and minerals. It is also beneficial to those suffering from atherosclerosis and diabetic heart disease [5].

In context of declaration of the year 2023 as the International Year of Millets by FAO, valorising the millet crop sorghum into a commercial product was aimed in this study. Sorghum (Sorghum *bicolor*) is the most drought-tolerant cereal grain crop that requires little input during growth and yields better with good husbandry (ICRISAT/FAO, 1996). Sorghum is emerged as a highly viable alternative for gluten-free foods, mainly because it presents a variety of bioactive compounds, a lower production cost, and a neutral flavour, which is an excellent advantage for the food industry [6]. Sorghum is high in phenolics and has antioxidant activity that outperforms wheat, barley, millet, and rye [7]. The antioxidant activity of phenolic compounds may offer potential health benefits such as protection against oxidative and radical damage of tissues responsible for health complications such as cancers, cardiovascular disease, and ageing among others [8].

Spices are dried seeds, fruits, roots barks, or flowers of plants or herbs used in small quantities as seasoning [9]. They have many applications as flavouring agents, medicinal, preservative, natural antioxidant, antibacterial, antifungal, and anti-viral activities. They not only have their own therapeutic value but also enhance the absorption and utilization of other therapeutic substances [10].

A combination of millets and spices led to the development of functional foods, which provide benefits beyond basic nutrition. A major development in the field of functional foods is relevant not only in meeting food security but also to provide entrepreneurship opportunities to the global youth. As globalization and urbanization are leading the world, the availability of ready-to-eat millet foods becomes more important. Considering the importance of millets and spices in their contribution towards nutritional and functional food benefits, the present work attempted to develop sorghum-based cookies flavoured with cardamom and black pepper, to evaluate the physical and biochemical properties of developed cookies and to carry out storage studies of the developed cookies under two types of packaging materials.

2. Materials and Methods

2.1 Preparation of Sorghum Millet Cookies

Dry ingredients used for the development of sorghum millet cookies such as sorghum millet flour, baking powder, custard powder, guar gum, and salt were weighed as per the formulation and were thoroughly mixed and sieved using a hand sieve. Margarine and powdered sugar were beaten until a cream-like consistency is attained. Vanilla essence was added to this. The cream thus formed was mixed with the sieved ingredients followed by the addition of milk to get the dough of the required consistency. The dough was kept covered and allowed to rest for a period of 15 min at room temperature. The dough was then flattened manually to make a sheet of desired thickness (approximately 0.4 cm) and cut into the desired shape using a mould. It was then transferred to previously greased baking trays. The trays were then placed in a preheated (130 °C for 5 min) commercial baking oven for baking at 130 °C (top tray) and 120 °C (bottom tray) for 20 minutes. After baking the trays were taken out of the oven, cooled and packed in two types of packaging materials namely – biaxially oriented polypropylene (BOPP) and metallised polyester packages.

2.2 Development of Spice Flavoured Sorghum Millet Cookies

Spices were added to enhance the flavour of the cookies. To select the most suitable spice to be added for the preparation of spice-based sorghum millet cookies, cardamom and black pepper were experimented. Dried cardamom and black pepper were powdered in a mixer grinder (Preethi Popular, 750 W, 230 V AC, 50 Hz) and the powders was used for the development of sorghum millet cookies. Cardamom and black pepper powder at a level of 3% was added to sorghum flour based on preliminary experiments. Three replicates of each treatment were taken for the evaluation of its physical, biochemical and organoleptic qualities.

2.3 Quality Analysis of Sorghum Millet Cookies

Quality analysis involved assessing various aspects of developed cookies to ensure their safety, nutritional value, and overall quality. The sorghum millet cookies prepared were tested for various physical (moisture content, diameter, thickness, spread ratio, hardness and colour), biochemical (peroxide value, free fatty acids,) and organoleptic quality parameters.

2.4 Physical properties of spice flavoured sorghum cookies

2.4.1 Moisture content

The moisture content of the prepared sorghum millet cookies was determined by using digital moisture meter (Model: MA37-1, Make: Sartorius, Germany). Four grams of grounded sample was placed in the moisture meter pan and the moisture meter was programmed to run at 130 °C [11].

2.4.2 Diameter

The diameter of cookies was measured along two axis and the average of the readings was taken as the final diameter of cookies based on the previous works and preliminary investigations [12].

2.4.3 Thickness

The total thickness was measured in centimetres with the help of a 15 cm stainless steel ruler. This process was repeated thrice to get an average value and results were reported in mm.

2.4.4 Spread ratio

Spread ratio of cookies can be defined as a ratio of average diameter to average height. The spread ratio of the cookies was determined according to the method followed by [13] by using the formula.

$$\text{Spread ratio} = \frac{\text{Diameter(cm)}}{\text{Thickness(cm)}}$$

2.4.5 Hardness

Hardness is a highly valued and universally accepted character which signifies the freshness and quality of cookies. The hardness of spice-flavoured sorghum millet cookies was evaluated in terms of the breaking force. Texture Analyser (Model: EZ-LXHS, Make: Shimadzu) was used to determine the breaking force. The test was performed on each cookie, mounted over two pointed supports and using a probe (3-point bend Stainless steel probe) in the texture analyser. The texture analyser was calibrated at the beginning of each test using a 500 N load cell and procedure as outlined in the data acquisition software (Trapezium). The texture analyser measures force, distance, and time, thus providing three-dimensional product analysis. The probe forces the cookies resulting in fracture of the cookies at a speed of 1 mm s^{-1}.

2.4.6 Colour

Colour is one of the important quality attributes for consumer acceptance of bakery products. The colour Measurement (ΔE) of cookies during the storage period was studied by using the Hunter colour lab colour meter with using the parameters L* (lightness), a* (red/green intensity), and b* (yellow/blue intensity). All analyses were performed in triplicates.

2.5 Biochemical analysis

2.5.1 Free fatty acid

The standard method of [14] was used to determine free fatty acids present in the cookies. Ten grams of ground sample from the stored cookies was taken in a conical flask. To the sample, 50 ml of neutral alcohol was added and allowed to boil in a water bath. The well-boiled sample was filtered and the filtrate was taken. Phenolphthalein was used as the indicator and titrated against 0.02N KOH till the appearance of pale pink colour. The FFA analysis was performed in triplicate.

$$\text{FFA (\%)} = \frac{282 \times 0.02\text{NKOH} \times \text{ml of alkali used} \times 100}{1000 \times \text{Weight of sample taken} \times 100}$$

2.5.2 Peroxide value

Peroxide value was determined by the iodometric titration method of [15]. Five-gram sample from the stored cookies was taken in a 250 ml iodine flask and followed by addition of 50 ml chloroform. The mixture was allowed to shake well in a mechanical shaker for one hour and then filtered through Whatman No.1 filter paper. Filtrate (20 ml) was transferred to an iodine flask containing 30 ml of acetic acid and 1 ml of saturated potassium iodide solution and kept in dark for 30 min. Distilled water (50 ml) was added and titrated against sodium thiosulphate solution (0.02N) using starch (1%) as an indicator. The endpoint is the colour change from blue to colourless.

$$\text{Peroxide value} = \frac{\text{Titre value} \times \text{Normality of sodium thiosulphate solution} \times 100}{\text{Weight of fat}}$$

2.6 Microbial Analysis

Microbial examinations of spice-flavoured sorghum cookies were performed to assess bacterial, fungal and yeast load under laboratory conditions. This technique is generally used to count viable microorganisms in the given sample by enumerating the total number of colony-forming units (CFUs) on the surface of the solid medium. The media and equipment were sterilized by steam at 15 psi for 20 minutes at 121˚C in an autoclave. For analysis, 10 gm of TPC agar was aseptically weighted and diluted to 1:10 (10 gm in 90 ml) with sterilized distilled water and thoroughly mixed. Total plate count (TPC) was estimated by decimal dilution technique followed by the pour plate method.

$$\text{CFU/mL} = \frac{\text{Total number of colonies obtained}}{\text{Volume of Specimen used(aliquot)}} \times \text{dilution factor}$$

2.7 Estimation of Secondary Metabolites

Spices are known for their distinct flavour, aroma, and medicinal properties which are attributed due to the presence of secondary metabolites namely essential oil and oleoresin. The essential oil was extracted by hydro-distillation of the powdered sample by modified Clevenger apparatus (ASTA, 1968). 25g of each sample was taken in a 1 L round bottom flask with water added up to the mark. The trap and the condenser tube were placed in position. The oil being lighter than water was collected over water. The trap was filled with water and the distillation was carried out for 3 hours. The oil is lighter and immiscible with water forming a separate layer on top of the water. The oil yield can be noted from the trap itself. The oil was stored in a refrigerator with the addition of a small amount of anhydrous sodium sulphate. The distillation period was 3 hours and oil recovery from different plant parts is calculated in percentage using the formula,

$$\text{Essential oil percentage} = \frac{\text{Oil extracted in volume(ml)}}{\text{Weight of the fresh sample(g)}} \times 10$$

Oleoresin content was estimated by the ASTA method (1968). 10 g of each sample was taken in a column packed with cotton at the bottom. 50 ml of solvent acetone was added and kept for 24 hours. The extract was drained into a pre-weighed beaker and again 30 ml solvent was added and held for 1 hour and drained. The solvent was then evaporated to dryness in a water bath. The difference in the weight of the beaker gave the amount of oleoresin present in the sample.

$$\text{Oleoresin content (\%)} = \frac{\text{Volume of oleoresin collected in a beaker(g)} \times 10}{\text{Weight of sample taken (g)}}$$

2.8 Organoleptic Quality

Sensory methods are a fast and simple method that provides immediate quality information. Sensory evaluation was conducted on sorghum millet cookie samples using quantitative analysis. Sensory evaluation was conducted by semi-trained panel. The attributes evaluated for the sorghum millet cookies were colour, flavour, taste, texture, and overall acceptability using 9-point Hedonic scale.

2.9 Packaging and storage studies of Cookies

Biaxially oriented polypropylene (outer 12µ polyester and inner 35µ BOPP) and metallised polyester packages (outer 12µ metallized polyester and inner 40 µ LDPE) were used in the present study based on literature reviews [16-17]. Optimized sorghum millet cookies were packed (2 cookies in one packet) in two packaging materials, heat sealed using a heat-sealing machine, and stored for 120 days at room temperature around (25-30 °C). Cookies were taken periodically over a sequence of 60 days for analysis as per the work plan.

2.10 Experimental Design

Three factor completely randomised design was used to evaluate storage studies of spice flavoured sorghum millet cookies in three replications. Detailed experimental design is as follows:

Detailed experimental design is as follows

Independent variables	:	Flour (T), packaging materials (P: BOPP and metallised polyester package), Storage period (S: 0,60,120)
Treatments	:	T1-Sorghum cookies, T2-Control (refined wheat flour cookies), P1: Biaxially oriented poly propylene package, P2: Metallised polyester package, S1: Storage period (0 days), S2: Storage period (60 days), S3: Storage period (120 days)
Dependent variables		Moisture content (%), hardness (N), colour (ΔE)Free fatty acid (%), peroxide value (%), antioxidant activity (%), total plate count (cfu/ml)

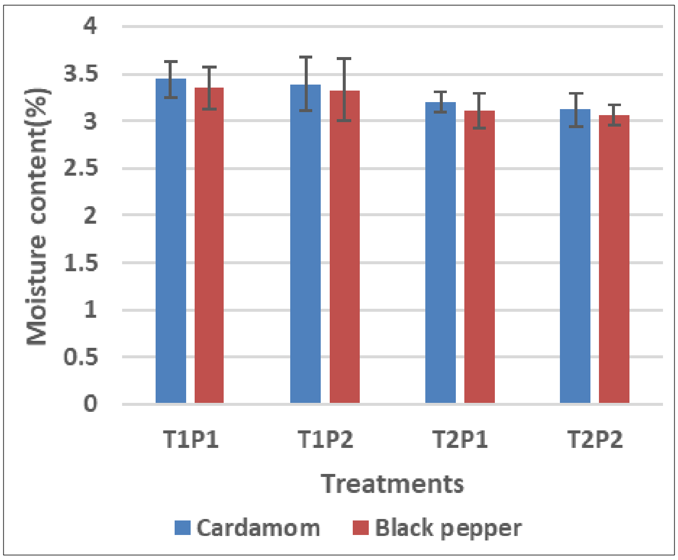
3. Results and Discussion

3.1 Physical properties of spice flavoured sorghum cookies

3.1.1 Moisture content

Moisture content is one of the important parameters which influences the shelf life or storage stability of cookies. The observations on the effect of two packaging materials (BOPP and metallised polyester) packages on the moisture content of cardamom and black pepper flavoured sorghum millet cookies during 120 days of storage at ambient conditions are presented in Fig. 1(a-c). It was observed that two packaging materials significantly affected the moisture content during storage. The highest average moisture value of 4.66 and 5.99% were observed for cardamom and black pepper flavoured control cookies packaged in BOPP packages while the lowest average moisture values of 4.54 and 4.46% were observed for cardamom and black pepper flavoured control cookies packed in metallised polyester package. The results indicated that the cookies packed in biaxially oriented polypropylene packages have the highest moisture content than those packed in metallised polyester packages. The gain in the moisture might be due to the hygroscopic nature of the dried product, the storage environment (temperature and relative humidity) as well as the nature of the packaging material [18].

The maximum limit of moisture content is 5% as recommended by Bangladesh Standards and Testing Institution (BSTI) for biscuits (BDS383 2001) [19]. also reported that the migration of water vapour from the storage environment into the packaging material increased the moisture content of jackfruit powder packaged in both aluminium laminated polyethylene and metallized co-extruded biaxially oriented polypropylene packaging materials.



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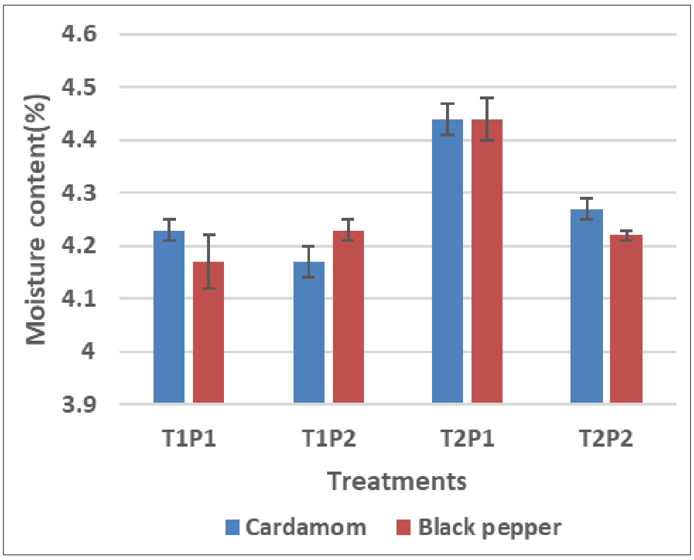

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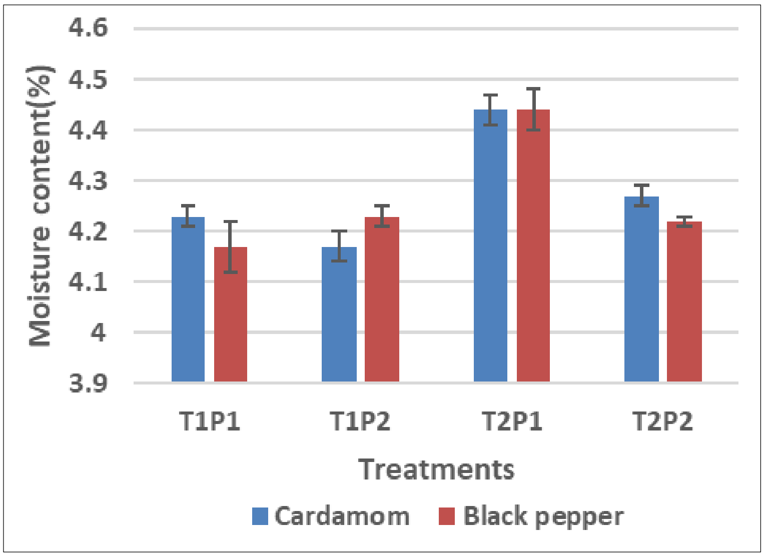

C

Fig 1(a-c): Moisture content of fresh cardamom and black pepper flavoured sorghum cookies during 0, 60 and 120 days of storage

Statistical analysis of cardamom flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the moisture content of cookies (Table 1). The effect of packaging material is significant on moisture content of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the moisture content of cookies. The interaction between flour and packaging material on moisture content of cookies was not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was not significant on moisture content of the cookies.

Statistical analysis of black pepper flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the moisture content of cookies (Table 2). The effect of packaging material is not significant on moisture content of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the moisture content of cookies. The interaction between flour and packaging material on moisture content of cookies was highly significant ($p \leq 0.01$), whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was highly significant ($p \leq 0.01$) on moisture content of the cookies.

Table 1: ANOVA for moisture content of cardamom flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	4.4944	4.4944	41.5646	1.147e-06	***
Packaging material(P)	1	0.4807	0.4807	4.4457	0.04562	*
Storage period (S)	2	30.6514	15.3257	141.7333	5.116e-14	***
Flour × Packaging material	1	0.3520	0.3520	3.2557	0.08374	.
Flour × Storage period	2	6.4462	3.2231	29.8073	3.127e-07	***
Packaging material × Storage period	2	0.2168	0.1084	1.0024	0.38186	
Flour × Packaging material × Storage period	2	0.6067	0.3034	2.8056	0.08037	.
***Significant at 0.1%, **Significant at 1%, * Significant at 0.5						

Table 2: ANOVA for moisture content of Black pepper flavoured cookie

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	3.379	3.3795	30.4274	1.132e-05	***
Packaging material (P)	1	0.001	0.0006	0.0056	0.9408246	
Storage period (S)	2	50.472	25.2360	227.2150	2.539e-16	***
Flour × Packaging material	1	1.686	1.6857	15.1771	0.0006854	***
Flour × Storage period	2	5.618	2.8088	25.2897	1.233e-06	***
Packaging material × Storage period	2	0.048	0.0241	0.2173	0.8062648	
Flour ×Packaging material × Storage period	2	4.636	2.3180	20.8704	5.604e-06	***
***Significant at 0.1%, ** Significant at 1%, * Significant at 0.5%						

3.1.2 Diameter

The diameter of both cardamom and black pepper flavoured sorghum cookies was 40 mm. The diameter of the cookies prepared from refined wheat flour with cardamom and pepper flavour was 41 mm. The results showed that the diameter of sorghum-based cookies was significantly lower than the control. Also, the diameter of the cookies was not influenced by the addition of spices (Fig. 2).

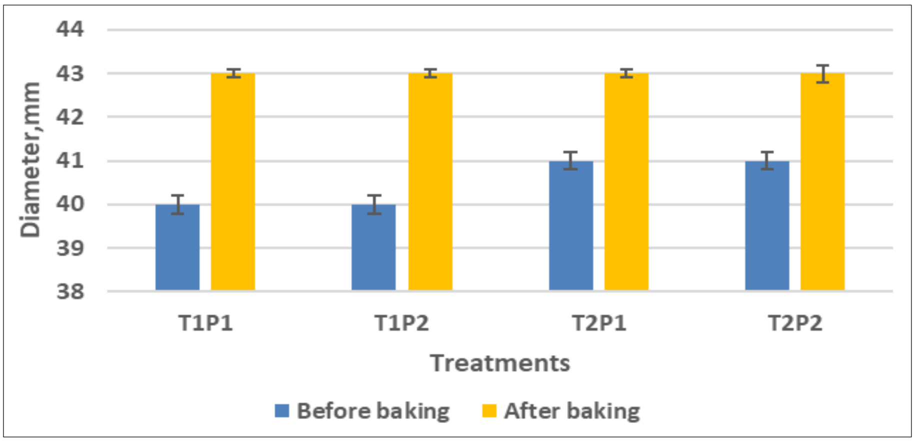

Fig 2a: Effect on physical parameters of cardamom flavoured cookies during baking

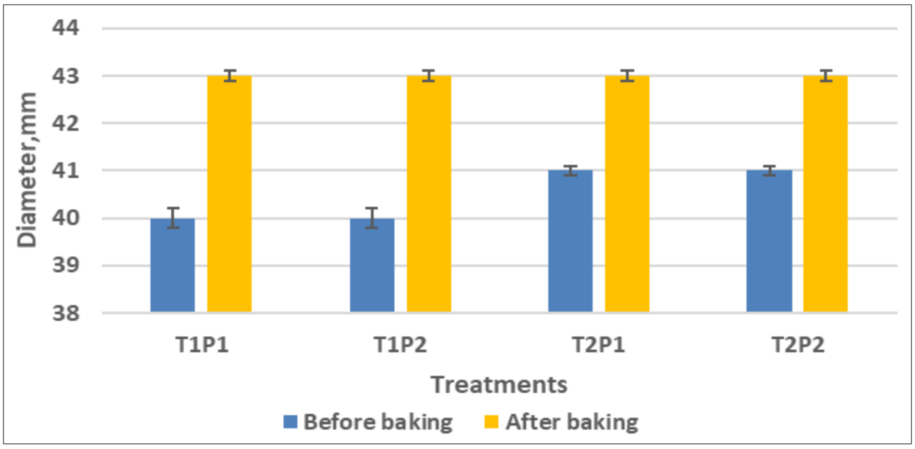

Fig 2b: Effect on physical parameters of black pepper flavoured cookies during baking

3.1.3 Thickness

The thickness of sorghum millet cookies was presented in Fig. 3. It is observed that the thickness of cardamom flavoured sorghum and control cookies increased from 7 to 9 mm and 8 to 10 mm respectively. Black pepper flavoured sorghum millet and control cookies increased from 7 to 9 mm respectively [20] reported that the incorporation of jering seed flour increased the thickness of the composite cookies from 6.19 mm (5% substitution level) to 6.69 mm (100% substitution level) without any significant change in the diameter of the cookies, which further validates the present study [21] observed a similar trend in cookies produced from cassava/soyabean/mango composite flours and attributed it to the hydrophilic nature of the flour used in producing the biscuit, which caused a reduction in spread, thus leading to an increase in thickness of the cookies. Cookies made with fermented sorghum flour showed similar results [22].

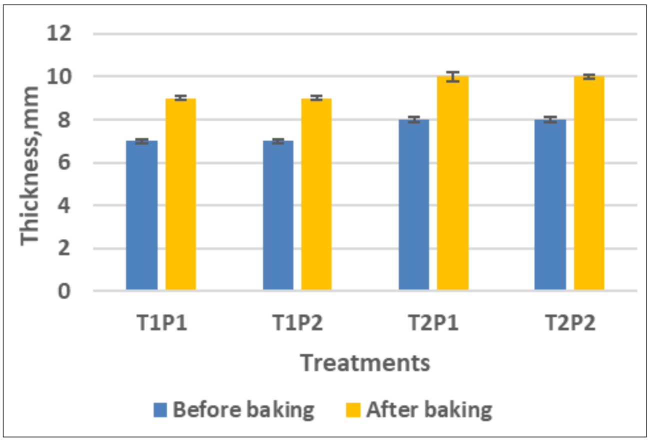

Fig 3a: Effect on physical parameters of cardamom flavoured cookies during baking

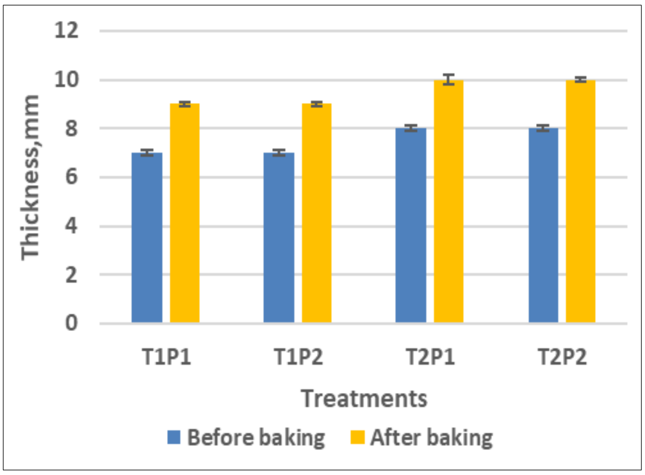

Fig 3b: Effect on physical parameters of black pepper flavoured cookies during baking

3.1.4 Spread ratio

The spread ratio of cookies is an important parameter for evaluating the rising ability of cookies. Cookies with a low spread ratio will have better-rising ability than those with a high spread ratio [23].

The spread ratio of black pepper and cardamom enriched sorghum millet cookies were presented in Fig.4. The results showed that the spread ratio of developed sorghum millet cookies was not significantly influenced by the spices used. The spread factor is an important aspect in determining the cookie quality. The spread factor is significantly dependent on the viscosity of the dough [24, 25] similarly reported an increase in spread factor with increasing sugar levels and attributed it to an increase in dough fluidity, allowing for the production of two-dimensional extensible films rather than three-dimensional elastic networks. The viscosity of the dough determines the rate of cookie spread. Sugar influences dough viscosity and dough expansion during baking.

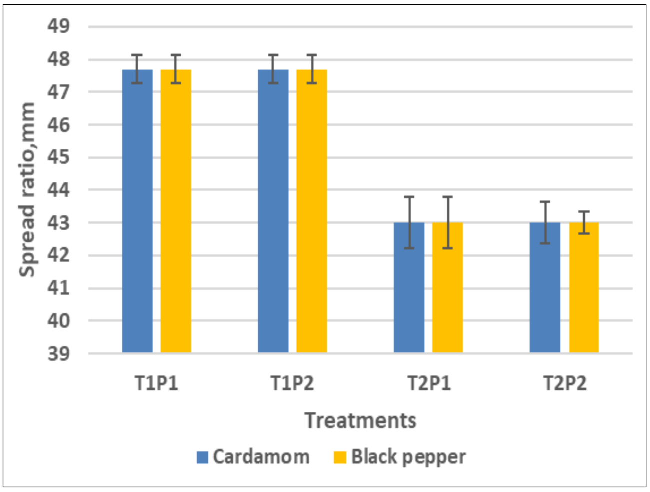

Fig 4: Spread ratio of cookies

3.1.5 Hardness of cookies

Hardness is the main criterion for assessing the overall quality of end products like cookies. The present observation on the effect of the hardness of sorghum millet cookies is shown in Fig.5. From the figure it is observed that the hardness of the developed sorghum millet cookies was found to decrease with an increase in storage period.

The hardness of cardamom flavoured sorghum millet cookies decreased from 16.63 to 4.70 N and 17.34 to 4.22 N in BOPP package, metallised polyester package respectively. Hardness value of cardamom flavoured control cookies decreased from 14.56 to 12.59 N and 16.03 to 8.36 N in BOPP and metallised polyester packages respectively.

The hardness of pepper flavoured sorghum millet cookies decreased from 20.009 to 4.06 N and 16.94 to 3.64 N in BOPP and metallised polyester package respectively. Whereas the hardness of black pepper flavoured control cookies decreased from 16.30 to 12.93 N and 18.17 to 14.06 N in BOPP and metallised polyester package respectively [26]. also reported that hardness of cookies decreased with an increase in storage time of cookies packed in both metalized polyethylene terephthalate-polyphenylene ether and low-density polyethylene-packed, whereas, the decrease was more in cookies packed in low-density polyethylene-packed laminate as compared to cookies packed in metalized polyethylene terephthalate-polyphenylene ether laminated.

Water content influences the texture and consumer acceptance of a food product, which is important in determining the ultimate quality of baked goods [27]. The interaction of the components, the water activity, and the moisture content of the cookies are influenced by both ambient conditions and packaging material [28]. The cookies become soft and squishy due to their hygroscopic nature and prolonged exposure to ambient and accelerated conditions. Furthermore, hardening is the primary cause of cookie quality decreases. Even after hours of baking, the cooked cookies transform from soft to firm and subsequently crumble [29].

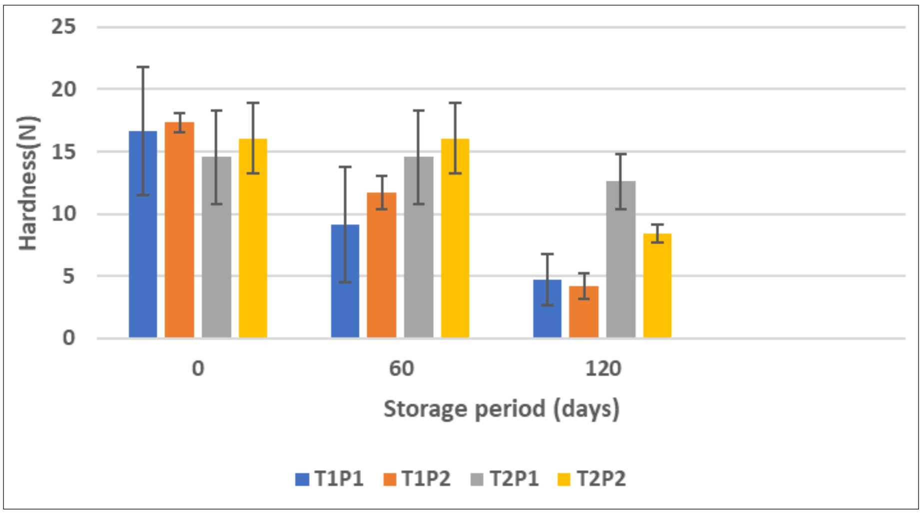

Fig 5a: Hardness of cardamom flavoured cookies during storage

Statistical analysis of cardamom flavoured cookies showed that the effect of flour is significant ($p \leq 0.05$) on the hardness of cookies. The effect of packaging material is not significant on hardness of cookies (Table 3). The effect of storage days was highly significant ($p \leq 0.01$) on the hardness of cookies. The interaction between flour and packaging material on hardness of cookies was not significant, whereas the interaction between flour and storage period was significant ($p \leq 0.05$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was not significant on hardness of the cookies.

Table 3: ANOVA for Hardness of cardamom flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	85.07	85.069	9.6333	0.004844	**
Packaging material (P)	1	0.54	0.543	0.0615	0.806248	
Storage period (S)	2	460.31	230.154	26.0628	9.642e-07	***
Flour × Packaging material	1	4.09	4.092	0.4634	0.502545	
Flour × Storage period	2	104.01	52.003	5.8889	0.008302	**
Packaging material × Storage period	2	31.74	15.869	1.7970	0.187388	
Flour × Packaging material × Storage period	2	7.76	3.880	0.4394	0.649512	
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

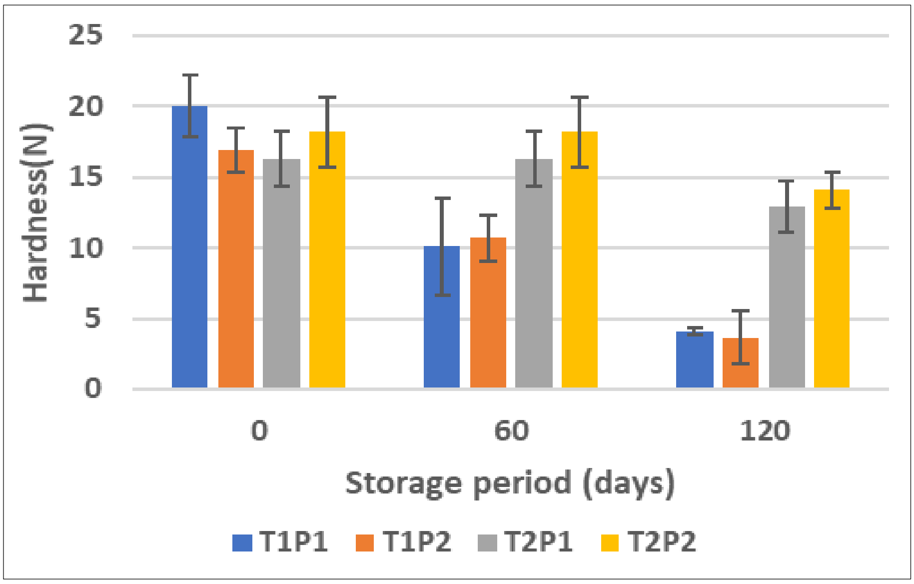

Fig 5b: Hardness of black pepper flavoured cookies during storage

Statistical analysis of black pepper flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the hardness of cookies. The effect of packaging material is not significant on the hardness of cookies (Table 4). The effect of storage period was highly significant ($p \leq 0.01$) on the hardness of cookies. The interaction between flour and packaging material on hardness of cookies was not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was not significant on hardness of the cookies.

Table 4: ANOVA for Hardness of black pepper flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	232.55	232.548	56.1968	9.779e-08	***
Packaging material (P)	1	0.99	0.994	0.2403	0.62844	
Storage period (S)	2	508.17	254.087	61.4019	3.645e-10	***
Flour × Packaging material	1	14.93	14.925	3.6068	0.06963	.
Flour × Storage period	2	191.66	95.828	23.1575	2.500e-06	***
Packaging material × Storage period	2	5.09	2.544	0.6149	0.54902	
Flour ×Packaging material × Storage period	2	6.28	3.139	0.7586	0.47920	
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

3.1.6 Colour

The lightness value of cardamom flavoured sorghum millet cookies ranged from 54.32 to 47.45 and 51.45 to 50.85 in BOPP package and metallised polyester packages. The lightness value for cardamom flavoured control cookies decreased from 77.90 to 75.27 and 77.92 to 74.78 in BOPP and metallised polyester package. Lightness value of black pepper flavoured sorghum millet cookies packed in BOPP and metallised polyester package were 49.81 to 43.21 and 48.18 to 44.95 respectively. Black pepper flavoured control samples exhibited lightness values of 70.40 - 69.72 and 74.07 to 69.06 in BOPP and metallised polyester packages. Biaxially oriented polypropylene and in metallised polyester packages. A positive a* value indicates a red colour and a positive b* value indicates a yellow colour. Variation in colour values of cookies is shown in Fig. 6-8.

The decrease in lightness of samples can be attributed to the higher temperatures of baking to which the cookies are exposed. The decrease in lightness value with increasing temperature, time, and sugar content could be due to the caramelization of sugar and Maillard browning reactions, which cause melanoid production during heating, resulting in product darkening [30]. The findings are consistent with those of [31], who found that when temperature and sugar content increased, it also affected the colour (L value) of cookies [32]. showed a similar declining trend for cake colour value.

Table 5: ANOVA for L* value of cardamom flavoured cookie

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	6180.1	6180.1	20816.642	< 2.2e-16	***
Packaging material(P)	1	7.4	7.4	24.982	4.176e-05	***
Storage period (S)	2	49.2	24.6	82.843	1.683e-11	***
Flour×Packaging material	1	19.0	19.0	64.129	3.100e-08	***
Flour × Storage period	2	6.3	3.2	10.674	0.0004828	***
Packaging material× Storage period	2	60.8	30.4	102.374	1.779e-12	***
Flour×Packaging material× Storage period	2	33.2	16.6	55.846	9.373e-10	***
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

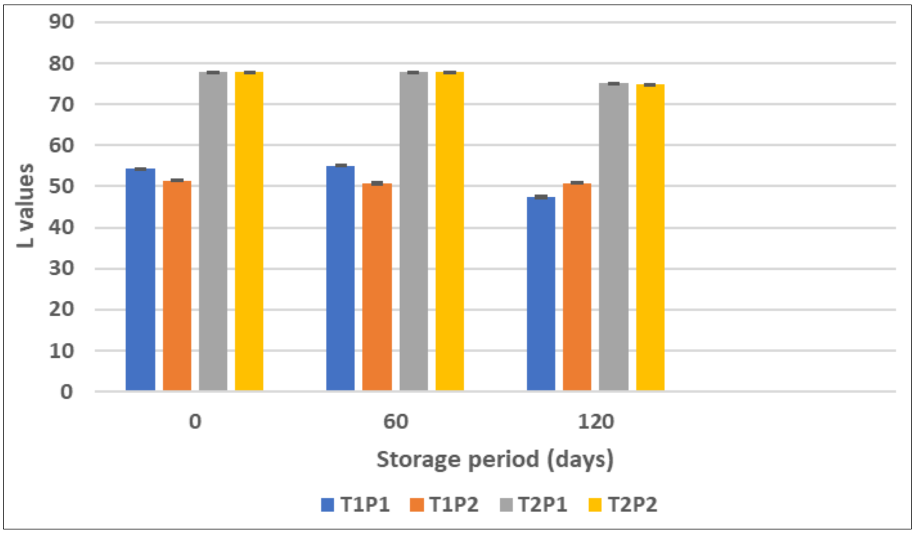

Fig 6a: Lightness (L*) of cardamom flavoured cookies during storage

Statistical analysis of cardamom flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the lightness of cookies (Table 5). The effect of packaging material is highly significant ($p \leq 0.01$) on lightness of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the lightness of cookies. The interaction between flour and packaging material on lightness of cookies was highly significant ($p \leq 0.01$), whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period was highly significant ($p \leq 0.01$). Also, the interaction between flour, packaging material and storage period was highly significant ($p \leq 0.01$) on lightness of the cookies.

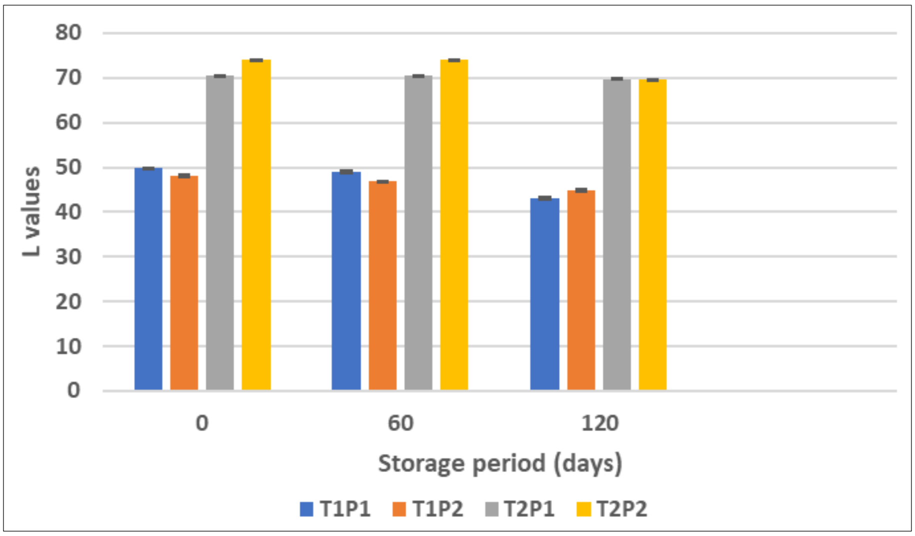

Fig 6b: Lightness (L*) of black pepper flavoured cookies during storage

Table 6: ANOVA for Lightness (L*) values of black pepper flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	5345.8	5345.8	1.6930e+05	< 2.2e-16	***
Packaging material(P)	1	7.4	7.4	2.3517e+02	6.701e-14	***
Storage period (S)	2	98.0	49.0	1.5526e+03	< 2.2e-16	***
Flour×Packaging material	1	20.8	20.8	6.5807e+02	< 2.2e-16	***
Flour × Storage period	2	8.5	4.3	1.3519e+02	8.620e-14	***
Packaging material × Storage period	2	0.1	0.0	9.4560e-01	0.4024	
Flour×Packaging material × Storage period	2	26.4	13.2	4.1836e+02	< 2.2e-16	***
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

Statistical analysis of black pepper flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the lightness of cookies (Table 6). The effect of packaging material is highly significant ($p \leq 0.01$) on lightness of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the lightness of cookies. The interaction between flour and packaging material on lightness of cookies was highly significant ($p \leq 0.01$), whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period was not significant. Also, the interaction between flour, packaging material and storage period was highly significant ($p \leq 0.01$) on lightness of the cookies.

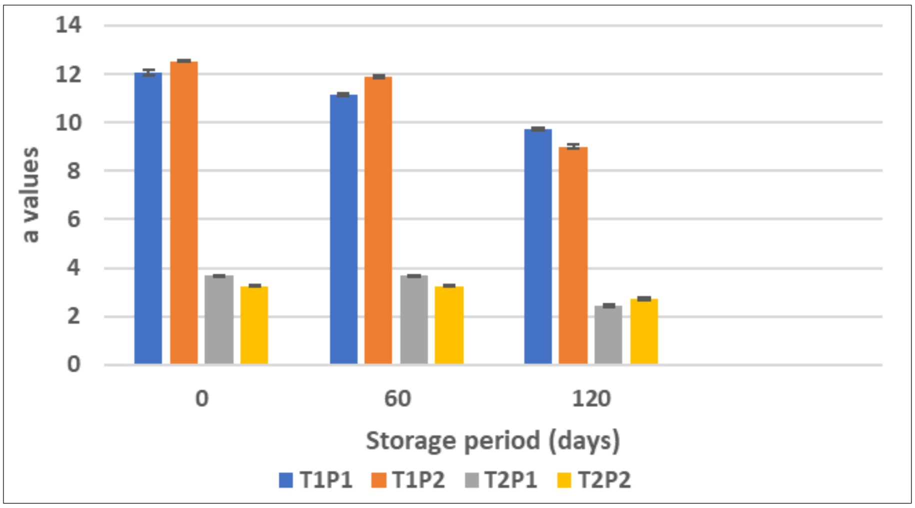

Fig 7a: Hunter color a* value of cardamom flavoured cookies during storage period

Statistical analysis of cardamom flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the redness of cookies (Table 7). The effect of packaging material is highly significant ($p \leq 0.01$) on the redness of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the redness of cookies. The interaction between flour and packaging material on redness of cookies was not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period was highly significant ($p \leq 0.01$). Also, the interaction between flour, packaging material and storage period was highly significant ($p \leq 0.01$) on redness of the cookies.

Table 7. ANOVA Hunter color a* value of cardamom flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	581.69	581.69	1.5076e+05	< 2.2e-16	***
Packaging material(P)	1	0.22	0.22	5.7660e+01	7.846e-08	***
Storage period (S)	2	26.93	13.47	3.4903e+03	< 2.2e-16	***
Flour× Packaging material	1	0.00	0.00	6.9190e-01	0.4137	
Flour × Storage period	2	7.40	3.70	9.5888e+02	< 2.2e-16	***
Packaging material× Storage period	2	0.18	0.09	2.2929e+01	2.703e-06	***
Flour×Packaging material× Storage period	2	1.35	0.67	1.7460e+02	5.003e-15	***
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

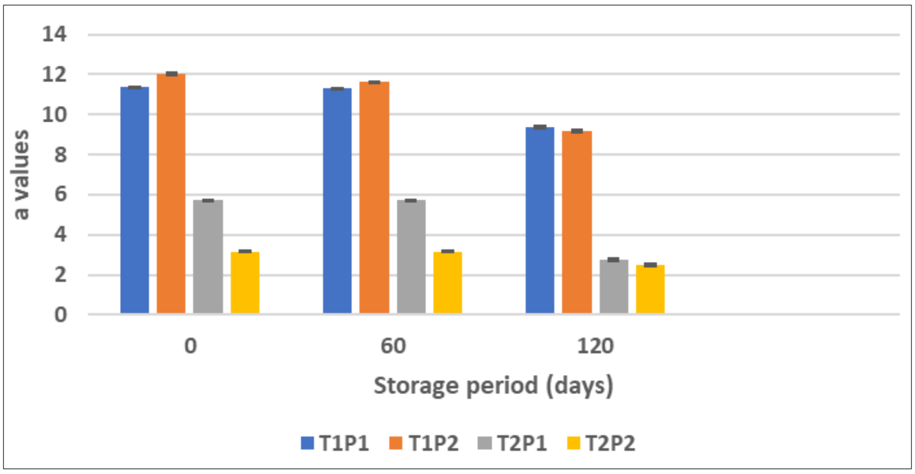

Fig 7b: Hunter color a* value of black pepper flavoured cookies during storage

Statistical analysis of black pepper flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the redness of cookies (Table 8). The effect of packaging material is highly significant ($p \leq 0.01$) on redness of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the redness of cookies. The interaction between flour and packaging material on redness of cookies is not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period was highly significant ($p \leq 0.01$). Also, the interaction between flour, packaging material and storage period was highly significant ($p \leq 0.01$) on redness of the cookies.

Table 8: ANOVA Hunter color a* value of black pepper flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	581.69	581.69	1.5076e+05	< 2.2e-16	***
Packaging material(P)	1	0.22	0.22	5.7660e+01	7.846e-08	***
Storage period (S)	2	26.93	13.47	3.4903e+03	< 2.2e-16	***
Flour×Packaging material	1	0.00	0.00	6.9190e-01	0.4137	
Flour × Storage period	2	7.40	3.70	9.5888e+02	< 2.2e-16	***
Packaging material× Storage period	2	0.18	0.09	2.2929e+01	2.703e-06	***
Flour×Packaging material × Storage period	2	1.35	0.67	1.7460e+02	5.003e-15	***
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

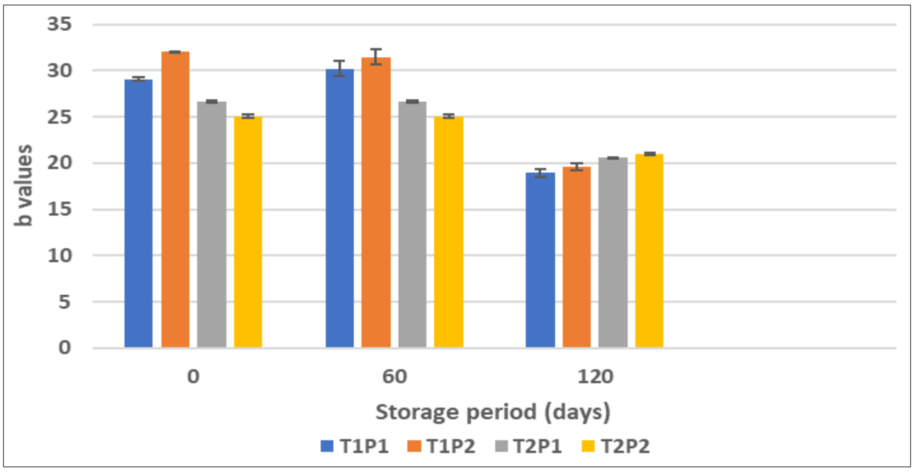

Fig 8a: Hunter color b* value of cardamom flavoured cookies during storage period

Statistical analysis of cardamom flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the yellowness of cookies (Table 9). The effect of packaging material is not significant on the yellowness of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the yellowness of cookies. The interaction between flour and packaging material on yellowness of cookies was not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was highly significant ($p \leq 0.01$) on the yellowness of the cookies.

Table 9: ANOVA for Hunter color b* value of cardamom flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	66.94	66.940	448.3151	< 2.2e-16	***
Packaging material(P)	1	1.09	1.085	7.2670	0.01263	*
Storage period (S)	2	549.96	274.982	1841.6380	< 2.2e-16	***
Flour × Packaging material	1	14.61	14.605	97.8150	6.096e-10	***
Flour × Storage period	2	80.28	40.138	268.8133	< 2.2e-16	***
Packaging material × Storage period	2	1.13	0.565	3.7862	0.03723	*
Flour × Packaging material × Storage period	2	6.85	3.425	22.9391	2.694e-06	***
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

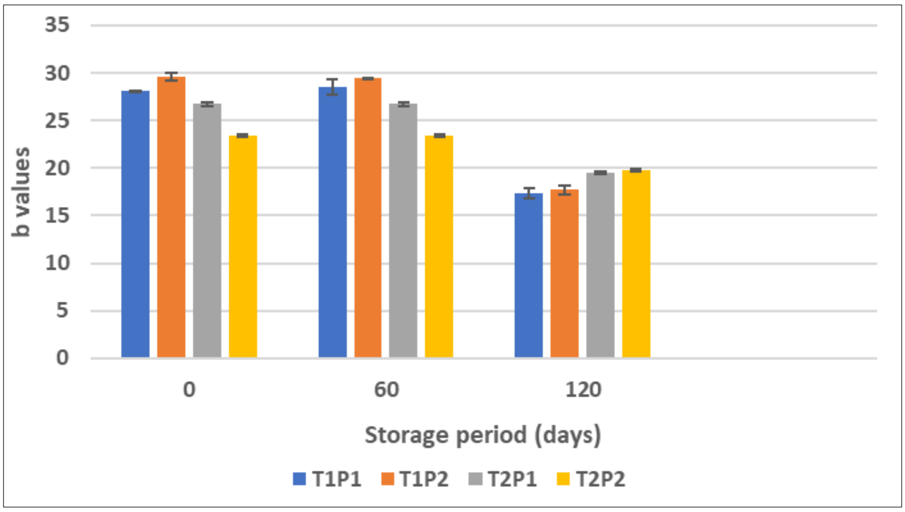

Fig 8b: Hunter color b* value of black pepper flavoured cookies during storage period

Statistical analysis of black pepper flavoured cookies showed that the effect of flour is highly significant ($p \leq 0.01$) on the yellowness of cookies (Table 10). The effect of packaging material was highly significant ($p \leq 0.01$) on the yellowness of cookies. The effect of storage period was highly significant ($p \leq 0.01$) on the yellowness of cookies. The interaction between flour and packaging material on yellowness of cookies was highly significant ($p \leq 0.01$), whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period was highly significant ($p \leq 0.01$). Also, the interaction between flour, packaging material and storage period was highly significant ($p \leq 0.01$) on yellowness of the cookies.

Table 10: ANOVA for Hunter color b* value of black pepper flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	30.31	30.305	238.993	5.619e-14	***
Packaging material(P)	1	3.39	3.392	26.748	2.691e-05	***
Storage period(S)	2	563.89	281.946	2223.504	< 2.2e-16	***
Flour × Packaging material	1	21.02	21.022	165.787	2.868e-12	***
Flour × Storage period	2	71.01	35.506	280.011	< 2.2e-16	***
Packaging material × Storage period	2	3.89	1.943	15.324	5.148e-05	***
Flour ×Packaging material × Storage period	2	9.73	4.863	38.349	3.360e-08	***
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

3.2 Biochemical Analysis

3.2.1 Free fatty acid

The free fatty acid content of cookies indicates the rancidity of foods. The lowest free fatty acid content was observed on 0 days of storage while the highest free fatty acid content was found on the 120th day of storage. It was observed that an increase in the free fatty acid content of cookies packed in BOPP was more compared with the metallised polyester package.

The free fatty acid content in cardamom flavoured sorghum millet cookies was increased from 0.27 to 0.50 and 0.47 respectively in BOPP and metallized polyester packages. The FFA of control increased from 0.22 to 0.58 and 0.44 in BOPP and metallized packages respectively. 0.22 to 0.58 and 0.22 to

0.44. Pepper flavoured sorghum millet cookies packed in BOPP showed an increase from 0.24 to 0.50 and 0.43 respectively in BOPP and metallized polyester packages. Free fatty acid content of control samples was found to be 0.58 and 0.55 under BOPP and metallized polyester packages respectively.

Statistical analysis of cardamom flavoured cookies showed that the effect of flour is not significant on the free fatty acid content of cookies. Table 11 shows that the effect of packaging material is not significant on free fatty acid content of cookies. The effect of storage days was highly significant ($p \leq 0.01$) on the free fatty acid content of cookies. The interaction between flour and packaging material on free fatty acid content of cookies was not significant, whereas the interaction between flour and storage period is not significant. The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was not significant on free fatty acid content of the cookies.

Table 11: ANOVA for free fatty acid content of cardamom flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	0.00871	0.008711	3.4236	0.07662	.
Packaging material(P)	1	0.00250	0.002500	0.9825	0.33146	
Storage period(S)	2	0.37642	0.188211	73.9694	5.471e-11	***
Flour × Packaging material	1	0.00284	0.002844	1.1179	0.30090	
Flour × Storage period	2	0.01609	0.008044	3.1616	0.06043	.
Packaging material × Storage period	2	0.02407	0.012033	4.7293	0.01855	*
Flour ×Packaging material × Storage period	2	0.00569	0.002844	1.1179	0.34340	
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

Statistical analysis of black pepper flavoured cookies showed that the effect of flour is not significant on the free fatty acid content of cookies (Table 12). The effect of packaging material is not significant on free fatty acid content of cookies. The effect of storage days was highly significant ($p \leq 0.01$) on the free fatty acid content of cookies. The interaction between flour and packaging material on the free fatty acid content of cookies was not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was not significant on free fatty acid content of the cookies.

Table 12: ANOVA for free fatty acid content of black pepper flavoured cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	0.00422	0.004225	1.7707	0.1958	
Packaging material(P)	1	0.00062	0.000625	0.2619	0.6135	
Storage period(S)	2	0.49827	0.249136	104.4109	1.440e-12	***
Flour × Packaging material	1	0.00047	0.000469	0.1967	0.6613	
Flour × Storage period	2	0.09282	0.046408	19.4494	9.524e-06	***
Packaging material × Storage period	2	0.00802	0.004008	1.6799	0.2076	
Flour ×Packaging material × Storage period	2	0.00094	0.000469	0.1967	0.8227	
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

3.2.2 Peroxide value

Peroxide value is the measure of fat stability in food and is an index of its shelf stability [33]. The peroxide value increased with an increase in storage time and reached to a maximum value after 120 days of storage. From the present experiment, the peroxide value (PV) of cardamom flavoured sorghum millet cookies was increased from 1.56 to 2.48% and 1.56 to 2.8% in BOPP package and metallised polyester package respectively. The control samples with cardamom flavour showed PV values of 1.4 to 3.8% and 1.4 to 3.4% respectively. Black pepper flavoured sorghum millet cookies packed in BOPP and metallised polyester package indicated PV values of 1.66 to 2.76% and 1.66 to 2.4% respectively. The control cookies with black pepper flavour showed PV values of 1.23 to 3.4% in both BOPP and metallised polyester packages.

It was observed that two packaging materials significantly affected the peroxide value during storage. The highest average peroxide value observed was 3.8 and 3.4% respectively in cardamom and black pepper-flavoured sorghum cookies packed in BOPP packages [34]. also reported that the peroxide value of cookies increases during storage up to 120 days, due to an increase in their moisture content which promoted fat hydrolysis during storage.

Statistical analysis of cardamom flavoured cookies showed that the effect of flour is not significant on the peroxide value of cookies (Table 13). The effect of packaging material is not significant on peroxide value of cookies. The effect of storage days was highly significant ($p \leq 0.01$) on the peroxide value of cookies. The interaction between flour and packaging material on peroxide value of cookies was not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was not significant on the peroxide value of the cookies.

Table 13: ANOVA for peroxide value of cardamom flavoured sorghum cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	0.0201	0.0201	0.3647	0.55160	
Packaging material(P)	1	0.0084	0.0084	0.1527	0.69944	
Storage period(S)	2	17.6638	8.8319	160.4742	1.287e-14	***
Flour × Packaging material	1	0.0951	0.0951	1.7274	0.20117	
Flour × Storage period	2	4.0860	2.0430	37.1208	4.518e-08	***
Packaging material × Storage period	2	0.0043	0.0022	0.0391	0.96170	
Flour ×Packaging material × Storage period	2	0.2976	0.1488	2.7040	0.08728	.
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

Statistical analysis of black pepper flavoured cookies showed that the effect of flour is not significant on the peroxide value of cookies. Table 14 shows the effect of packaging material is not significant on peroxide value of cookies. The effect of storage days was highly significant ($p \leq 0.01$) on the peroxide value of cookies. The interaction between flour and packaging material on peroxide value of cookies was not significant, whereas the interaction between flour and storage period was highly significant ($p \leq 0.01$). The interaction between packaging material and storage period is not significant. Also, the interaction between flour, packaging material and storage period was not significant on peroxide value of the cookies.

Table 14: ANOVA for peroxide value of black pepper flavoured sorghum cookies

Treatments	Df	Sum	Mean sq.	F value	Pr (>F)	
Flour (T)	1	0.0136	0.0136	0.2970	0.5908	
Packaging material (P)	1	0.1225	0.1225	2.6727	0.1151	
Storage days (S)	2	15.6772	7.8386	171.0242	6.311e-15	***
Flour × Packaging material	1	0.0336	0.0336	0.7333	0.4003	
Flour × Storage days	2	3.3006	1.6503	36.0061	5.951e-08	***
Packaging material × Storage days	2	0.0617	0.0308	0.6727	0.5197	
Flour × Packaging material × Storage days	2	0.0672	0.0336	0.7333	0.4908	
***- Significant at 0.1%, ** - Significant at 1%, * - Significant at 5%,						

3.2.3 Anti-oxidant activity

In the present study, the antioxidant activity of cardamom flavoured sorghum millet cookies decreased from 56.05%to 46.72% and 56.37% to 49.46% in BOPP and metallised polyester packages. The control samples with cardamom flavour indicated antioxidant activity of 53.06% to 48.20% and 54.88%to 51.58% in BOPP and metallised polyester packages respectively. Black Pepper flavoured sorghum millet cookies packed in BOPP and metallised polyester package were 54.69% to 49.34% and 54.64% to 51.84% respectively, in the control samples with black pepper flavour showed antioxidant activity of 53.07% to 48.22% and 53.24 to 49.20% respectively in BOPP and metallised polyester packages.

The antioxidant activity decrease during storage may be attributed to the dilution of antioxidant components by increased moisture and also to the possible oxidation of antioxidant components under favourable conditions during storage [35] while working on lipid oxidation in extruded corn reported that phenolic components are lost during storage.

3.3 Microbial Analysis

The microbial count of the cookies is shown in Tables 15 and 16. The data given shows the mean total plate count of the microbial load in cookies on Nutrient Agar. A similar analysis was found in microbial load in cookies on Nutrient Agar and Fungi count on Potatoes Dextrose Agar. The maximum value for total microbial load on nutrient agar (4.62×10^2) was observed in (6% Whey Protein concentrate supplemented cookies) and the minimum value of (3.05×10^2) was observed in (control cookies), while the maximum value for fungal count (0.13×10^3) was observed in (6% Whey protein concentrate supplemented cookies) while lowest value of (0.5×10^1) was found in (control cookies). Results revealed that the total microbial load of all samples does not differ significantly (Fig. 9 and 10).

The increase in microbial load of cookies as compared to control may be due to an increase in moisture content with increasing supplementation level. The microbial load of given cookie samples was compared with microbiological standards of fortified blended products and was found that the total plate count was less than 10^5 cfu/g. It was observed that the value is still within the acceptable limit.

Table 15: Microbial analysis of the cardamom flavoured sorghum cookies

Experiment 1	
Samples	**Total Plate Count (CFU/g)**
T1P1	2×10^4
T1P2	10×10^4
T2P1	4×10^4
T2P2	1×10^4

Table 16: Microbial analysis of the black pepper flavoured sorghum cookies

Experiment 2	
Samples	**Total Plate Count (CFU/g)**
T1P1	3×10^4
T1P2	3×10^4
T2P1	1×10^4
T2P2	2×10^4

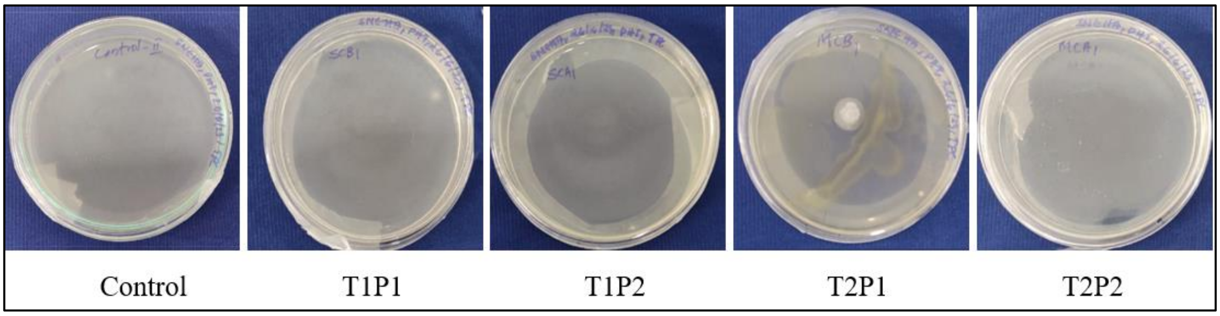

Fig 9: Microbial analysis of cardamom flavoured sorghum cookies

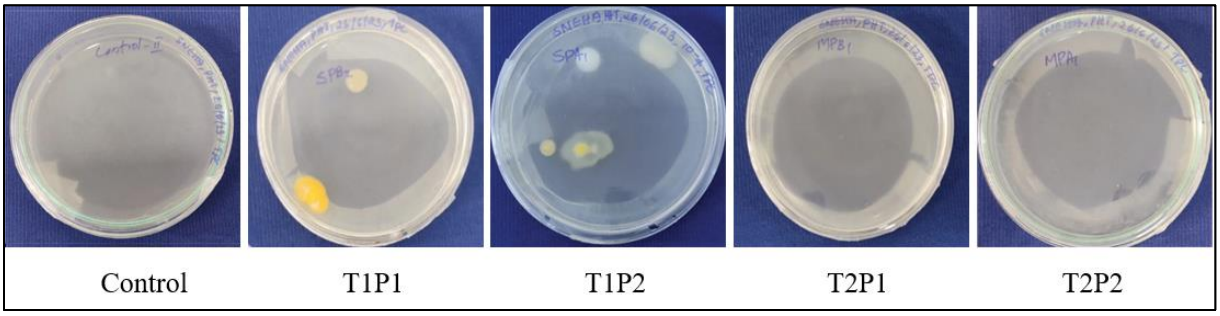

Fig 10: Microbial analysis of black pepper flavoured sorghum cookies

3.5 Analysis of Secondary Metabolites

Secondary metabolites such as essential oil and oleoresin content were evaluated and observed that cardamom had the highest essential oil content of (5.4%) followed by black pepper (2.8%). Out of the two spices used the highest oleoresin content of 8.2% was exhibited by cardamom black pepper (6.83%).

3.6 Organoleptic Characteristics of Developed Sorghum Millet Cookies

The spice-enriched sorghum millet cookies were evaluated to study organoleptic properties like colour, flavour, taste, texture, and overall acceptability. The data on the effect of the addition of cardamom and black pepper powder on the organoleptic characteristics of sorghum millet cookies are presented in Fig. 11 and 12.

The results showed that the colour, flavour, taste, texture, and overall acceptability of developed sorghum millet cookies were found to increase initially and then decrease during storage. From the figure it is observed that in cookies the colour score was found to decrease when the storage duration of cookies increased. Similar to colour, the flavour, taste, and texture of developed cookies decreased. The flavour was found good in cardamom flavoured sorghum millet cookies compared to pepper flavoured and control. The texture was found good in cardamom flavoured sorghum millet cookies compared to pepper flavoured. During storage, the hardness decreased from 5.7 to 4.7 and 5.7 to 5.2 in pepper and cardamom flavoured sorghum millet cookies in metallised polyester packages. Whereas in BOPP package, the hardness value decreased from 7 to 6 and 6.8 to 5.1 for cardamom and pepper cookies respectively. The results showed that the overall acceptability of developed cookies was increased initially and then decreased from (7.8 to 5.6) in cardamom flavoured sorghum millet cookies. (6.8 to 5.1) in pepper flavoured sorghum millet cookies. From the present observation, the highest overall acceptability was found in cardamom flavoured sorghum millet cookies packed in metallised polyester packages. Photographs of the developed cookies are shown in Fig.

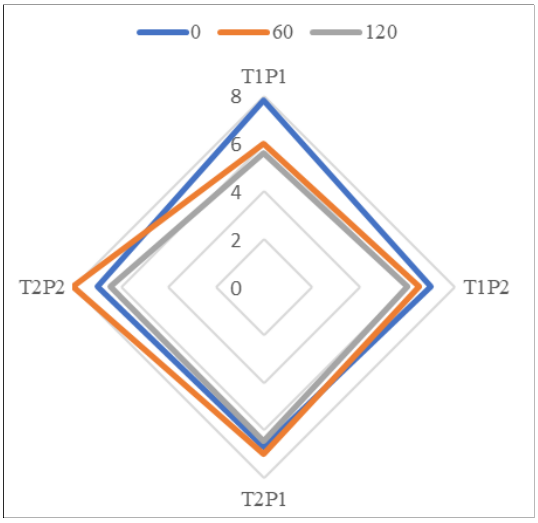

Fig 11: Organoleptic characteristics of cardamom flavoured sorghum millet cookies on 120 days of storage

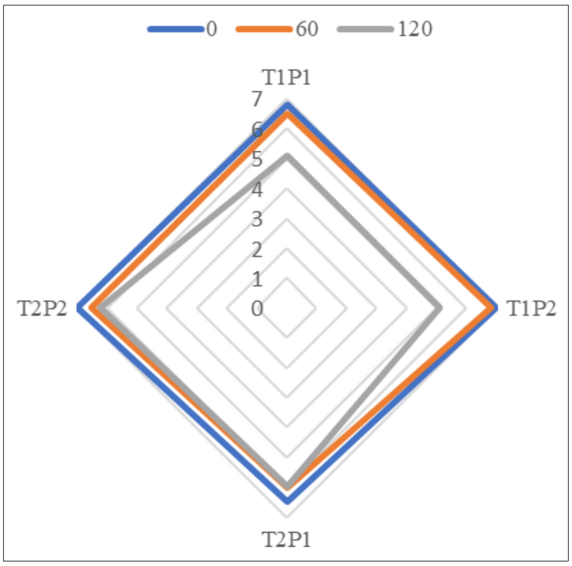

Fig 12: Organoleptic characteristics of black pepper flavoured sorghum millet cookies on 120 days of storage

Cardamom flavoured sorghum cookie in BOPP package

Cardamom flavoured sorghum cookie in metallized polyester package

Control cardamom cookie in BOPP package

Control cardamom cookie in metallized polyester package

Black pepper flavoured sorghum cookie in BOPP package

Black pepper flavoured sorghum cookie in metallized polyester package

Control black pepper cookie in BOPP package

Control black pepper cookie in metallized polyester package

Fig 13: Photographs of spice flavoured sorghum cookies

4. Conclusion

The highest average overall acceptability was observed for both cardamom and black pepper flavoured sorghum cookies packed in metallised polyester packages. The spread ratio of the developed spice flavoured and control cookies were 47.7 mm and 43.3 mm respectively. The hardness of cardamom flavoured sorghum millet cookies decreased from 16.63 to 4.70 N and 17.34 to 4.22 N in BOPP and metallized polyester packages respectively. Whereas the hardness of pepper flavoured sorghum millet cookies decreased from 20.09 to 4.06 N and 16.94 to 3.64 N in BOPP and metallized polyester packages respectively. The peroxide value of cardamom flavoured sorghum cookies increased from 1.56 to 2.48 and 2.8% respectively in BOPP and metallized polyester packages. Peroxide value of black pepper flavoured sorghum cookies increased from 1.66 to 2.76 and 2.40% in BOPP and metallized polyester [packages. However, highest peroxide value observed were 3.8 and 3.4% respectively in BOPP and metallized polyester at the end of storage period. Antioxidant activity of cardamom cookies at the end of storage were 46.72 and 49.46% respectively in BOPP and metallized polyester packages. Black pepper flavoured cookies exhibited antioxidant activity of 49.34 and 51.84% respectively in BOPP and metallized polyester packages. Free fatty acid content of cardamom cookies was 0.5 and 0.47 in BOPP and metallized polyester packages respectively. Black pepper cookies shoed free fatty acid content of 0.5 and 0.43 respectively in BOPP and metallized polyester packages. Hence it is concluded that metallized polyester packages (outer 12µ metallized polyester and inner 40 µ LDPE) can enhance the shelf life of spice flavoured sorghum cookies to a maximum of 120 days under ambient conditions. The output of the study can be further used in commercialization of sorghum cookies and thereby promote entrepreneurship development.

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