

DEVELOPMENT, STANDARDISATION AND SHELF-LIFE  
EVALUATION OF A GHEE-ENRICHED SEED BUTTER  
PREPARED FROM PUMPKIN (*CUCURBITA PEPO*) AND  
CUCUMBER (*CUCUMIS SATIVUS*) SEEDS:  
PHYSICOCHEMICAL AND SENSORY QUALITY ASSESSMENT

S.K. Mathanghi<sup>1\*</sup> and Sheetal Kumari<sup>2</sup>

Dept of Poultry Engineering  
College of Poultry production and Management  
Tamil Nadu Veterinary and Animal Sciences University  
Hosur

ABSTRACT

*Seed butters have emerged as a nutritionally rich and functionally versatile alternative to conventional nut-based spreads, garnering increasing attention in the food industry owing to their favourable fatty acid profiles, high protein content, and bioactive compound diversity. The pumpkin seeds from Cucurbita pepo and cucumber seeds from Cucumis sativus are abundant in dietary fiber, protein, good fats, vitamins, minerals, and bioactive substances and are beneficial to health when added in daily diets due to their nutraceutical components. The combinations of pumpkin seeds and cucumber seeds are used along with ghee for the standardization of seed butter. Sensory evaluation of the developed seed butter packaged in PET plastic was conducted over five-time intervals — Day 0, 15, 30, 45, and 60 — across five attributes: colour, texture, taste, spreading ability, and overall acceptability, rated on a 9-point hedonic scale. All attributes recorded high mean scores ranging from 8.2 to 8.9 at 0<sup>th</sup> day, reflecting excellent initial product quality. Scores remained statistically stable from 0<sup>th</sup> day through 45<sup>th</sup> day, indicating the absence of any significant difference across these time points. At day 60, a uniform and statistically significant decline was observed across all five sensory attributes ( $p < 0.05$ ) confirming a critical quality transition beyond 45 days of storage. The standardized seed butter stored at room temperature of 30°C was microbiologically safe till the end of storage days, and the impact of packaging material analysis on organoleptic parameters confirmed the most suitable package for this product was PET jars.*

**Key words:** seed butter, shelf -life, pumpkin seeds, ghee

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<sup>1</sup>Associate Professor, \* Corresponding Author Email: mathanghisoma@gmail.com

<sup>2</sup>UG Scholar, College of Food and Dairy Technology, TANUVAS, Koduveli, Chennai

## INTRODUCTION

Seed butter is a semi perishable food made by blending the roasted seeds. The sweetness and saltiness are added by the different natural and artificial additives. Pumpkin seeds have beneficial effect on health as it possesses most phytochemicals required for healthy life (Šamec *et al.*, 2022). The presence of polyphenols, phytoestrogens and squalene, minerals in cucumber seeds contribute to goodness of health (Mukherjee *et al.*, 2013; Wang, 2021).

Simple processing methods are involved like roasting, grinding, homogenisation and tempering and chilling. The roasting helps in oil capsules to open up and helps in flavour development (Gorrepati *et al.*, 2015). The optimal colour and desirable flavours are obtained during roasting (Zhang *et al.*, 2019). The seed of pumpkin (*Cucurbita* spp.) are generally considered to be agro – industrial wastes and discarded. The health-conscious consumers are shifting to nutritionally sound and economically feasible products as snack alternatives. Pumpkin seeds are one such alternative to gain importance in snack industry. Sesame seeds possess good amount of  $\gamma$ -tocopherol (Morsy *et al.*, 2022) and acts as a antioxidant and helps in prevention of many ailments and it is used in many culinary and medicated preparations in South India. Ghee besides being a traditional milk product, finding extensive uses in food industry and traditional system of medicine that originated in India (Kataria and Singh, 2024). Recently the research focus of ghee with its beneficial impact on CVD, skin

ailments and wound healing properties. Also, the per capita consumption of ghee and butter in India has increased to 12.3 g/person/day in the year 2020 from 7.4 g/person/day in 2007 (Muehlhoff *et al.*, 2013). Due to the increased milk production in India, the abundance of milk-based products is engaging to go for new product developments. With all these perspectives, a seed-based butter was developed in this study and the storage analysis was done.

## MATERIALS AND METHODS

### Preparation of seed butter

Pumpkin (*Cucurbita pepo*) and cucumber (*Cucumis sativus*) seeds were obtained from fresh mature fruits sourced from a local market. Seeds were manually separated, washed thoroughly under running water to remove adhering pulp residues, and spread uniformly on stainless steel trays for surface drying at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 24 hours prior to roasting. The weighed quantity of seeds was roasted separately to open up the fat molecules and allowed to cool. Cleaned and dried seeds were roasted in a pre-heated convection oven at  $160^\circ\text{C}$  for 15 minutes for pumpkin seeds and  $150^\circ\text{C}$  for 12 minutes for cucumber seeds, conditions optimised to achieve uniform browning, enhanced flavour development, and adequate moisture reduction without inducing excessive char or bitterness. Immediately following roasting, seeds were removed from the oven and transferred onto clean stainless steel wire mesh trays. Cooling was carried out at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 20 minutes until the seed temperature reached approximately

30–32°C. Then the first grinding was done separately using high speed kitchen mixer (Kenwood Processor Pvt. Ltd) operated at a fixed speed of 1400 rpm for 3 minutes to induce phase transition from solid to semi-liquid stage. The second grinding was done after 10 min interval with other essential ingredients for homogenization in the same mixer. The two-stage grinding helped to obtain creamy consistency of the seed butter and enhances the stability of phase transition. Ghee was added gradually during fine grinding stage and Salt was incorporated, added in the final 60 seconds of grinding. Tempering of the seed butter is an essential step to get the conformational crystal changes (Oseni *et al.*, 2019) and it was done at 5 °C for 72 hours to get good crystallisation of fat and it helps to stabilize the product. The different combinations of pumpkin and cucumber seed butter was mixed with Ghee and added with INS 471 stabilizer.

The treatments consisted of different proportions of pumpkin seed and cucumber seed incorporation. The combinations were designated as follows: T1 (40:25%), T2 (35:30%), T3 (30:35%), and T4 (25:40%), representing the respective ratios of pumpkin seed to cucumber seed. The level of addition of Ghee, sugar, salt and stabilizer were kept constant based on the pilot trials (Ghee: 15 %, Sugar: 15 %, Salt: 3 % and stabilizer: 2%) in all the formulations. The standardization of the seed butter was based on the organoleptic evaluations and further analysis was made. Higher addition of pumpkin as well as cucumber seeds beyond 40 percent leads to slight curdling

and taste differences in the final product. The maximum limit of both pumpkin seed and cucumber was taken as 40 percent in the formulation.

### **Proximate analysis of the standardized seed butter**

The seed butter incorporated with pumpkin and cucumber seed were analyzed for proximate composition such as protein (AOAC (2019) methods 984.13), fat (AOAC (2019) Method 920.39), fibre (AOAC (2019) method 978.10) and ash (AOAC (2019) Method 900.02) and moisture content using hot air oven method (Mathanghi *et al.*, 2021).

### **Physicochemical analysis of seed butter**

The standardized seed butter was tested for primary oxidation products such as free fatty acid (FFA), peroxide value, to determine the shelf-life and sensory properties using standard procedures on the 0th day and end of storage days. The content of FFA in seed butter was estimated by titrating it against KOH in the presence of phenolphthalein indicator. The Iodometric Titration method was used to measure the peroxides contained in a sample of seed butter, expressed as milli-equivalents of peroxide per kg of the material. The pH and titrable acidity were also measured on the 0th day and end of storage days.

### **Storage analysis of seed butter**

The standardized seed butter was subjected to storage analysis for 60 days at room temperature (approximately @ 30°C) in two different packaging material. One

with four layered metallized pouch and another material selected was PET jar. Both the packaging material was selected for their qualities against air and light permeation as well as for ease of handling by the consumers and tested for organoleptic evaluations. A 9-point hedonic scale was employed for scoring, where 9 = like extremely and 1 = dislike extremely. Sensory attributes evaluated included colour, texture, taste, spreading ability, and overall acceptability by a semi-trained sensory panel comprising 20 panelists recruited from the faculty, research scholars, and postgraduate students of the Department.

### **Microbial analysis of the standardized seed butter**

The total plate count was enumerated using Plate Count Agar medium. 10 grams of the sample were mixed homogeneously and serially diluted using sterile water at 10<sup>-3</sup> for and 0.1 mL of appropriate dilutions were poured into plates in duplicates and further incubated aerobically at 30°C for 48–72 h (ISO, 2019). Finally, colonies were enumerated using colony counter.

### **Statistical Analysis**

All data collected in the present study were subjected to appropriate statistical analyses using standard procedures to ensure rigorous interpretation of results. ANOVA is widely employed in food science research to evaluate the effect of a single independent variable — in this case, storage duration — on multiple continuous dependent variables simultaneously (Montgomery, 2017). One way ANOVA was performed to determine

significant differences among treatment groups or storage intervals and Tukey's Honest Significant Difference (HSD) post hoc test was applied to perform pairwise multiple comparisons between group means. For sensory evaluation data, the non-parametric Kruskal-Wallis H test was employed (Lawless and Heymann, 2010). The test statistic H was evaluated against a chi-squared distribution with k–1 degrees of freedom, where k denotes the number of groups, at a significance level of  $p < 0.05$ . All statistical analyses were performed using SPSS Statistics (Version 19.0, IBM Corp., Armonk, NY, USA).

## **RESULTS AND DISCUSSION**

### **Standardization of seed butter**

The standardization of seed butter was based on 9-pt hedonic sensorial rating method, where 20 semi-trained panelists from the institute were asked to rate the seed butter samples. The sample T2 scored the highest among the panel members and it was comparable to peanut butter available in the market. The organoleptic scores are given in table 1, where the overall acceptability was shown to be  $8.89 \pm 0.04$  for T2. The treatment T1 showed moderately high scores and was statistically lower than T2 but higher than T3 and T4 for most attributes. The lower scores observed for T3 and T4 indicate comparatively inferior sensory quality, particularly in body and texture, where T4 recorded the lowest value (7.0), suggesting poor structural consistency. Higher the quantity of cumber seeds than pumpkin seed was not acceptable due to bitterness formation and quick off flavor

development during pilot study. Similar effect of bitterness was observed by (Aygün and Emirmustafaoglu, 2025) in the pumpkin seed pudding at higher incorporation levels. Also, the oil separation was observed when the quantity of cucumber seeds was high.

### Proximate analysis of the seed butter

The sample T2 was subjected to proximate analysis and moisture content was found to be  $6.39 \pm 0.01$ g and other nutritional parameters were carbohydrates ( $76.83 \pm 0.07$ ), protein ( $4.44 \pm 0.22$  g), fat ( $4.21 \pm 0.03$  g), fibre ( $0.18 \pm 0.02$ ) and ash ( $8.1 \pm 0.02$ ) in 100 g of seed butter. The ash content was higher than the products available as peanut butter in the market. Pumpkin seeds have high phosphorous, potassium and magnesium content (Batool *et al.*, 2022)

### Physico-chemical analysis of seed butter

The observed percentage of changes in physico chemical parameters values of seed butter are portrayed in table 2 and the values are represented in fig.1. The peroxide value (PV) increased substantially from  $34.08 \pm 1.21$  to  $67.52 \pm 4.06$  meq  $O_2$ /kg over the 60-day storage period, representing a 98.1% increase. The peroxide value is an indication of primary lipid oxidation and can be taken as useful indicator of fat spoilage (He *et al.*, 2023). The near-doubling of PV at Day 60 confirms active oxidative deterioration of the unsaturated fatty acids present in the seed butter matrix. This finding is consistent with previous studies on high-fat seed-based products stored at ambient temperatures, where elevated peroxide values were

attributed to exposure to oxygen, light, and temperature fluctuations through packaging (Gómez-Caravaca *et al.*, 2016). During the storage study the 0th day value for FFA was 0.41 and it was found to be well within the normal limits given by FSSAI. Shibli *et al.*, (2019) studied that the free fatty acid of peanut butter should 0.02-0.6% in order to be fit for human consumption. The FFA between Day 0 and Day 60, corresponding to a 51.2% increase. These values are comparable to findings reported by Onsaard *et al.* (2010) in stored sesame-based products, where FFA content increased steadily with storage duration under ambient conditions. While the pH remained within a mildly acidic range throughout the storage period, the downward trend signals progressive chemical deterioration of the lipid fraction in the seed butter sample. Titratable acidity is a comprehensive measure of all acidic components present in a food matrix and serves as a reliable indicator of overall acid development during storage (Nielsen, 2017) and it was increased by 25.6 % in the sample due to effects of FFA and titratable acidity.

### Impact of package materials during real time storage

The standardized treatment T2 stored in PET jars scored good than the seed butter stored in 4 layered aluminum pouches throughout the study period of 60 days. The off-flavour development was observed in pouch packs at the end of 45th day and there was a sudden decline in the sensorial scores. Whereas in the PET jars there was a gradual decline overall acceptability till 60th day. Both the seed butter samples showed rancid smell formation after 60 days. The shelf life

of the seed butter is greatly influenced by the temperature and proper handling and the data on sensorial scores are given in table 3 and 4. In both the packaging the organoleptic scores started near 8.2–8.3, but PET shows a sharper early dip at Day 15 that partially recovers, while aluminium stays stable until a drop at Day 60 (6.9 vs 6.3). there existed a slightest change in the texture but after 45th day there was a declining trend observed. Similar fashion of data was observed with taste and spreadability. PET bottles showed relatively more consistent overall acceptability at Day 60. The storage stability of the seed butter is greatly influenced by the PUFA content of the seeds, the pumpkin seeds contain 45-55 % of Total PUFA and cucumber seeds has 55-65 % total PUFA (Li *et al.*, 2020). Neither packaging maintains scores above 7.0 across all attributes beyond 45 days at room temperature. The seed butter stored in PET jar showed better performance than 4 layer pouches as per this study and it was supported by Muttagi *et al.* (2014) in their study on storage stability of sunflower seed chikki.

#### **Microbial analysis of peanut butter incorporated with cucumber and pumpkin seed**

The microbial analysis of the developed seed butter revealed a total plate count of 2.6 log CFU/g on the 0th day, which increased to 5.4 log CFU/g on the 60th day in the product stored in PET jars. In comparison, samples stored in aluminum pouches exhibited a total plate count of 3.4

log CFU/g on the 0th day and 6.3 log CFU/g on the 60<sup>th</sup> day. The observed microbial counts were within acceptable limits as per prescribed food safety standards. Coliform count in the product was not detected which indicate the quality of the developed product. The rate of degradation was higher for samples packed in aluminum pouch than those packed in PET jars.

#### **CONCLUSION**

The pumpkin seed butters add variety in the daily diets as well as it can be considered for valorising under-utilised agricultural products. Owing to their healthy fatty acid composition and functional properties, they may offer potential health benefits, particularly in supporting heart health and overall well-being. The storage study clearly indicated a significant ( $p < 0.05$ ) increase in peroxide value from  $34.08 \pm 1.21$  to  $67.52 \pm 4.11$  meq O<sub>2</sub>/kg over 60 days, confirming progressive lipid oxidation; however, the values remained within acceptable limits for consumption. Other physicochemical parameters showed non-significant (NS) or marginal changes, indicating overall product stability. Sensory scores exhibited a slight decline during storage but remained within the acceptable range, supporting consumer acceptability up to the end of the storage period. The PET jars were the best container for the developed nutritionally valuable; shelf- stable pumpkin – cucumber seed butter, which had a 60-day shelf life and was microbiologically safe when kept at room temperature.

**Table.1. Standardization of seed butter**

Sensory Attribute	T1	T2	T3	T4	F-value
Colour/appearance	8.0±0.03 <sup>b</sup>	8.6±0.01 <sup>a</sup>	7.4±0.02 <sup>c</sup>	7.6±0.01 <sup>c</sup>	152.4
Taste	8.2±0.02 <sup>b</sup>	8.8±0.03 <sup>a</sup>	7.6±0.04 <sup>c</sup>	7.8±0.03 <sup>c</sup>	168.7
Body and texture	8.2±0.02 <sup>b</sup>	8.3±0.03 <sup>a</sup>	7.6±0.01 <sup>c</sup>	7.0±0.01 <sup>d</sup>	210.6
Spreading ability	7.8±0.11 <sup>b</sup>	8.8±0.04 <sup>a</sup>	7.6±0.02 <sup>c</sup>	7.4±0.02 <sup>c</sup>	134.9
Overall acceptability	8.05±0.07 <sup>b</sup>	8.89±0.04 <sup>a</sup>	7.57±0.03 <sup>c</sup>	7.45±0.03 <sup>c</sup>	189.3

Data are mean ±SD. (n=6)using one-way ANOVA followed by post hoc comparison (Tukey test).

**Table.2. Change in Physicochemical parameters of the standardized seed butter**

Parameter	0th Day (Mean ± SD)	60th Day (Mean ± SD)	% Change	Interpretation
Peroxide Value (meq O <sub>2</sub> /kg)	34.08 ± 1.21	67.52 ± 4.06	↑ 98.1%	Significant oxidation increase
Free Fatty Acid (%)	0.41 ± 0.30	0.62 ± 0.08	↑ 51.2%	Lipid hydrolysis increased
pH	6.29 ± 0.44	5.80 ± 0.32	↓ 7.8%	Slight acidification
Titrateable Acidity (%)	4.3 ± 0.2	5.4 ± 0.7	↑ 25.6%	Increased acidity

**Table.3. Shelf-life analysis of developed seed butter packaged in 4 layered Aluminum packs**

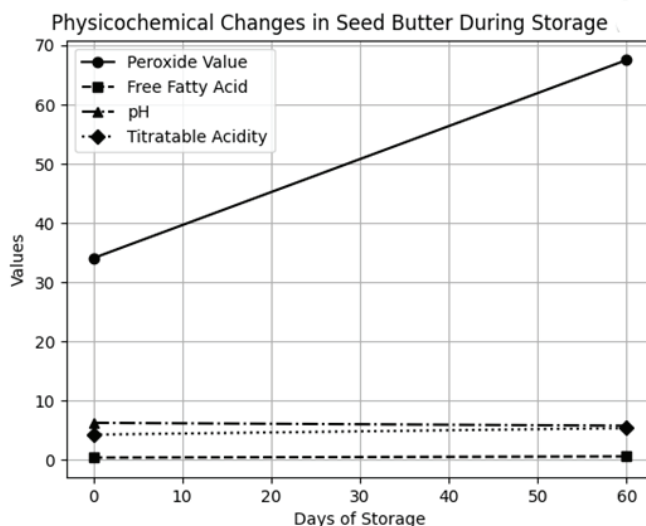
Sensory attribute	0 days	15 days	30 days	45 days	60 days	H- Statistic
Colour	8.3±0.23 <sup>a</sup>	8.4±0.94 <sup>a</sup>	8.3±0.54 <sup>a</sup>	8.4±0.77 <sup>a</sup>	6.9±0.43 <sup>b</sup>	16.48*
Texture	8.7±0.32 <sup>a</sup>	8.2±0.65 <sup>b</sup>	8.3±0.37 <sup>c</sup>	8.3±0.22 <sup>d</sup>	5.5±0.82 <sup>c</sup>	21.96**
Taste	8.8±0.43 <sup>a</sup>	8.4±0.40 <sup>a</sup>	8.4±0.38 <sup>b</sup>	8.4±0.88 <sup>a</sup>	5.7±0.12 <sup>c</sup>	19.28**
Spreading ability	8.8±0.11 <sup>a</sup>	8.7±0.35 <sup>a</sup>	8.8±0.84 <sup>a</sup>	8.7±0.24 <sup>a</sup>	6.3±0.72 <sup>b</sup>	16.88*
Overall acceptability	8.9±0.21 <sup>a</sup>	8.3±0.65 <sup>b</sup>	8.3±0.17 <sup>c</sup>	8.2±0.22 <sup>d</sup>	6.6±0.21 <sup>c</sup>	25.29**

Data are mean ± SD. Different superscript letters within the same row indicate significant differences ( $p < 0.05$ ) based on Kruskal-Wallis test with pairwise Mann-Whitney U comparisons

**Table.4. Shelf-life analysis of developed seed butter packaged in PET bottles**

Sensory attribute	Day 0	Day 15	Day 30	Day 45	Day 60	H- Statistic
Colour	8.2±0.21a	7.3±0.21b	7.8±0.21c	7.3±0.21d	6.3±0.54e	21.746**
Texture	8.2±0.30a	8.6±0.32b	8.3±0.32a	8.0±0.11d	6.5±0.12c	17.760**
Taste	8.1±0.44a	7.9±0.32a	7.4±0.54b	7.4±0.52c	5.7±0.34d	16.547NS
Spreading ability	8.1±0.44a	8.1±0.21a	8.1±0.11a	8.0±0.32a	7.3±0.23b	11.780NS
Overall acceptability	8.9±0.80a	8.3±0.11a	8.2±0.87a	8.2±0.21b	7.6±0.21c	12.353NS

Data are mean ± SD. Different superscript letters within the same row indicate significant differences ( $p < 0.05$ ) based on Kruskal-Wallis test with pairwise Mann-Whitney U comparisons.



**Fig.1. Impact of storage on physicochemical parameters**

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