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## Effect of PP Nanocomposite Multi-layered Film on Physicochemical Properties of Ready-To-Cook (RTC) Idli Batter and Prepared Idli Cake

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#### Authors' contributions

This work was carried out in collaboration among all authors. Author PSG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BKY and SA managed the analyses of the study. Authors ML and SS managed the literature searches. All authors read and approved the final manuscript.

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## **ABSTRACT**

**Aim:** The current study focuses on the effect of PP nanocomposite multi-layered film on physicochemical properties of stored idli batter and prepared idli cakes.

Place and Duration of Study: Department of Food Packaging and System Development at Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India, between Aug 2018 and Dec 2019.

**Methodology:** The developed PP nanocomposite multi-layered film composed with depositing silicon dioxide (SiO<sub>2</sub>) on polypropylene (PP) film (0.010 mm) and attached with ethylene-vinyl alcohol (EVOH 32%) film (0.066 mm). The Physico-chemical properties of developed multi-layered and commercial PP film stored idli batter with different blend ratios of rice to black gram (3:1 and 4:1) were evaluated in terms of pH, percent titrable acidity (% TA), colour value ( $\Delta$ E), viscosity (cP)

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and volume measurement (ml). Idli cake prepared with batter stored in multi-layered film and evaluated in terms of colour, texture and percent pore characteristics. The percent pore presents in per square inches of prepared idli cake with different blend ratios and fermentation time were analysed using the grayscale image in MATLAB.

Results and Discussion: The pH value of stored batter ranges from 6.7 to 4.5 or lower, to reach pH below 4.5 or lower commercially stored batter took 24 h and batter stored in the multi-layered film took 36 h at ambient temperature. The multi-layered stored batter showed better results of physicochemical properties in terms of fermentation time. Idli cake prepared with 3:1 blend ratio showed good results in terms of textural properties with compared to 4:1 blend ratio. Idli cake prepared with 3:1 blend ratio showed more pore percentage (21.88-36.80%) as compared to 4:1 blend ratio (18-32.98%) in per square inch. Due to higher barrier properties of the multi-layered film, the bacterial count increases at a slow rate and help to extend the shelf life of packaged batter more than 30 h at ambient temperature.

Keywords: Idli batter; PP nanocomposite multi-layered film; silicon dioxide; per cent pore; shelf life.

#### **ABBREVIATIONS**

ANOVA: Analysis of Variance
EVOH: Ethylene-vinyl alcohol
LAB: Lactic acid bacteria
LbL: Layer-by-layer

OTR : Oxygen Transmission Rate % TA : Percent titrable acidity

PP : Polypropylene
RTC : Ready-to-cook
SiO<sub>2</sub> : Silicon dioxide
SD : Standard Deviation
TPA : Texture Profile Analysis

WVTR : Water Vapour Transmission Rate

## 1. INTRODUCTION

Idli is a spontaneously fermented steam-cooked breakfast food item, popular in southern India among the several traditional foods and consumed all over India, Sri Lanka, Malaysia and Singapore [1,2]. Traditionally, idli batter is mainly prepared with soaking parboiled rice (*Oryza sativa L*.) which is rich in carbohydrates and decorticated black gram dal (*Phaseolus mungo L*.) rich in proteins. The idli batter fermentation was carried out for 12-18 h by the conventional way using rice to black gram ratios of 3:1 and 4:1 with the addition of 2% of salt (w/w) and allow it to ferment for overnight at ambient condition. The fermented batter dispensed into idli moulds in the form small cake and steamed [3-6].

Idli is well-known for its soft and spongy texture, sour taste, soft mouthfeel, attractive appearance, and digest easily. The packaged idli batter has a very short shelf life due to its high amount of moisture content and the presence of live fermentation. Generally, idli batter can be stored up to one day at room temperature while in the refrigerated condition, it can be stored up to 1

week [7]. Nowadays in the market, there is a huge demand for ready-to-cook packaged idli batter which have a moderate shelf life. For extending the shelf life of packaged idli batter there are few thermal preservation techniques used which improves the shelf life of idli batter more than two to three days at room temperature and up to one month in refrigerated conditions [8-11]. Those techniques are not viable it coagulates the idli batter or due to heat applications idli will be formed. Hence, there is a requirement to establish a method which will help extend the shelf life of packaged idli batter without coagulating it.

Now day consumer demands are increasing towards fresh and safe food products. New innovative packaging technologies have been developed and it shows active and intelligent function to extend the shelf life and quality of fresh, processed and refrigerated foods [12]. Active or smart packaging is used for preservation of foods rather than providing an external and inert barrier to the packaged food product.

Polypropylene (PP) is commonly used packaging material for the food packaging application, due to its higher melting temperature, high crystallinity, tensile strength, barrier properties and cheap to manufacture [13-15]. Nowadays in the food industry, silicon dioxide (SiO<sub>2</sub>) coated packaging materials are using due to its high barrier properties against the oxygen. The strength of SiO<sub>2</sub> coated packaging materials can be improved with an increase or decrease in the coating thickness [16-17].

Ethylene-vinyl alcohol (EVOH) is mostly used for the multilayer packaging materials due to its strong barrier properties against the oxygen [15]. Layer-by-layer (LbL) coating of packaging materials will improve the barrier properties, optical properties and wettability of developed packaging material [15,18,19]. In LbL multilayer packaging, acrylic acid (5 %) is used as an adhesive to join two or more different layers together [20].

The multilayer packaging films and coating of films have been developed through the surface modification with the help of plasma deposition, plasma treatment and polymerization [21], layer-by-layer (LbL) electrostatic deposition, electrospinning/electro - hydrodynamic [22], vacuum deposition technologies [23], magnetron sputtering, electron beam evaporation, sol-gel techniques and polymeric composites sandwiching [24]. The purpose of multilayer and coating packaging includes improving packaging performance [19], protection of antimicrobial agents from the external environment and controlling its release for effective activity [18].

In this study, effort was made to develop a suitable packaging film to protect the batter from interdiffusion of gases and extend the shelf life of RTC package idli batter without affecting its physicochemical properties of stored batter and prepared idli cake.

## 2. MATERIALS AND METHODS

## 2.1 Raw Material

EVOH resin (32% of ethylene content) was provided by Kuraray Co., Ltd. (Tokyo, Japan). Parboiled rice (*Oryza sativa*) variety, namely, IR 20 (idli rice) and black gram (*Phaseolus mungo*) of the variety of Aduthurai 3 (ADT 3) were taken as a major raw material for the preparation of idli batter and procured from the local market of Thanjavur, Tamil Nadu, India. All the chemicals used for this study were of reagent grade and procured from Merck, Sigma, (Chennai, Tamil Nadu, India). All the materials in this study were used without further purification.

# 2.2 Development of Multilayer Packing Material

The packaging film selected for this study was commercial (PP) film and PP nanocomposite multi-layered film. The PP nanocomposite multi-layered film was developed using PP as an inner layer (gas permeable) with compositing SiO<sub>2</sub> (100 nm) and EVOH as an outer layer (light transparent and the barrier to water and gas).

The commercial film having water vapour transmission rate (WVTR) of 23.03±3.38 g/m<sup>2</sup>.day and developed PP nanocomposite multi-layered film having WVTR of 1.07±0.16 g/m<sup>2</sup>day. The oxygen transmission rate (OTR) values for commercial and PP nanocomposite multi-layered film respectively, were 1274.57±155.25 and 452.46±33.94 cc/m<sup>2</sup>/24h. The characteristics of commercial and PP nanocomposite multi-layered film were mentioned in the previous paper (in press) [25].

## 2.3 Preparation of Idli Batter

The RTC idli batter was prepared by soaking parboiled rice and decorticated black gram dhal in water separately followed by the grinding process with the occasional addition of water at 1.5 to 2.0 times of the initial weight. The slurries of rice and black gram dhal are prepared with different blend ratios (3:1 and 4:1) and mixed with 1-2% of salt [26,27]. The prepared fresh batter about 150±5 g was stored in commercial and PP nanocomposite multi-layered film and allowed to ferment for different fermentation time up to the spoilage of idli batter at ambient condition. The quality evaluation of stored batter and prepared idli cakes with different fermentation were analyzed.

## 2.4 Quality Evaluation and Physicochemical Properties of Packaged Idli Batter

Quality evaluation and physicochemical properties of commercial (PP 0.010 mm) and PP nanocomposite multi-layered packaged idli batter sample was analyzed from the initial hour of experiment and thereafter every 3 h of intervals which were determined using the standard procedure as described below.

#### 2.4.1 pH of stored idli batter

Stored Idli batter about 10 ml was mixed with 100 ml of distilled water and centrifuged the mixture for 20 min at 17,880 g. The collected supernatant was separated and the pH of batter was determined with pH meter (Susima AP-1 plus, Chennai) [5].

## 2.4.2 Percent Titrable Acidity (%TA) of stored idli batter

The prepared idli batter (5g) was diluted with distilled water (10 ml) and titrated against 0.1 (N) NaOH and phenolphthalein as an indicator [28].

Total acidity of batter was calculated using the following formula

% Titrable acidity = 
$$\frac{Equivalent\ weight\ of\ acid\ X\ N\ of\ NaoH\ X\ Titer\ value}{weight\ of\ the\ sample\ X\ 1000}\ x\ 100$$
 (1)

### 2.4.3 Color value of stored idli batter

The colour value of prepared and stored batter in different packaging films was measured using a Hunter lab colourimeter (CFEX-0925, USA). The colour meter shows L\*, a\* and b\* values which denote to whiteness to darkness (L\*), green (-a\*) to red (+a\*) and yellow (+b\*) to blue (-b\*). Three measurements were performed and the results were averaged. The colour value was calculated by the following formula.

$$\Delta \mathbf{E} = [(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2]^{1/2}$$
 (2)

## 2.4.4 Viscosity of stored idli batter

The viscosity of prepared and stored batter (100 ml) in different packaging films were measured at 100 rpm using Viscometer (Brookfield DV-E) attached with LV-4 (S-64) spindle [7]. The torque reading was not below 10% and readings were taken after 1 min of revolution.

## 2.4.5 Volume measurement of stored idli batter

The prepared idli batter (150 g) stored in different packaging (commercial and PP nanocomposite multi-layered) films at ambient condition and measured the changes in volume as fermentation time increases were calculated as follows [7].

% Decrease in volume = 
$$\frac{Initial\ volume-final\ volume}{initial\ volume}\ X\ 100 \tag{3}$$

## 2.5 Quality Evaluation and Physicochemical Properties of Prepared Idli Cakes

The idli cake's prepared with a different blend ratio of stored batter into PP nanocomposite multi-layered film was analyzed for physicochemical analysis, namely,  $\Delta E$  value, texture, percent pore formation. Physicochemical analysis of prepared idli cakes was carried out for every 6 h of intervals from the minimum fermentation period (12 h) to till the end of the fermentation period.

## 2.5.1 Colour value of prepared idli cake

The colour measurements of prepared idli with different time intervals (12, 18, 24, 30 and 36 h) was calculated, as mentioned in section 3.2.8.3.

### 2.5.2 Texture of prepared idli cake

The texture of idli cake is one of the important parameters as per consumers point of view to judge the softness and fluffiness of prepared idli cakes [29]. The texture profile analysis (TPA) of prepared idli cake with different time intervals (12, 18, 24, 30 and 36 h) was measured using Texture Analyzer (Model TA-XT), Stable Micro Systems, Surrey, UK. The prepared idli was cut into the desired shape (20 mm X 20 mm) and the texture of idli was analysed by using a probe SMS/75mm. The test speed was set at 5mm/sec and penetration of probe of 50% of the sample's original height. Analysis of Variance (ANOVA) is a proficient statistical decision-making tool used to test the suitability for the responses in experiments. Based on TPA (force deformation) curve. several parameters (adhesiveness, springiness, cohesiveness, chewiness, and resilience) were calculated. All the TPA experiments were conducted in triplicates and data were statistically analyzed using One-way ANOVA analysis test carried out in SPSS 25.0.

### 2.5.3 Ink print test of prepared idli cake

The ink print test was carried out to identify the numbers of pores present in the internal structure of prepared idli using ink print photography [5]. The number of pores present in per square inch was analysed for different time intervals (12, 18, 24, 30 and 36 h) of prepared idli, which indicates the softness of idli with compared to time.

## 2.5.4 Estimation of pore percent of prepared idli cake

The pore present in prepared idli at different fermentation time was calculated by capturing the image of ink print test using SONY Cybershot digital camera (Model No. DSC-W570) with a non-reflecting white cloth-covered on the table. Ink print test paper was uniformly illuminated and the camera was placed vertically with 40 cm distance over the ink print test paper. All the images were taken with a natural illuminating lighting source.

In the image analysis, the first step was segmentation to separate the background image. To avoid the segmentation, the images were taken by focusing the camera on the boundaries of ink print test paper, which helps to eliminate the background image. The pixel intensity values of the boundaries of ink print test paper were calculated using MATLAB R2019b (MathWorks, INC., USA). Initially, the RGB image of the ink print test was converted into a grayscale image and pore % were calculated using the following formula:

Pore % = 
$$\frac{\text{Pore area } (no.of \ pixel)}{\text{Non-pore area } (no.of \ pixel) + \text{Pore area } (no.of \ the \ pixel)} X \ 100(4)$$

## 2.6 Statistical Analysis

The experimental data were analysed statistically, the linear and non-linear equation was developed using SPSS 25.0 (regression analysis) and Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA) for Windows 10.0. The means and standard deviation (SD) values were mentioned in figures and tables. The image acquisition for identification of pore space present in prepared idli was analysed using MATLAB R2017a (MathWorks, INC., USA).

### 3. RESULT AND DISCUSSION

## 3.1 Quality Evaluation and Physicochemical Properties of Packaged Idli Batter

Idli batter prepared with different blend ratios (3:1 and 4:1) of rice to the black gram was stored in commercial and developed PP nanocomposite multi-layered film for the physicochemical analysis, namely, pH, titrable acidity (% TA), colour value ( $\Delta E$ ), viscosity and volume measurement were monitored for every 3 h of intervals and results were described as follows.

#### 3.1.1 pH value of stored idli batter

The pH value of stored batter in commercial and PP nanocomposite multi-layered film with different blend ratios are shown in Fig. 1. It is obvious from Fig. 1, the pH values of batter stored in commercial and PP nanocomposite multi-layered film found to be decreased from 6.7±0.1 to below 4.5±0.2 and similar results were also observed by Balasubramanian and Viswanathan, with idli batter. According to Balasubramanian and Viswanathan [26] and Soni and Sandhu [30], the increase in the fermentation time help to decrease the pH of stored batter which was due to increase in the

lactic acid content produced by lactic acid bacteria (LAB).

The variation in pH during storage of RTC idlibatter in commercial, as well as PP nanocomposite multi-layered film, showed nonlinear nature (2<sup>nd</sup> order polynomial equation). The relationship can be expressed with the eg. 5

$$x = at^2 - bt + c (5)$$

Where,

a, b and c = Constant t = Time

The values of a. b and c for commercially stored batter were 0.0056, 0.2214 and 6.6879 for 3:1 batter and the corresponding values of pH for 4:1 batter was respectively, 0.006, 0.2331 and 6.6752 with an R<sup>2</sup> of 0.99 for both the blend ratios. The constant values a, b and c for the batter stored in F were respectively, 0.0021, 0.1345 and 6.7113, and 0.0021, 0.1344 and 6.655 for blend ratios 3:1 and 4:1 with an R<sup>2</sup> value of 0.99. The pH of batter stored in commercial and PP nanocomposite multi-layered film packaging film could estimate from storage duration which is evident from the close to unity slope (1.00) and high R<sup>2</sup> (0.99) value of the straight line fitted between actual and estimated values.

The batter stored in the commercial package with both the blend ratios took 24 h to reach pH below 4.5 and spoiled at ambient temperature. The batter stored in PP nanocomposite multi-layered film with both the blend ratios showed the better range of pH in terms of fermentation time and it took 36 h to reach pH below 4.5 which was due to the higher barrier properties of PP nanocomposite multi-layered film.

During idli batter fermentation, LAB breaks down the available carbohydrates into aldehyde, ketone, acids and ethanol which increases yeast activity to increases the CO<sub>2</sub> gas concentration with lowering the pH and deteriorate the quality of stored batter [26,27,31, 32]. The LAB, namely, *Streptococcus faecalis and Pediococcus cerevisiae* mostly responsible for lowering the pH of stored batter [32]. The bacterium *S. faecalis* produces low acid concentration at the initial stage of fermentation while bacterium *P. cerevisiae* produces high acid at the end of fermentation period which helps to lower the pH of stored batter and spoil it [26,32].

#### 3.1.2 %TA of stored batter

The %TA of batter prepared with different blend ratios and stored in commercial and PP nanocomposite multi-layered film are calculated after every 3 h of intervals and results are shown in Fig. 2. The %TA of batter stored in PP nanocomposite multi-layered film varies from 0.43±0.16% to 0.86±0.03% for 3:1 batter and 0.43±0.18% to 0.90±0.02% for 4:1 batter. The corresponding values for batter stored in commercial film were respectively, 0.43±0.19% to 0.87±0.02% for 3:1 batter and 0.43±0.20% to 0.94±0.02% for 4:1 batter.

The difference between %TA concerning fermentation time of batter stored in commercial and PP nanocomposite multi-layered film showed non-linear (2<sup>nd</sup> order polynomial equation) curve (Fig. 2). The relationship can be expressed using eq. 5. The values of a, b and c for batter stored in the commercial film were -0.0006, 0.031 and 0.4516 for 3:1 batter and the corresponding values of %TA for 4:1 batter was respectively, -0.0007, 0.0351 and 0.4523 with an R<sup>2</sup> value of 0.98 for both the blend ratios of batter. The constant values a, b and c for PP nanocomposite multi-layered film stored batter were respectively. -0.0002, 0.0179 and 0.4609. and -0.0002, 0.0204 and 0.4659 for both the blend ratios with an R<sup>2</sup> value of 0.98. The straight line fitted between actual and estimated %TA values showed close to unity slope (1.02) with an R<sup>2</sup> value of 0.98 for batter stored in commercial and PP nanocomposite multi-layered film.

From Fig. 2 it was observed that the batter stored in commercial and PP nanocomposite multi-layered film with different blend ratios showed a gradual increase of % TA at the beginning stage of fermentation and it might be due to the presence of bacterium S. faecalis which produces low acid concentration at the beginning stage of fermentation. The % TA of stored batter increases with increases in the fermentation time and spoil the batter with increasing acidity content and similar results were also reported by Balasubramanian and Viswanathan [26] and Sridevi, et al. [27].

From the visual inspection, it was observed that the stored batter goes TA above 0.90% showed whey formation on the top layer of the batter which indicate the spoilage of batter. To reach TA above 0.90%, the batter stored in the commercial package with both the blend ratios took 21-24 h at ambient temperature and batter

stored in PP nanocomposite multi-layered film with both the blend ratios took 33-36 h at ambient temperature.

From Fig. 2 it was observed that the acidity of the stored batter has a direct relationship with pH of stored batter. According to Balasubramanian and Viswanathan [26] as the fermentation time increases the acidity of stored batter increases with decreasing pH of stored batter and it was due to the increasing growth of LAB (*P. cerevisiae*).

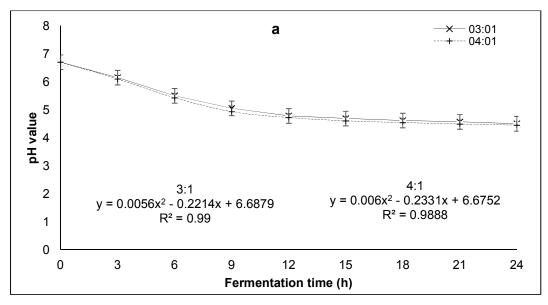
#### 3.1.3 Color value of stored idli batter

The colour ( $\Delta E$ ) value of commercial and PP nanocomposite multi-layered film stored batter with different blend ratios concerning different fermentation time are calculated using Eq. 2 and results are presented in Fig. 3. From Fig. 3 it is obvious that the  $\Delta E$  value for commercially packaged idli batter sample varies from 1.21±0.01 to 3.72±0.04 for 3:1 ratio and 1.28±0.07 to 4.11±0.10 for 4:1 ratio. The corresponding values for batter stored in PP nanocomposite multi-layered film were respectively, 0.94±0.01 to 4.54±0.01 for 3:1 ratio and 1.03±0.01 to 4.62±0.02 for 4:1 ratio.

The variation in  $\Delta E$  value of RTC batter stored in commercial and PP nanocomposite multi-layered film showed a non-linear curve (Supplementary Tables 1,2 and Fig. 3). The batter stored in commercial film and as well as PP nanocomposite multi-layered film follows 3<sup>rd</sup> order polynomial equation. The relationship between  $\Delta E$  concerning fermentation time can be expressed using eq. 6 for commercial and PP nanocomposite multi-layered stored batter (Supplementary Table 3).

$$x = at^3 - a_1t^2 - bt + c (6)$$

The constants values of a, a<sub>1</sub>, b and c for batter stored in the commercial film were -0.001, 0.0487, 0.5372 and 2.5648 for 3:1 batter and the corresponding values of  $\Delta E$  for 4:1 batter was respectively, -0.0012, 0.0571, 0.6013 and 2.7782 with an R2 of above 0.90 for both the blend ratios. The constant values a, a<sub>1</sub>, b and c for PP nanocomposite multi-layered stored batter were respectively, -0.0004, 0.0244, 0.2892 and 1.9107 and -0.0004, 0.0242, 0.2816 and 1.9444 for both the blend ratios with an R2 value of above 0.98. The straight line fitted between actual and estimated  $\Delta E$  values showed close to unity slope (1.0) with a high  $R^2$  value of above 0.90 for the commercially stored batter and 0.94 for PP nanocomposite multi-layered stored batter.



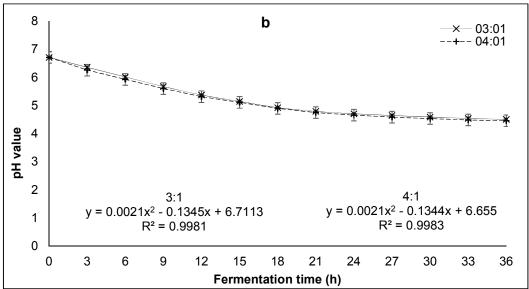
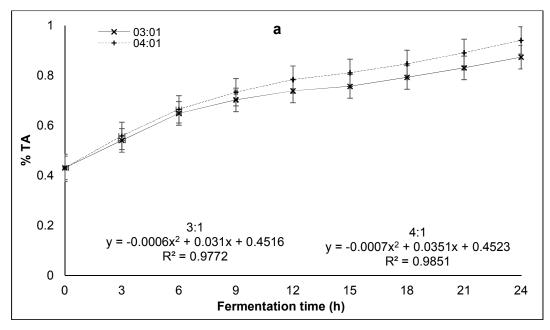


Fig. 1. pH value of different blend ratios (3:1 and 4:1) of idli batter stored in different packaging film (a: commercial film; b: PP nanocomposite multi-layered film)

The L\*, a\* and b\* value of commercial and PP nanocomposite multi-layered film stored batter with different blend ratios and fermentation time are summarized in Supplementary Tables 1 and 2. The commercial and PP nanocomposite multi-layered film stored batter with different blend ratios showed a decrease in L\* and increase in b\* value as fermentation time increases, which indicates a decrease in whiteness and increase in yellowness of stored batter and it might due to increasing in the LAB count of stored batter. The batter stored in PP nanocomposite multi-layered film with different blend ratios (3:1 and 4:1)

showed a steady decrease of L\* (white) value with steady increasing of b\* (yellow) value as compared to commercially stored batter with different blend ratios. From Fig. 3 it was observed that the commercially packaged batter with different blend ratios spoiled quickly at 24 h and batter stored in PP nanocomposite multilayered film with different blend ratios spoiled at 36 h due to its higher barrier properties. The batter stored in PP nanocomposite multi-layered film help to extend the shelf life of batter more than 12 h as compared to commercially stored batter at ambient temperature.



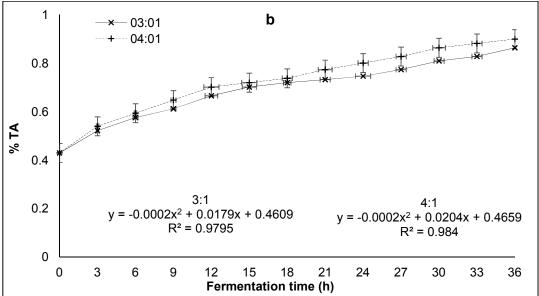


Fig. 2. % TA of different blend ratios (3:1 and 4:1) of idli batter stored in different packaging film (a: commercial film; b: PP nanocomposite multi-layered film)

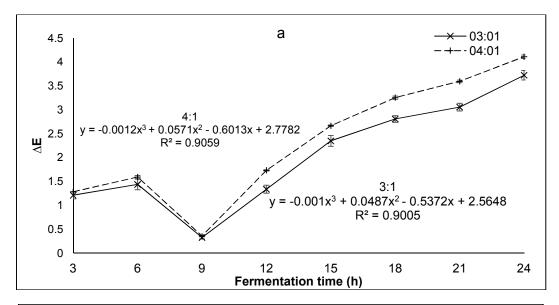
### 3.1.4 Viscosity of stored idli batter

The viscosity of commercial and PP nanocomposite multi-layered film stored batter with different blend ratios and fermentation time are measured at 100 rpm and results are represented in Fig. 4. The viscosity of commercially stored batter varies from 6369.87±166.81 to 2250.16±54.79 cp for 3:1 and 6336.05±202.42 to 2136.97±50.58 cp for 4:1 ratio. The corresponding values for PP

nanocomposite multi-layered film store batter were respectively, 6375.08±80.20 to 2556.17±228.17 cp for 3:1 and 6422.22±90.17 to 2117.34±123.25 for 4:1 ratio. The commercially stored batter with both the blend ratios showed lower in viscosity value as compared to PP nanocomposite multi-layered film stored batter with both the blend ratios due to increase in the fermentation period which makes the harder texture of the batter.

The difference in cP values of RTC batter stored in commercial and PP nanocomposite multi-layered film showed a non-linear curve in nature (Fig. 4). The relationship can be expressed using eq. 5 and 6. The constants values of a, b and c for commercially stored batter were 1.4905, 218.34 and 6523.3 for 3:1 batter and the corresponding values of cP for 4:1 batter was respectively, 5.5524, 307.99 and 6408.6 with an R<sup>2</sup> of 0.99 for both the blend ratios. The constant values a, a<sub>1</sub>, b and c for PP nanocomposite

multi-layered film stored batter were respectively, -0.2384, 9.419, 137.87 and 6299.2 for 3:1 blend ratio with an  $\mbox{R}^2$  value of 0.99. The constant values a, b and c for 4:1 batter stored in PP nanocomposite multi-layered film were 0.9897, 152.25 and 6422.6 with an  $\mbox{R}^2$  value of 0.99. The straight line fitted between actual and estimated cP showed close to unity slope (0.99) with a high  $\mbox{R}^2$  value of 0.99 for commercial and PP nanocomposite multi-layered film stored batter.



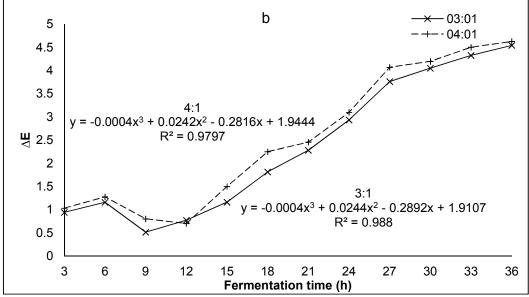


Fig. 3.  $\Delta$  E value of different blend ratios (3:1 and 4:1) of idli batter stored in different packaging film (a: commercial film; b: PP nanocomposite multi-layered film)

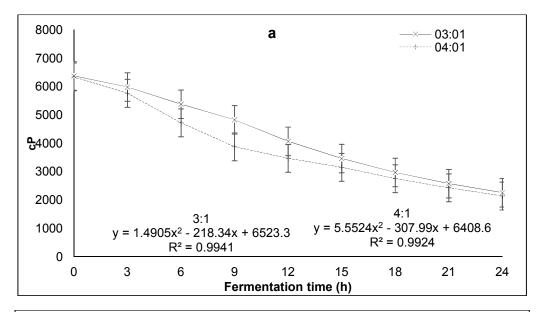
Table 1. TPA mean values of prepared idli cake with different blend ratios and fermentation time for all the responses using SPSS

Ratios	Hours	Hardness (Force N)	Adhesiveness (N s)	Springiness	Cohesiveness	Chewiness	Resilience
3:1	12	22.20±1.41 <sup>e</sup>	-0.05±0.005 <sup>a</sup>	0.924±0.003 <sup>a</sup>	0.769±0.005 <sup>d</sup>	1119.90±4.44 <sup>e</sup>	0.475±0.004 <sup>e</sup>
	18	43.51±1.83 <sup>d</sup>	-0.19±0.007 <sup>b</sup>	0.934±0.003 <sup>cd</sup>	0.775±0.004 <sup>d</sup>	1291.11±4.31 <sup>d</sup>	0.497±0.004 <sup>d</sup>
	24	85.39±4.38 <sup>c</sup>	-0.64±0.005 <sup>c</sup>	0.953±0.021 <sup>bc</sup>	0.795±0.005 <sup>c</sup>	1383.75±4.00 <sup>c</sup>	0.528±0.004 <sup>c</sup>
	30	134.57±3.43 <sup>b</sup>	-1.14±0.003 <sup>d</sup>	0.959±0.004 <sup>ab</sup>	0.837±0.006 <sup>b</sup>	1588.97±4.52 <sup>b</sup>	0.556±0.003 <sup>b</sup>
	36	175.33±3.49 <sup>a</sup>	-2.16±0.006 <sup>e</sup>	0.981±0.003 <sup>a</sup>	$0.859 \pm 0.005^{a}$	1794.49±3.85 <sup>a</sup>	0.588±0.005 <sup>a</sup>
4:1	12	23.53±1.58 <sup>e</sup>	-0.06±0.003 <sup>a</sup>	0.931±0.004 <sup>d</sup>	0.787±0.004 <sup>e</sup>	1209.68±4.85 <sup>e</sup>	0.486±0.003 <sup>e</sup>
	18	45.12±2.34 <sup>d</sup>	-0.21±0.005 <sup>b</sup>	0.944±0.002 <sup>d</sup>	0.813±0.007 <sup>d</sup>	1386.12±2.91 <sup>d</sup>	0.510±0.004 <sup>d</sup>
	24	91.14±4.50 <sup>c</sup>	-0.66±0.004 <sup>c</sup>	0.964±0.003 <sup>c</sup>	0.832±0.004 <sup>c</sup>	1459.42±4.63 <sup>c</sup>	0.528±0.003 <sup>c</sup>
	30	145.68±3.94 <sup>b</sup>	-1.16±0.006 <sup>a</sup>	0.979±0.004 <sup>b</sup>	0.854±0.004 <sup>b</sup>	1679.05±4.92 <sup>b</sup>	0.585±0.004 <sup>b</sup>
	36	195.51±4.10 <sup>a</sup>	-2.26±0.001 <sup>e</sup>	0.995±0.004 <sup>a</sup>	0.875±0.003 <sup>a</sup>	1879.17±2.62 <sup>a</sup>	0.618±0.006 <sup>a</sup>
Total mean		95.59	-0.8545	0.9664	0.8164	1475.92	0.5353

\*Different letters are significantly different

Table 2. ANOVA for TPA analysis of prepared idli cakes with different blend ratios

Response	3:1				4:1					
-	Sum of Squares	df	F	Sig.	R <sup>2</sup>	Sum of Squares	df	F	Sig.	R <sup>2</sup>
Hardness	106189.69	17.00	2156.34	0.00	99.89	128425.99	17.00	1763.60	0.00	99.85
Adhesiveness	20.56	17.00	123336.67	0.00	99.74	19.10	17.00	114619.20	0.00	99.65
Springiness	0.01	17.00	19.12	0.00	98.79	0.02	17.00	105.32	0.00	98.77
Cohesiveness	0.03	17.00	169.77	0.00	98.61	0.02	17.00	141.60	0.00	98.33
Chewiness	1411750.86	17.00	14805.67	0.00	99.98	1341024.52	17.00	15806.06	0.00	99.98
Resilience	0.05	17.00	364.80	0.00	99.35	0.07	17.00	592.00	0.00	99.60



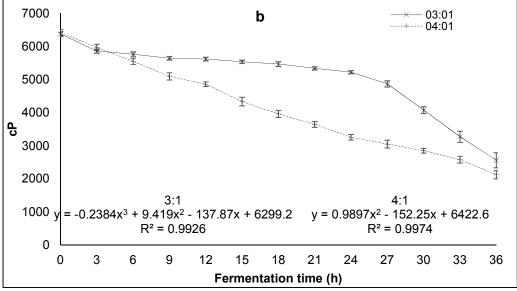


Fig. 4. Viscosity of different blend ratios (3:1 and 4:1) of idli batter stored in different packaging film (a: commercial film; b: PP nanocomposite multi-layered film)

From Fig. 4 it was observed that the batter stored in commercial and PP nanocomposite multi-layered film showed a decreasing trend of viscosity as the increase in the fermentation time and it was might be due to increasing growth of LAB which makes batter harder to spoil it. The viscosity of commercially packaged batter with different blend ratios started decreasing after the 12 h of the fermentation and batter stored in PP nanocomposite multi-layered film showed a steady decline of viscosity which was due to its higher barrier properties and helps to extend the shelf life of packaged batter.

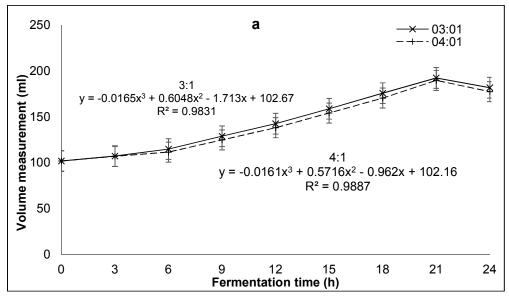
## 3.1.5 Volume measurement of stored idli batter

The changes in volume measurement of stored batter in commercial and PP nanocomposite multi-layered film are shown in Fig. 5. The values of volume measurement of batter stored in commercial package range from 101.67±1.53 ml to 181.67±3.21 ml for 3:1 batter and 4:1 batter it ranges from 101.67±1.53 ml to 177.33±3.21 ml. The corresponding values of volume measurement for PP nanocomposite multi-layered film stored batter were respectively,

 $101.67\pm1.53$  ml to  $180.33\pm1.52$  ml for 3:1 batter and  $101.67\pm1.53$  ml to  $176.33\pm2.52$  ml for 4:1 batter.

The difference in volume measurement (ml) values of RTC batter stored in commercial and PP nanocomposite multi-layered film showed non-linear (3<sup>rd</sup> order polynomial equation) curve (Fig. 5). The relationship can be expressed using eq. 6. The constants values of a, a<sub>1</sub>, b and c for commercially stored batter were -0.0165, 0.6048, 1.713 and 102.67 for 3:1 batter and the corresponding values of volume measurement

for 4:1 batter were respectively, -0.0161, 0.5716, 0.962 and 102.16 with an  $R^2$  of 0.99 for both the blend ratios. The constant values a, a<sub>1</sub>, b and c for PP nanocomposite multi-layered film stored batter were respectively, -0.0071, 0.4236, 3.842 and 104.61, and -0.0061, 0.3805, 3.6309 and 104.67 for both the blend ratios with an R2 value of above 0.99. The straight line fitted estimated between actual and measurement (ml) showed close to unity slope (0.99) with a high R<sup>2</sup> value of above 0.98 for commercial and PP nanocomposite multi-layered film stored batter.



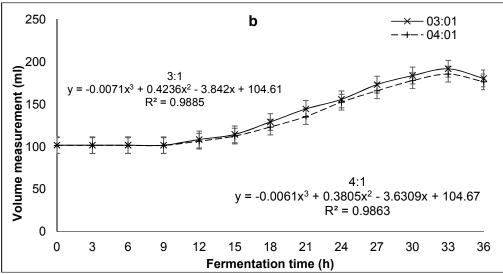


Fig. 5. Rate of change in volume of different blend ratios (3:1 and 4:1) of idli batter stored in different packaging film (a: commercial film; b: PP nanocomposite multi-layered film)

From Fig. 5 it was observed that the volume of commercially stored batter with different blend ratios started increasing after the 6 h and increases up to 21 h and after that, it started decreasing up to 24 h at ambient temperature. Similarly, the volume of PP nanocomposite multilayered film stored batter with different blend ratios started increasing after 9 h and it increases gradually up to 33 h, later it found to be decreased up to 36 h at ambient temperature. The decrease in the volume measurement which indicates the end of the fermentation period and it was due to the increase in the acidity content of the stored batter. The batter stored in PP nanocomposite multi-layered film showed a gradual increase of batter volume due to higher barrier properties of the film which multiply the LAB at a slow rate up to optimum count.

## 3.2 Quality Evaluation and Physicochemical Properties of Prepared Idli Cake

From the above result, it was observed that the batter stored in PP nanocomposite multi-layered film showed better results and help to extend the shelf life of packaged batter more than 12 h as compared to commercially stored batter at ambient condition. Hence, it was decided that the physicochemical analysis of prepared idli cake with different blend ratios was carried out using batter stored in PP nanocomposite multi-layered film.

Generally, in India the household vendors allow the batter to ferment for overnight (12 h) and poured into idli moulds to steam for 15 min to achieve fluffy idli cakes. Considering this into the mind we allowed batter to ferment at least for 12 h and after every 6 h of intervals (18, 24, 30 and 36 h) up to the spoilage of batter the physicochemical analysis, namely, colour value, textural properties and pore per cent of prepared idli cakes were analyzed as followed.

## 3.2.1 Colour (△E) of prepared idli cake

The plot of  $\Delta E$  of cooked idli as compared to control, i.e., idli cook from 12 h stored batter in PP nanocomposite multi-layered film with storage duration is shown in Fig. 6. The  $\Delta E$  value of idli cake ranges from 0.25±0.05 to 2.90±0.06 and 0.31±0.07 to 3.30±0.0.08 respectively, for the batter prepared with a blend ratio of 3:1 and 4:1.

The difference in  $\Delta E$  values of prepared idli cakes stored in PP nanocomposite multi-layered

film with both the blend ratios showed non-linear ( $3^{rd}$  order polynomial equation) curve shown in Fig. 6. The relationship can be expressed using eq. 6. The constants values of a, a<sub>1</sub>, b and c for commercially stored batter were respectively, -0.0009, 0.0625, 1.1783 and 6.9343, and -0.0004, 0.0232, 0.257 and 0.6879 for both the blend ratios with an  $R^2$  value of above 0.98. The straight line fitted between actual and estimated  $\Delta E$  values of prepared idli cakes and it showed close to unity slope with an  $R^2$  value of above 0.98 for both the blend ratios.

The prepared idli cakes with different blend ratios showed an increasing trend of  $\Delta E$  value with increasing fermentation time (Fig. 6). The idli cake prepared with 3:1 blend ratio of stored batter showed lower in  $\Delta E$  value as compared to idli cake prepared with 4:1 blend ratio of stored batter which was due to different blend ratios of rice to black gram used for the preparation of batter.

According to Nisha, et al. [7] the L\*, a\* and b\* value of prepared idli cake should be within a range of L\* value 75 and above, a\* value below 0 and b\* value below 13. The L\*, a\* and b\* value of prepared idli cakes with both the blend ratio of rice to the black gram with different time intervals are shown Supplementary Table 3. The idli cake prepared after 36 h of fermentation with both the blend ratios showed a decrease in L\* (white) value and increase in and b\* (yellow) which indicates the spoilage of prepared idli cake and it might be due to the growth of LAB to spoil the idli batter/cake.

## 3.2.2 Texture Profile Analysis (TPA) of prepared idli

The texture of idli, namely, hardness, firmness, sponginess and stickiness are mainly affected by the starch content present in the idli batter [2]. The values of all the responses of TPA for idli cake prepared with both the blend ratios and different time intervals are summarized in Table 1 and results of ANOVA for all the responses are shown in Table 2.

The hardness of prepared idli cakes with different blend ratios (3:1 and 4:1) was calculated in force (N) required to compress the prepared idli cakes using TPA are summarized in Table 1. The hardness value of prepared idli cakes varied from 22.20±1.41 N to 175.33±3.49 N for 3:1 ratio and 23.53±1.58 N to 195.51±4.10 N for 4:1 ratio. The maximum value for the hardness of prepared idli cakes with both the

blend ratios was observed at 36 h which indicate the spoilage of prepared idli cakes. The prepared idli cakes with 3:1 blend ratio required less force to compress prepared idli cakes as compared to 4:1 blend ratio. The variations in the force to compress idli cakes were due to the different blend ratios and fermentation time of idli batter and similar results were also confirmed by Durgadevi and Shetty [2].

From Table 1 it is obvious that the hardness of prepared idli cakes with both the blend ratios with different time intervals showed an increasing trend of force to compress the prepared idli cakes which indicate the harder texture of idli cakes. The total mean value for the hardness for both the blend ratios was found to be 95.59 (N).

The adhesiveness of prepared idli cakes with different blend ratios and fermentation time measured using a negative peak in the TPA graph and mean values for the adhesiveness are given in Table 1. The adhesiveness values for prepared idli cakes range from -0.05±0.005 to -2.16±0.006 for 3:1 blend ratio and -0.06±0.003 to -2.26±0.001 for 4:1 blend ratio. The prepared idli with both the blend ratios showed lower in (Table adhesiveness The lowest 1). adhesiveness value of prepared idli cakes was observed at 36 h for both the blend ratio of prepared idli cakes and maximum adhesiveness observed at 12 h in both the blend ratios of prepared idli cakes (Table 1). According to Durgadevi and Shetty [2] and Ghasemi, et al. [33] the higher the value of adhesiveness indicates the more stickiness in prepared idli cakes. It is obvious from Table 1 the lower value of adhesiveness showed a harder texture of prepared idli cakes which indicates the spoilage of idli cakes.

The springiness of prepared idli cakes with different blend ratios showed an increasing trend with increasing fermentation time and results are presented in Table 1. The springiness value of prepared idli cakes ranges from 0.931±0.004 to 0.995±0.004 for 4:1 blend ratio and 0.924±0.003 to 0.981±0.003 for 3:1 blend ratio. The idli cakes prepared with 4:1 blend ratio showed higher springiness as compared to idli cakes prepared with 3:1 blend ratio. The highest springiness value was observed at 36 h and lower springiness was observed at 12 h in both the blend ratios of prepared idli cakes (Table 1). According to Durgadevi and Shetty [2], the springiness of idli cakes are depends on the amount of black gram dhal used for the preparation of idli batter.

The cohesiveness value of prepared idli cakes with different blend ratios and fermentation time are tabulated in Table 1. The cohesiveness value of prepared idli cakes varied from 0.769±0.005 to 0.859±0.005 for 3:1 blend ratio and 0.787±0.004

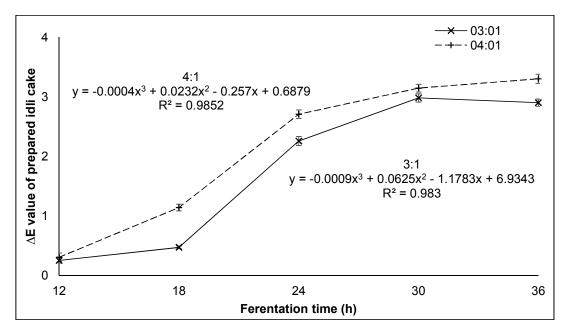
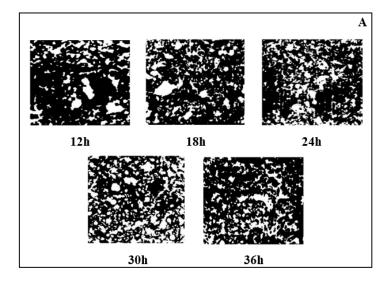


Fig. 6.  $\triangle$  E value of different blend ratios (3:1 and 4:1) with respect to different fermentation time for prepared idli cakes

Table 3. Pore percentage of prepared idli cake with different blend ratios and fermentation time

Ratio of idli cake	Time (h)	Number of pixel / in <sup>2</sup> in non-pores area (A)	Number of pixel / in <sup>2</sup> in pores area (B)	% pores /in² in total area (B/(A+B)*100)
4:1	12	706338	197807	21.88
	18	884147	299470	25.30
	24	1218195	491686	28.76
	30	1173531	683454	36.80
	36	722325	235412	24.58
3:1	12	649384	142561	18.00
	18	724465	231752	24.24
	24	822221	322516	28.17
	30	780345	384080	32.98
	36	914937	232040	20.23



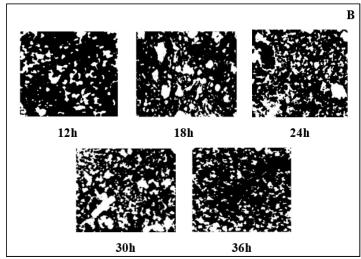


Fig. 7. Grayscale image of prepared idli cakes with different blend ratios and time intervals, A, 3:1 and B, 4:1 ratio of idli cake

to 0.875±0.003 for 4:1 blend ratio. The prepared idli cakes with different blend ratios showed an

increasing trend of cohesiveness with increasing fermentation time. The maximum cohesiveness

value was observed at 36 h and minimum cohesiveness value observed at 12 h in both the blend ratios of prepared idli cakes (Table 1). The cohesiveness of idli showed positive force area in the second compression to that of the first compression and similar results were also observed by Durgadevi and Shetty [2].

The chewiness of prepared idli cakes with different blend ratios and fermentation time showed increasing trend are shown in Table 1. The chewiness values for prepared idli cakes range from 1119.90±4.44 to 1794.49±3.85 for 3:1 blend ratio and 1209.68±4.85 to 1879.17±2.62 for 4:1 blend ratio. The idli cakes prepared with both the blend ratio at 12 h showed minimum chewiness value. According to Durgadevi and Shetty [2] the lower value of chewiness indicates the softness of prepared idli cakes.

The resilience of idli cake prepared with different blend ratios showed an increasing trend with increasing fermentation time are shown in Table 1. The resilience of prepared idli was measured in terms of speed and force recovers from the deformation of idli cakes. The value of resilience varied from 0.475±0.004 to 0.588±0.005 for 3:1 blend ratio and 0.486±0.003 to 0.618±0.006 for 4:1 blend ratio. The idli cakes prepared with 3:1 blend ratio showed lower in resilience value as compared to 4:1 blend ratio. The minimum resilience value for prepared idli cake was observed at 12 h and maximum resilience value observed at 36 h for both the blend ratios.

## 3.2.3 Estimation of pore per cent of prepared idli cakes

The ink print test performed to calculate the per cent of pores present in per square inch of prepared idli cakes with different blend ratios during storage from 12 h onwards are shown in Table 3. The softness of idli can also be judged by calculating percent of pores present in per square inch of prepared idli cakes. The % pore value for prepared idli cakes ranged from 21.88 to 36.82 %/in² area for 3:1 blend ratio and 4:1 blend ratio is varied from 18 to 32.98 %/in² area. It is obvious from Table 3 that the idli cakes prepared with 3:1 blend ratio showed higher numbers of percent pore as compare to 4:1 blend ratio and it was due to the different blend ratios used for the preparation of batter.

The grayscale image of prepared idli cakes with different blend ratios and fermentation time are shown in Fig. 7. From Fig. 7 it was observed that the increases in the fermentation time which

resulted from the increase in the percent pore formation up to 30 h and later it was found to be decreased as fermentation period gets over (36 h) and reaches close to an initial value of percent pore formation. The percent pore formation was higher at 30 h in both the blend ratios (36.80 and 32.98 %/in<sup>2</sup> area for 3:1 and 4:1 blend ratio) of prepared idli cakes. The idli cake prepared with 36 h of fermentation showed a decrease in percent pore which was due to the collapse in air bubbles and it might be due to an increase in the acidity content. According to Balasubramanian and Viswanathan [26] the presence of bacterium P. cerevisiae which reduces CO2 concentration and produces more acidity to affect the final texture of idli cakes. From Table 3 it was observed that the idli prepared with 3:1 blend ratio of rice to the black gram produces fluffier idli cakes compared to the 4:1 blend ratio.

### 4. CONCLUSION

This study was carried out to study the effect of PP nanocomposite multi-layered film on the quality of stored batter and prepared idli cake with different fermentation time. The developed PP nanocomposite multi-layered film showed better barrier properties as compared to commercial package and help to extend the shelf life of stored batter more than 30 h at ambient condition. The physicochemical properties of idli batter, namely, pH, %TA,  $\Delta E$ , viscosity and volume measurement was found to acceptable with batter stored in nanocomposite multi-layered packaging film. The textural characteristics of prepared idli cake with 3:1 blend ratio of rice to the black gram showed better results as compared to the 4:1 blend ratio. The ink print test showed that 4:1 blend ratio of rice to black gram has a lower pore formation as compared to 3:1 blend ratio and it was due to the different properties of rice to the black gram used for the preparation of idli batter. The present work suggests that the developed PP nanocomposite multi-layered film help slow down the rate of microbes and extend the shelf life of RTC packaged idli batter at ambient condition.

### **DISCLAIMER**

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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