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## Development and quality evaluation of retort processed ready-to-drink blended vegan milk

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### Abstract

Sterilization of a food product ensures maximum sterility along with extended shelf life. Retort processing is a technique that employs sterilization after the product is packed in flexible pouches or cans thus avoiding contamination during storage. In this study a blend of coconut and foxtail millet milk was retorted with different processing temperature and its quality was evaluated during storage. The raw materials were selected based on their nutrient profile. The plant-based beverage was processed at 75°C, 85°C, and 95°C for 15 minutes. The control sample packed in a retort pouch without processing and stored in normal atmospheric condition. The samples processed at a higher temperature maintained maximum sterility while bacterial growth was observed from day 28 in samples processed at lower temperatures. The color value of sample processed at 85°C implied minimal changes with L\* (75.54 ± 0.33), a\* (-0.61 ± 0.02) and b\* (12.70 ± 0.24). Among the treatments, the best result was observed in the sample processed at 85°C as it retained maximum nutrient and commercial sterility without much thermal quality degradation.

**Keywords:** Retort processing, vegan milk, quality, shelf-life extension, thermal treatment, functional food

### 1. Introduction

The consumption of vegan products has gained momentum in recent years due to an increase in the consumption choice of health benefitting functional foods among the consumers. There is a growing demand of Ready-to-Drink products from the consumer end. The market for such food products is also increasing due to the changing lifestyle patterns of the consumers. Ready-to-Drink (RTD) and Ready-to-Eat (RTE) foods are also preferred due to the busy working life of a majority of the population. The market for vegan milk is gaining popularity in many developed countries [1]. Plant-based milk resembles dairy-based milk in appearance but is extracted fine particle suspension of chosen raw material [2].

Coconut is a tropical plant largely grown in tropical and sub-tropical regions [3]. It is a member of the Palmaceae family and is widely used in a majority of Asian and Thai dishes due to its unique flavor and nutrient content [4]. It is often regarded as a “tree of life” owing to the health benefits acquired on its consumption [3, 5]. Coconut milk is obtained by mechanically pressing tender grated coconut meat. Coconut milk is rich in polyunsaturated fatty acids (PUFA) [4] while foxtail millet is rich in protein profile (12.3%) [6]. Foxtail millet is rich in protein and helps in the treatment of blood pressure reduction, depletion of fat accumulation when consumed with daily routine meals [7]. It belongs to the Poaceae family. Foxtail millet milk was obtained by grinding overnight soaked millets. Beverage from millets is obtained by grinding soaked millets with water at desired proportion and filtering through a fine mesh. The extracts obtained from millets are rich in nutrition profile thus proving to be a better substitute for carbonated or sugar-rich beverages.

The major disadvantage observed in vegan milk is its reduced shelf life. The milk gets spoiled easily when left in the open within few hours of extraction. Thus, processing becomes a major step in the preservation and commercial production of vegan milk. UHT treatment and retort processing are majorly used thermal processing techniques. Since retorting can be done in flexible metalized pouches, the processing remains comparatively less expensive [8]. Retorting is a thermal processing technique that reduces the presence of spoilage organisms as sterilization is done after packing the food products in flexible retort pouches thus aiding in the extension of shelf-life as human intervention is avoided. Retort pouches exhibit a greater heat transfer are compared to metal cans during thermal processing thus reducing the time required for processing and preserving the nutrients.

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With the growing millet-based food market in India, the study was focused on millet-based beverage with high nutritional value was chosen for thermal processing using a still retort. Coconut and foxtail millet were chosen as the raw material for the preparation of RTD beverages owing to their respective nutritional properties.

In the present study, retort processing is opted to extend the shelf life of prepared blended vegan milk. The present study aims to develop and evaluate retort processed blended vegan milk in retort pouches. The process is carried out at three different temperatures to evaluate the quality and nutritional changes.

## 2. Materials and Methods

### 2.1 Procurement of raw materials

The coconut and semi-polished foxtail millet required for the study was bought from a local market in Thanjavur, Tamil Nadu. Retort sterilization chamber and metalized pouches were purchased from M/s. Lakshmi Engineering Works, Ambattur, Chennai. Analytical chemicals for analytical analysis were purchased from M/s. Suresh Scientific Company, Trichy, Tamil Nadu.

### 2.2 Method of preparation of Blended Vegan milk

The samples for retort processing were prepared by blending coconut and millet milk. Coconut milk was obtained by filtering grated coconut meat blended with warm water (1:3) using a Philips food processor through a double-layered muslin cloth. Foxtail millet was soaked in warm water (32°C) overnight after which the soaked millets were washed thoroughly in running tap water. Millet milk was obtained by grinding the soaked millets with water in the ratio of 1:4 in a colloidal ball mill for 20 mins. The extract was filtered to obtain millet milk. The water to product ratio was optimized based on reduced physical separation of the constituent particles during preliminary trials. Sucrose was added to the blended beverage at 10% to get required sweetness and act as a binding agent to reduce gravitational separation. After the addition of sucrose, the beverage was homogenized at 14000 rpm using an IKA T-18, Germany Digital Ultra Turrax homogenizer for 15 mins. The samples were heated to 60°C for 10 mins before filling into retort pouches.

### 2.3 Filling and sealing

100mL of blend vegan milk was filled in multilayered retort pouch (120 µm) and sealed using an EPCOMB FR-900 continuous band sealing machine. Before sealing, the air trapped inside the pouch was manually released to avoid the bursting of pouches during retort processing. Hot filling of samples into pouches was carried out to reduce microbial contamination from the ambient atmosphere.

### 2.4 Thermal processing

The retort chamber used for the study was a pilot-scale steam-air still overpressure chamber (Fig 1). The retort chamber can maintain a maximum temperature of 121°C and withstand 3.5 bar pressure. The chamber is insulated to reduce heat transfer by surroundings. The chamber consists of 8 RTD thermocouples to check product temperature at each tray and a retort thermocouple placed inside in contact with water at the bottom of pressure vessel to evaluate the temperature of the chamber. The capacity of the chamber is 25-30 pouches per cycle. The packed samples were retort sterilized at three different temperatures, namely, 75°C, 85°C, and 95°C for 15

minutes. The chamber was pre-heated to 50 °C after which sterilization temperature and pressure were maintained at the desired level. After completion of sterilization, the pouches were cooled to 45 °C by a fine mist of cold-water spray. A heat-penetration study was carried out by placing a thermocouple in a reference pouch in contact with the core region. The processing time was calculated by the method followed by [9]. The time and temperature profiles were recorded in real-time by Delta data logger in-built in the retort sterilizing unit. After processing, F<sub>0</sub> values were calculated using MS Excel software. Thermally processed pouches were examined for sterility and quality during storage period.

### 2.5 pH, TSS, and titratable acidity

The pH of the sample was measured using Wensar 5-point calibration electronic pH meter. The pH meter was first calibrated using pH 4, 7, and 10 standard solutions. 10 mL of sample was taken into which the electrode was inserted to record the pH value [10]. The TSS was measured using an Atago PAL-1 series hand-held pocket refractometer. The refractometer was first calibrated with distilled water to obtain zero Brix value after which 2 mL of sample was placed in the sample well to obtain TSS value [11]. The titratable acidity of the samples was measured by titrating 10 mL of the sample against 0.1N sodium hydroxide solution using 300 µL phenolphthalein as an indicator. The appearance of pale pink color was observed to be the endpoint and acidity was measured using the following calculation given by [12]:

$$\text{Titratable acidity (\%)} = \frac{V * N * M * 100}{S} \quad (\text{Equation 2.1})$$

#### Where

V = volume of NaOH consumes on titration, in mL  
N = normality of prepared NaOH solution, usually 0.1N  
M = molecular equivalent of NaOH, and  
S = volume of the sample taken, in mL

### 2.6 Color

The color values of the samples were measured using Hunter Colour flex EZ colorimeter. The colorimeter was standardized by placing black tile and white tile at the sample port before placing the sample [13]. 50 mL of the sample was transferred to the cup which was then placed at the sample port and covered with a black cup. L\*, a\*, and b\* color values were noted during storage period.

### 2.7 Proximate analysis

The blended vegan milk samples were analyzed for moisture content, fat, protein, ash, carbohydrate, and energy value. The protein, carbohydrate, moisture and ash content of the samples were determined by the method suggested by [13]. Free fat analysis was performed according to the method suggested by [14] where the fat present in the sample was extracted using petroleum ether by a separating funnel. The extracted fat is dried to determine the amount present in the sample. The carbohydrate and energy value were determined from the formula,

$$\text{Carbohydrate} = 100 - (\text{Protein} + \text{Fat} + \text{Moisture} + \text{Ash}) \quad (\text{Equation 2.2})$$

$$\text{Energy (Kcal)} = 4 * (\text{Protein}) + 4 * (\text{Carbohydrate}) + 9 * (\text{Fat}) \quad (\text{Equation 2.3})$$

## 2.8 Microbial analysis

The samples were analyzed microbiologically to check commercial sterility and evaluate the shelf life during storage. Bacterial colonies were isolated using plate count agar while yeast and molds were isolated using potato dextrose agar. The samples were serially diluted before plating. The spread plate method was followed for microbial analysis. The plates were incubated at 37°C for 24h for bacterial culture and 25°C for 3-5 days for yeast culture. Colony calculation and enumeration was performed by the method stated by [15].

## 2.9 Statistical analysis

The results obtained during the experiment were statistically analyzed and are presented in the format mean  $\pm$  standard deviation. All analyses were done in triplicates and average was considered as results. Analysis was performed with IBM SPSS 20.0 software on a Windows computer. Duncan's multiple range test was carried out to evaluate the statistical significance.

## 3. Results and Discussions

Thermal processing aids in ensuring the microbial safety of food products with reduced quality degradation [9]. It was thus selected as a processing condition for blended vegan milk as human interaction with the product after processing is avoided. The retort temperatures were maintained in the lower level as retort at higher temperatures causes instability and coagulation of the coconut milk as reported by [8]. It was observed that retorting at higher temperatures caused gelatinization of blended millet and coconut milk samples. Thus, retort temperature was fixed at lower range. The  $F_0$  values obtained were 1.34 min, 0.039 min, and 0.011 min at 95°C, 85°C, and 75°C for 15 min respectively. The values were enumerated by plotting logged T-t values on MS Excel. The  $F_0$  value was obtained from lethality graph. The greater the  $F_0$  value, the higher is the sterility of products processed. After retort processing, the pouches were cooled down using water spray inside the chamber. The pouches were cleaned, dried, labeled, and stored at ambient conditions for storage studies.

### 3.1 Effect of retort processing on pH, TSS and titratable acidity

pH is used to evaluate the onset microbial occurrence in food samples [16]. The lower values of pH, suggests the onset of microbial growth. The pH of samples decreased from  $6.18 \pm 0.01$  to  $5.98 \pm 0.01$ ,  $6.33 \pm 0.02$  to  $5.97 \pm 0.04$ , and  $6.56 \pm 0.01$  to  $5.99 \pm 0.02$  at 75, 85, and 95°C respectively. The change in pH values was perceived as an indicator of the onset of microbial spoilage. A similar trend was observed in pure coconut milk that was pasteurized and stored for 21 days [10]. A slight decrease in TSS is observed in storage. As the processed samples were stored in aluminum laminate pouches, there was no reduction in the solid content. Due to processing at various temperatures, the TSS of samples increased. The titratable acidity predicts the acid formation in the sample due to the growth of spoilage microorganisms. Table 1 shows that the values of titratable acidity of samples processed at 75°C, 85°C, and 95°C increases with an increase in storage time. The lowering of pH and increase in titratable acidity relates to the onset of fermentation. As acidity increases, the lactic acid bacteria feeds on soluble sugars thereby reducing the TSS value [17]. [18] Studied the storage period of retorted chennapoda where the acidity of the sample

was found to increase following a similar trend to retorted vegan beverage.

### 3.2 Effect of retort processing on the color of the samples

Color is an essential property to be maintained for consumer acceptance [19]. It can be observed from Table 1 that the  $L^*$  value of the samples increases,  $a^*$  value increases, and  $b^*$  decreases on storage. The initial differences in color values are observed due to the effect of processing temperatures. At higher temperatures, the products turn darker. In the present study, the samples exhibited similar L values as the process temperatures were maintained with a 10°C difference. A similar result was obtained in [20] where oat milk was pasteurized thermally and non-thermally to study the influence of processing on storage. The values corresponding to  $a^*$  reduce on storage and also due to process temperature, which implies a tinge of redness development in the samples.  $b^*$  values decrease with increase in the storage study period. The decrease in  $b^*$  value is due to initiation of Maillard reaction of protein compounds during processing. The change in color value might be attributed to gelatinization of starch present in samples during processing [21]. Table 1 shows the effect of processing on the color degradation of samples.

### 3.3 Effect of retort processing on proximate analysis

The amount of protein, ash, carbohydrate, and moisture was determined by the method followed by [22]. Figure 2 shows the effect of temperature on the nutrient profile of samples. It can be observed from the graph that protein degradation occurs on storage. The protein values decrease steadily with an increase in the storage period. A similar trend is observed in ash and carbohydrate. Ash content is greatly influenced by the mineral and starch compounds present. As the carbohydrate content decreases and protein degradation occurs, the ash content also reduces. During storage, hydrolytic rancidity initiates the breakdown of long-chain fatty acids into smaller units [9]. The free fat analysis proved that free fat present in samples increases with an increase in storage period. The free fat and peroxide content of the dairy products was found to increase with an increase in shelf life [18]. [23] Reported an increase in free fat content during the storage of coconut milk. Since the samples are stored in retort pouches, moisture transfer to the outside environment is reduced. There is not much significant difference in the moisture content of vegan beverage samples. The energy value increases due to an increase in free fat content in the sample. The fat content in vegan milk greatly influences the energy value [23]. Observed similar results on storing coconut milk after thermal processing. In a study conducted by [24], the changes in nutritional properties of RTE foxtail halwa were observed. It was reported that free fat content increases during storage due to fat hydrolysis at retort temperatures which leads to the breakdown of unsaturated fat linkages into monomer units. The flavor profile of samples was maintained throughout the storage period as retort pouch laminate retains maximum flavor without imparting off-odors as discussed by [25].

### 3.4 Effect of retort processing on microbial growth

The colonies were calculated according to the procedure given by [15]. Sample was serially diluted before plating. Plates were incubated in replicates of various dilutions under study. It can be understood from Table 2 that with an increase in processing temperature, the occurrence of bacterial and fungi colonies is reduced to a much greater extent. Retort

processing provides a highly sterile product with an extended shelf-life. The control sample started developing microbial growth from day 7 and gas formation of the pouch was observed from day 17 onwards. The processed samples did not show any gas formation even after 35 days of storage. The initiation of bacterial and mold growth was observed in sample processed at 75°C from day 21 onwards. In samples processed at 85°C, initial growth was observed from day 28. The increase in bacterial growth can be observed during the storage period. The processing of vegan milk samples at 95°C resulted in no growth of yeast and molds while an initial growth of bacterial colony was identified. Maximum mold growth was observed in samples having pH in the range 5-6 in processing of coconut milk study conducted by [10].



Fig 1: Retort sterilization chamber used under study

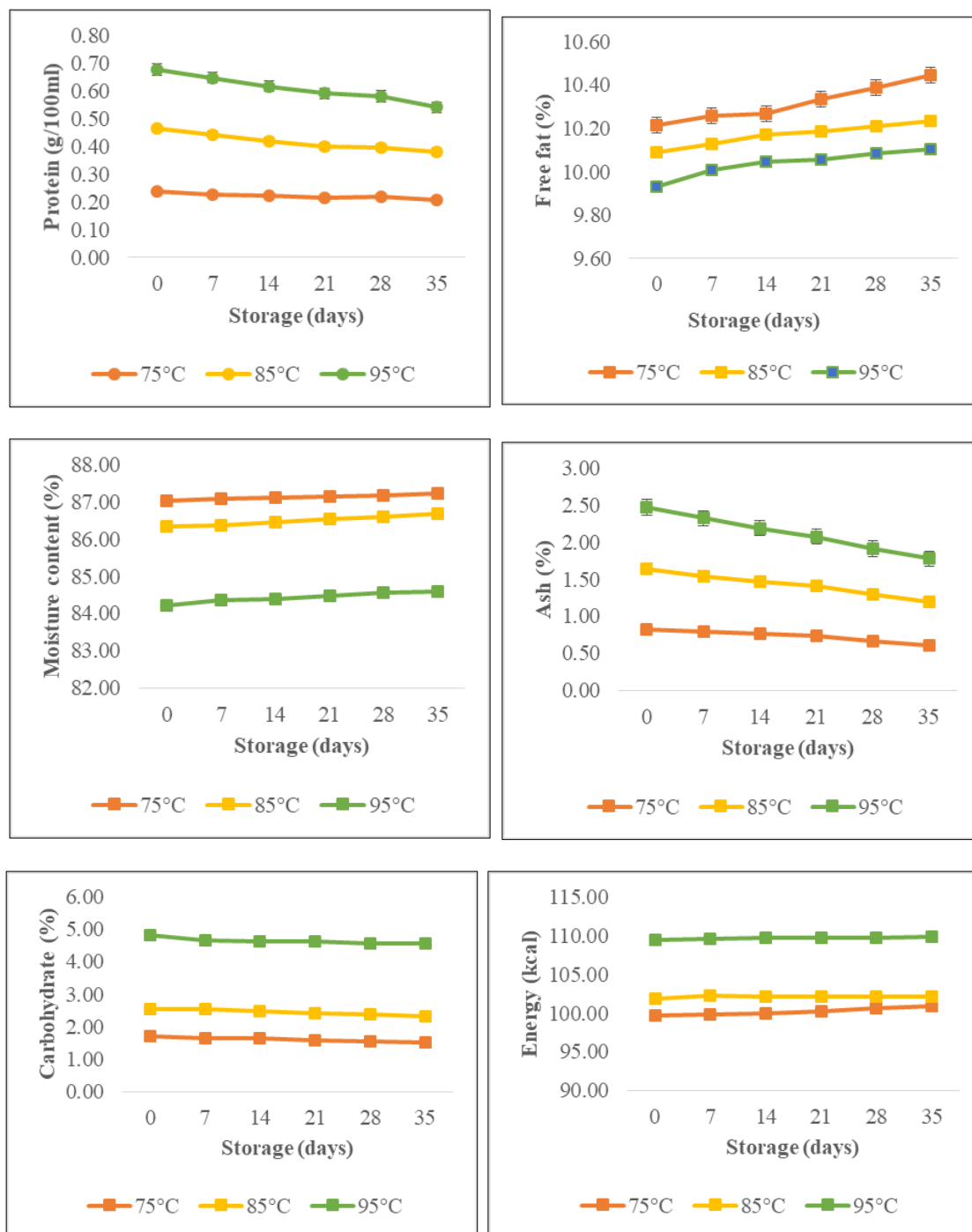


Fig 2: Effect of retort temperature on quality of vegan milk during storage at ambient conditions

**Table 1:** Effect of pH, TSS, titratable acidity and color of retort processed samples at 75 °C, 85 °C and 95 °C at ambient storage conditions

Storage Days	pH			TSS			Titratable acidity (%)			Color								
	75°C	85°C	95°C	75°C	85°C	95°C	75°C	85°C	95°C	L			a*			b*		
	75°C	85°C	95°C	75°C	85°C	95°C	75°C	85°C	95°C	75°C	85°C	95°C	75°C	85°C	95°C	75°C	85°C	95°C
0	6.18 ±0.01 <sup>a</sup>	6.33 ±0.02 <sup>a</sup>	6.56 ±0.01 <sup>a</sup>	13.33 ±0.15 <sup>ab</sup>	12.53 ±0.15 <sup>a</sup>	11.70 ±0.20 <sup>a</sup>	0.32 ±0.01 <sup>e</sup>	0.28 ±0.01 <sup>e</sup>	0.22 ±0.01 <sup>f</sup>	76.37 ±0.19 <sup>f</sup>	75.54 ±0.33 <sup>f</sup>	76.36 ±0.09 <sup>e</sup>	0.04 ±0.01 <sup>d</sup>	-0.61 ±0.02 <sup>e</sup>	-0.59 ±0.03 <sup>e</sup>	14.32 ±0.07 <sup>a</sup>	12.70 ±0.24 <sup>a</sup>	13.54 ±0.03 <sup>a</sup>
7	6.09 ±0.08 <sup>b</sup>	6.30 ±0.01 <sup>b</sup>	6.48 ±0.01 <sup>b</sup>	13.30 ±0.10 <sup>ab</sup>	12.73 ±0.12 <sup>a</sup>	11.77 ±0.06 <sup>a</sup>	0.36 ±0.01 <sup>e</sup>	0.30 ±0.01 <sup>e</sup>	0.31 ±0.02 <sup>e</sup>	76.81 ±0.09 <sup>e</sup>	76.10 ±0.05 <sup>e</sup>	77.05 ±0.03 <sup>d</sup>	0.06 ±0.01 <sup>cd</sup>	-0.59 ±0.01 <sup>e</sup>	-0.56 ±0.02 <sup>d</sup>	14.12 ±0.04 <sup>b</sup>	12.45 ±0.08 <sup>b</sup>	13.17 ±0.01 <sup>b</sup>
14	6.07 ±0.01 <sup>b</sup>	6.27 ±0.01 <sup>b</sup>	6.31 ±0.02 <sup>c</sup>	13.20 ±0.10 <sup>bc</sup>	12.73 ±0.21 <sup>a</sup>	11.53 ±0.06 <sup>ab</sup>	0.45 ±0.02 <sup>d</sup>	0.43 ±0.02 <sup>d</sup>	0.36 ±0.01 <sup>d</sup>	77.67 ±0.10 <sup>d</sup>	76.61 ±0.04 <sup>d</sup>	77.61 ±0.23 <sup>c</sup>	0.07 ±0.01 <sup>c</sup>	-0.55 ±0.03 <sup>d</sup>	-0.50 ±0.03 <sup>d</sup>	13.81 ±0.06 <sup>b</sup>	12.17 ±0.11 <sup>c</sup>	13.02 ±0.02 <sup>c</sup>
21	6.04 ±0.01 <sup>bc</sup>	6.23 ±0.01 <sup>c</sup>	6.23 ±0.03 <sup>d</sup>	13.10 ±0.10 <sup>c</sup>	12.67 ±0.06 <sup>a</sup>	11.63 ±0.06 <sup>a</sup>	0.54 ±0.04 <sup>c</sup>	0.56 ±0.02 <sup>c</sup>	0.48 ±0.01 <sup>c</sup>	78.23 ±0.02 <sup>c</sup>	77.35 ±0.12 <sup>c</sup>	77.83 ±0.08 <sup>c</sup>	0.09 ±0.02 <sup>b</sup>	-0.46 ±0.03 <sup>d</sup>	-0.43 ±0.03 <sup>d</sup>	13.52 ±0.06 <sup>d</sup>	11.71 ±0.14 <sup>d</sup>	12.85 ±0.05 <sup>d</sup>
28	6.01 ±0.01 <sup>c</sup>	6.20 ±0.01 <sup>d</sup>	6.10 ±0.01 <sup>e</sup>	13.40 ±0.10 <sup>a</sup>	12.60 ±0.10 <sup>a</sup>	11.50 ±0.10 <sup>ab</sup>	0.75 ±0.03 <sup>b</sup>	0.65 ±0.02 <sup>b</sup>	0.54 ±0.01 <sup>b</sup>	78.76 ±0.11 <sup>b</sup>	77.71 ±0.14 <sup>b</sup>	78.39 ±0.14 <sup>b</sup>	0.11 ±0.02 <sup>ab</sup>	-0.38 ±0.02 <sup>b</sup>	-0.34 ±0.01 <sup>a</sup>	12.56 ±0.08 <sup>e</sup>	11.43 ±0.12 <sup>e</sup>	12.53 ±0.01 <sup>e</sup>
35	5.98 ±0.01 <sup>c</sup>	5.97 ±0.04 <sup>e</sup>	5.99 ±0.02 <sup>f</sup>	13.30 ±0.10 <sup>ab</sup>	11.53 ±1.67 <sup>a</sup>	10.77 ±1.10 <sup>b</sup>	0.95 ±0.02 <sup>a</sup>	0.77 ±0.02 <sup>a</sup>	0.68 ±0.01 <sup>a</sup>	79.61 ±0.04 <sup>a</sup>	78.26 ±0.04 <sup>a</sup>	78.75 ±0.09 <sup>a</sup>	0.11 ±0.02 <sup>a</sup>	-0.24 ±0.01 <sup>a</sup>	-0.31 ±0.02 <sup>a</sup>	12.19 ±0.14 <sup>f</sup>	10.96 ±0.09 <sup>f</sup>	12.24 ±0.03 <sup>f</sup>

All values are represented as mean ± standard deviation.

All samples were analysed in triplicates.

Means followed by the same letter in the column do not differ by Duncan multiple range test ( $p < 0.05$ ).

**Table 2:** Effect of retort processing on microbial growth on storage

Storage days	Bacteria (cfu/ml)				Yeast & Mould (cfu/ml)			
	Control	75°C	85°C	95°C	Control	75°C	85°C	95°C
0	5.70*10 <sup>5</sup>	Nil	Nil	Nil	0.13*10 <sup>5</sup>	Nil	Nil	Nil
7	6.43*10 <sup>5</sup>	Nil	Nil	Nil	0.93*10 <sup>5</sup>	Nil	Nil	Nil
14	8.79*10 <sup>5</sup>	Nil	Nil	Nil	1.17*10 <sup>5</sup>	Nil	Nil	Nil
21	-	4.16*10 <sup>5</sup>	Nil	Nil	-	0.27*10 <sup>5</sup>	Nil	Nil
28	-	5.86*10 <sup>5</sup>	2.79*10 <sup>5</sup>	Nil	-	0.58*10 <sup>5</sup>	Nil	Nil
35	-	7.29*10 <sup>5</sup>	3.64*10 <sup>5</sup>	3.14*10 <sup>5</sup>	-	0.72*10 <sup>5</sup>	0.36*10 <sup>5</sup>	Nil

All samples were analysed in replicates

#### 4. Conclusion

The study was conducted to extend the shelf-life of nutrient-rich blended vegan milk prepared from coconut and foxtail millet. The quality changes observed after processing proved that high temperature and long-time processing affect the samples in terms of clumping and coagulation. Thus, reducing the temperature and increasing the processing time of the samples aided in maintaining the visual appearance of the products and also reduced the microbial load to some extent. Thus, from the study, it can be concluded that samples retorted at both 85°C and 95°C for 15 mins showed no significant results in terms of commercial sterility for a longer time but retaining of nutrients throughout the storage period was observed in samples processed at 85°C for 15 mins.

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