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Factors influencing the calorimetric determination of glass transition temperature in foods: A case study using chicken and mutton

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1. Introduction

Chicken and mutton are consumed for their taste and nutritive value, and their demand is ever increasing throughout the world. However, the safety of meat is of major concern to its manufacturers as well as end users and hence strict quality control protocols are required during in the processing and preservation. Freezing is one of the most reliable and easy methods for preserving meat during in the processing and preservation. Freezing is one of the most reliable and easy methods for preserving meat (Goff, 1995). However, the stability of frozen meat depends on the state of water in the product and the stability of ice crystals during frozen storage (Goff and Sahagian, 1996). Hence mechanisms that govern the formation of glassy state and glass transition concepts are very much relevant during frozen storage (Rahman, 1999). The concept of water activity alone is insufficient to predict shelf stability of frozen foods. The alternate complimentary ideas like storing meat below Tg, may help to improve the shelf stability.

Glass transition (Tg) is a second order thermodynamic transition, helpful in elucidating the time-temperature related properties as well as the state of water during freezing and storage. Molecular mobility of glassy materials is significantly reduced below Tg. This in turn delays various deteriorative changes such as texture loss, enzymatic spoilage, flavour loss etc. in foods during storage (Mitchell, 1998). Levine and Slade (1986) and Slade and Levine (1988) postulated that Tg influences the stability of foods. Below Tg, water in the concentrated phases becomes kinetically immobilized and therefore does not support or participate in deteriorative reactions. Studies indicated that the concept of Tg should be added along with the existing concept of water activity, to get a better understanding about the factors governing the stability of foods (Rahman et al., 2005; Sablani et al., 2007a,b). Rahman (2006) combined the glass transition and water activity concepts in the state diagram in order to determine the stability of foods.

The most commonly used method to determine Tg of foods is by using differential scanning calorimeter (DSC), which detects the change in heat capacity, occurring over a range of temperatures. A step change occurring in the thermogram line of heat capacity curve during heating of food samples can be taken as glass transition temperature. In food samples, the glassy state conditions depend on the formation of ice crystals during freezing. Therefore the large number of variables influencing the freezing of foods can also affect the determination of glass transition temperature (Champion et al., 2000). In order to determine Tg values effectively, conditions that promote maximum ice formation has to be employed. Hence annealing protocols during freezing are extremely important (Brake and Fennema, 1999).

Delgado and Sun (2002) reported the Tg of chicken meat (breast portion) as −16.83 °C using DSC by employing an annealing temperature of −20 °C for 1 h. However the Tg value can vary depending on annealing temperature and the duration employed (Brake and Fennema, 1999; Rahman et al., 2007). Parameters like annealing temperature and time, rate of heating, moisture content,
processing conditions etc. have been found to affect Tg values of various food systems like fish muscles, tomato, date flesh, carbohydrate solutions etc. (Hashimoto et al., 2004; Telis and Sobral, 2002; Rahman, 2004; Ribeiro et al., 2003; Carrington et al., 1994; Goff, 1994; Huang et al., 1994; Sahagian and Goff 1994). However reports on systematic study on the effect of individual parameters on Tg of food samples are scanty (Sablani et al., 2007a,b). The lack of common DSC protocols used in food systems are a hindrance in the comparison of Tg values between the samples. Hence the main objective of this study was to focus on how various parameters influence the Tg determination of chicken and mutton and thus frame a protocol for DSC analysis.

2. Materials and methods

2.1. Sample preparation

Boneless chicken breast meat (class A) was procured from the local market; trimmed of any visible fat, chopped with a knife into small strips (5 cm length, 3 cm width and 2 cm thickness). These strips were divided into two lots and the first lot was kept frozen until analyzed. While the second lot was freeze-dried, packed in Aluminium foil based packaging material and kept frozen at −18 °C until analyzed. Before any experiment, the meat samples were thawed in a refrigerator at 4 ± 1 °C. Small sample cubes weighing 6–10 mg were carefully cut from the strips and used for thermal analysis. The moisture, crude fat, and protein contents were determined using AOAC, 1984 procedures, (24.002, 24.005 and 24.027, respectively).

2.2. Freeze drying

Freeze drying was carried out in a pilot scale freeze dryer, Epsilon 1/60 (Martin Christ GmbH & Co KG, Osterode, Germany) equipped with rapid freezing and drying facilities. Samples (Chicken and mutton) were pre-frozen to −40 ± 2 °C for 4 h and dried under variable chamber pressure (100–300 Pa) and temperatures (30–60 °C). After freeze drying, the final moisture content of chicken and mutton samples was reduced to 2–3%. Vacuum was released and the product taken to low humidity room (23 ± 2%) for 40 ± 2 h as constant. The results have been given in Table 2. Statistical analysis was performed with SPSS software (SPSS Inc., 1996) and used to test the significant effect of various parameters on ΔH and Tg values at 1% level of significance (P < 0.01).

2.3. Measurement of Tg by DSC

DSC 2010 differential scanning calorimeter (TA Instruments, New Castle, DE, USA) was used to determine the Tg values. Samples were enclosed in hermetically sealed aluminum pans just before sampling (Chicklon 1/60 (Martin Christ GmbH & Co KG, Osterode, Germany) was used to determine the Tg values. Samples were annealed at 23 ± 2% for 60 min and held isothermally for 60 min. In this study, TGA was used to determine the accurate moisture content in all the samples by plotting percentage weight loss against time.

2.4. Thermo gravimetric analysis

Thermo gravimetric analyzer (TGA Q50, TA Instruments, DE, USA), was used in conjunction with a thermal analysis controller. TGA was employed to measure the amount and rate of change in weight of the material either as a function of increasing temperature or time, under a controlled atmosphere. Initial weight of each sample was approximately 20 mg. Samples were placed in platinum pans and heated in a furnace flushed with N2 gas at the rate of 40 ml/min and heated from 30 °C to 105 °C at a rate of 10 °C/min and held isothermally for 60 min. In this study, TGA was used to determine the accurate moisture content in all the samples by plotting percentage weight loss against time.

2.5. Statistical analysis

Statistical analysis was performed with SPSS software (SPSS Inc., 1996) and used to test the significant effect of various parameters on ΔH and Tg values at 1% level of significance (P < 0.01).

3. Results and discussion

The average chemical composition of chicken and mutton samples has been given in Table 1. Determination of Tg in food systems depends on the various parameters employed during DSC scan. Meat samples such as chicken and mutton exhibit Tg at subzero temperatures and it is influenced by ice crystal formation in the freezeable water present. The present study was aimed at determination of the temperature at which ice crystal formation takes place in meat samples and how it varies depending on the annealing conditions employed. Chicken and mutton samples were cooled using DSC from 20 °C to −30 °C at the rate of 2 °C/min. Fig. 1 shows the freezing curve of the fresh chicken and mutton samples. It indicates that the onset of enthalpy change corresponding to the ice crystallization starts around −11 °C and ends at around −23 °C. The enthalpy of ice crystallization (ΔH) was determined by integrating the heat flow curves, while peak maximum value was taken as the freezing point. In order to determine the effect of annealing on ΔH values, meat samples were annealed at different temperatures, in the range of −11 °C to −23 °C by keeping the annealing time of 60 min constant. The results have been given in Table 2. Statistical analysis (at 99% confidence level) showed that annealing temperature had a significant effect on the ΔH values. The ΔH values (134 ± 1.66 J/g) were found to be the minimum when the samples were annealed at −17 °C for chicken. This implies that maximum

### Table 1

Average chemical composition of chicken and mutton

<table>
<thead>
<tr>
<th>Composition (% w/w)</th>
<th>Chicken</th>
<th>Mutton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>71.4 ± 1.06</td>
<td>64.26 ± 1.23</td>
</tr>
<tr>
<td>Protein</td>
<td>26.04 ± 0.49</td>
<td>29.2 ± 0.39</td>
</tr>
<tr>
<td>Fat</td>
<td>2.01 ± 0.12</td>
<td>6.12 ± 0.22</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.
freeze concentration can be achieved by employing the same annealing temperature. This observation is in agreement with the reports of Brake and Fennema (1999), who suggested that the most effective annealing temperature employed should be near to the Tg of the food samples. The degree to which maximum freeze concentration (Levine and Slade, 1986) can be achieved in meat samples is influenced by exposure time at a particular temperature. Hence sufficiently long holding time (60 min) was employed as reported by Delgado and Sun (2002) at various annealing temperatures, so as to attain maximum freeze concentration. Storage of samples at annealing temperatures for long durations leads to spontaneous ice crystallization, which in turn reduces the enthalpy content. In the case of mutton, annealing at −20°C and time.

Table 2
Summary of enthalpy change and freezing point of chicken and mutton at various annealing temperatures

<table>
<thead>
<tr>
<th>Annealing temperature (°C)</th>
<th>Chicken</th>
<th>Freezing point (°C)</th>
<th>Mutton</th>
<th>Freezing point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−11</td>
<td>145 ± 1.6</td>
<td>−17.19 ± 0.21</td>
<td>170 ± 1.38</td>
<td>−12.12 ± 0.13</td>
</tr>
<tr>
<td>−14</td>
<td>143 ± 1.31</td>
<td>−16.86 ± 0.14</td>
<td>167 ± 1.49</td>
<td>−11.83 ± 0.72</td>
</tr>
<tr>
<td>−15</td>
<td>140 ± 1.98</td>
<td>−16.88 ± 0.86</td>
<td>164 ± 2.23</td>
<td>−9.12 ± 0.36</td>
</tr>
<tr>
<td>−16</td>
<td>137 ± 1.88</td>
<td>−16.89 ± 0.53</td>
<td>168 ± 1.33</td>
<td>−9.77 ± 0.28</td>
</tr>
<tr>
<td>−17</td>
<td>134 ± 1.66</td>
<td>−16.93 ± 0.15</td>
<td>171 ± 1.28</td>
<td>−9.32 ± 0.53</td>
</tr>
<tr>
<td>−18</td>
<td>130 ± 1.34</td>
<td>−15.63 ± 0.41</td>
<td>175 ± 1.39</td>
<td>−9.48 ± 0.45</td>
</tr>
<tr>
<td>−19</td>
<td>129 ± 1.21</td>
<td>−12.43 ± 0.32</td>
<td>174 ± 1.44</td>
<td>−9.78 ± 0.91</td>
</tr>
<tr>
<td>−20</td>
<td>148 ± 1.45</td>
<td>−11.70 ± 0.61</td>
<td>175 ± 1.67</td>
<td>−10.92 ± 0.71</td>
</tr>
<tr>
<td>−23</td>
<td>157 ± 1.48</td>
<td>−11.83 ± 0.71</td>
<td>176 ± 1.53</td>
<td>−10.01 ± 0.27</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.
* Values are statistically significant at P < 0.01.
* Peak maximum values.

3.1. Effect of annealing on glass transition

The effect of annealing on the Tg of chicken and mutton samples has been studied by employing various annealing temperatures and time. Figs. 2 and 3 depict the effect of annealing temperature on Tg values. As the annealing temperature was decreased from −11°C to −23°C, Tg values shifted towards lower temperatures. When annealing temperatures for chicken and mutton samples were around −17°C and −15°C, respectively, step changes occurring to heat flow curves were more prominent. This resulted in an easy determination of Tg. Similarly, various annealing times like 0, 15, 30, 45, 60, and 120 min were also employed by keeping the annealing temperature constant. The effect of annealing time on Tg values of fresh chicken samples has been shown in Fig. 4. It is evident that as the annealing time increases Tg becomes more visible and the values shift towards lower temperatures. Table 2 gives the effect of annealing time on Tg values of fresh and freeze-dried chicken and mutton samples. DSC studies revealed that the Tg was not traceable without sufficient annealing time and as the annealing time increased, the likelihood of detecting Tg also increased. Statistical analysis (P < 0.01) showed that the annealing time also...

Fig. 1. Freezing curves of fresh (a) chicken and (b) mutton.

Fig. 2. Effect of annealing temperature on ((a) −11°C, (b) −14°C, (c) −17°C, (d) −20°C, and (e) −23°C) Tg of fresh chicken.

Fig. 3. Effect of annealing temperature on ((a) −11°C, (b) −14°C, (c) −15°C, (d) −17°C, and (e) −20°C) Tg of fresh mutton.

Fig. 4. Effect of annealing time ((a) 0 min, (b) 15 min, (c) 30 min, (d) 45 min, (e) 60 min, and (f) 120 min) on glass transition temperature.
had a significant effect on Tg values up to 1 h. Hence the optimum annealing conditions were employed for fresh chicken and mutton samples before the measurement of Tg. Conditions employed were −17 °C for 1 h and −15 °C for 1 h for the fresh chicken and mutton, respectively. Tg values for the samples were −16.63 °C and −12.97 °C, respectively. Freeze-dried chicken and mutton samples were also subjected to DSC analysis at different annealing temperatures (Figs. 5 and 6) and annealing time (Table 3). Interestingly Tg values shifted to higher temperatures like −13.21 °C and −9.24 °C in case of freeze-dried chicken and mutton, suggesting that the moisture may have played a vital role in determination of Tg.

3.2. Effect of moisture content on glass transition

Effect of moisture content on Tg was determined by conducting DSC scans for freeze-dried samples with different moisture levels. In order to vary the moisture content of chicken and mutton, the samples were drawn at regular intervals from the freeze-drier. The moisture content of the samples was approximately 10%, 20%, 30%, 40%, 50%, and 60%. Actual moisture content of these samples was determined by thermo gravimetrically. Figs. 7 and 8 depict thermograms of the chicken and mutton samples drawn at various time intervals. It is clear from the figures that fresh chicken and mutton samples had moisture content of 71.74% and 64.26%, respectively, while those in case of freeze-dried samples were 2.376% and 2.78%. While conducting DSC scans for all the samples, other parameters like annealing temperature and time, rate of heating etc. were kept constant in order to keep the moisture content as the only variable. Fig. 9 depicts the variation of glass transition temperatures for the samples with different moisture contents. In both chicken and mutton samples, Tg values were found to decrease with increased moisture content. A similar result has been earlier reported during thermal analysis of tomatoes (Telis and Sobral, 2002). Generally water acts as a plasticizer and can thus affect the Tg of food samples. Water is a mobility enhancer, which leads to large increase in free volume and decreased viscosity. Slade and Levine (1991) reported that the direct plasticizing effect of increased moisture content leads to increased segmental mobility of chains in amorphous region of glassy and partially crystalline systems and tends to reduce the Tg value. In the present study, the Tg values decreased with increased moisture content. However, the effect of increased moisture content leads to increased segmental mobility of chains in amorphous region of glassy and partially crystalline systems and tends to reduce the Tg value.

![Fig. 5. Effect of annealing temperature on ((a) -11 °C, (b) -14 °C, (c) -17 °C, (d) -20 °C, and (e) -23 °C) Tg values of freeze-dried chicken.](image)

![Fig. 6. Effect of annealing temperature on ((a) -11 °C, (b) -14 °C, (c) -15 °C, (d) -17 °C, and (e) -20 °C) Tg values of freeze-dried mutton.](image)

**Table 3**

<table>
<thead>
<tr>
<th>Annealing time (min)</th>
<th>Tg values (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh chicken</td>
<td>FD chicken</td>
</tr>
<tr>
<td>Fresh mutton</td>
<td>FD mutton</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>0</td>
<td>Not traceable</td>
</tr>
<tr>
<td>15</td>
<td>−13.33 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>−10.49 ± 0.05</td>
</tr>
<tr>
<td>30</td>
<td>−14.31 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>−11.03 ± 0.01</td>
</tr>
<tr>
<td>45</td>
<td>−15.79 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>−11.42 ± 0.06</td>
</tr>
<tr>
<td>60</td>
<td>−16.63 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>−12.91 ± 0.04</td>
</tr>
<tr>
<td>120</td>
<td>−16.72 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>−12.93 ± 0.03</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.

* Values are statistically significant at P < 0.01.
study, the Tg values were also found to be influenced by the initial moisture content of chicken and mutton samples.

3.3. Effect of rate of heating on glass transition

Since the rate of heating is also a significant factor affecting the calorimetric determination of Tg (Rahman, 2006), six different heating rates viz; 0.5, 1, 2, 3, 4, and 5 °C/min were employed during DSC scan. Fig. 10 shows the effect of rate of heating on Tg values for fresh as well as freeze-dried chicken and mutton samples. As the rate of heating increased, Tg values also showed a linear increase. Rahman et al. (2007) have also reported a similar trend, while determining the Tg of spaghetti. In case of freeze-dried samples, Tg values changed marginally due to reduced moisture content. As rate of heating increases, the duration of exposure to a particular temperature decreases. So at lower heating rates (Fig. 10) the characteristic step change in the heat flow curves occurred at lower temperatures leading to lower Tg values. As the rate of heating decreases, this step change becomes more visible and thus any rate of heating at 2 °C/min or below can be recommended for an easy and accurate detection of Tg.

4. Conclusions

Various factors influencing the calorimetric determination of Tg in chicken and mutton samples were investigated. Freezing behaviors of these samples were studied by employing different annealing temperatures and time. Proper selection of annealing conditions is very critical in the determination of Tg. The present study revealed that an annealing temperature of −17 °C and an annealing time of 1 h were the best suited for chicken samples, as it helped to attain the maximum freeze concentration at reduced time interval. While in case of mutton, annealing conditions like −15 °C for 1 h gave similar results. The effect of moisture content as well as the rate of heating on Tg was also determined. The rate of heating like 2 °C/min or below was found to be acceptable, while moisture content had to be estimated correctly before reporting the Tg values. The various parameters influencing Tg determination were identified and it helped in framing a DSC protocol for determining Tg in the case of meat samples.

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References


Physico-Chemical Changes in Ready to Eat Pineapple Chicken Curry during Frozen Storage

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ABSTRACT

RTE Pineapple chicken curry, a traditional Kerala recipe, was prepared and standardized by using de-boned broiler meat chunks, pineapple and spices. The product having both meat and gravy (1:1.9) was packed in polyethylene pouches and stored at −18°C ± 2°C for 6 months. During frozen storage, the free fatty acid (FFA) values were 0.28 - 0.46 (as percentage oleic acid) and thiobarbituric acid (TBA) values were 1.68 - 2.45 (mg of malonaldehyde/Kg of sample) increased in both meat and gravy. Meat and gravy pH were in the range of 5.5 to 6. Marginal decrease in shear force values (43.4 - 39.6 N) were also observed. During storage the SPC was found to be decreasing over period of storage (100, 40, 20, <10 respectively). Psychrophiles were within acceptable limit and pathogens were absent. Microbiological data showed that the product was microbiologically safe. The sensory score indicated that the RTE pineapple chicken curry is acceptable after storage at −18°C ± 2°C for 6 months.

Keywords: Chicken Curry; Pineapple; Frozen Storage; GC

1. Introduction

Globally several chicken recipes are very popular. Indian traditional meat based recipes are well known for its delicious and appetizing taste worldwide, but these foods require number of unit operations and long preparation time [1]. In order to minimize the tedious operations involved in preparation of food and to save time including all the required nutrition and to fulfill satiety of consumer especially for working population there is a need to develop several products that involve minimum processing. Ready to eat product has a budding industry in India and overseas, since needs of population is changing with time. Quality and safety of food is a major concern of both consumer and manufacturer that is influenced by several factors.

Indians have a variety of meat preparations that involves profuse use of spices that adds to the flavor as well as imparts antioxidant, antibacterial and various other functional roles. There are a number of products that vary in terms of preparation, kinds of meat, spices etc. Selection of suitable packaging material has been a great area of interest in order to maintain the shelf life and produce better products. Foods that have a short shelf life are processed for longer shelf life in variety of packaging materials such flexible films, multilayer packages, glass, cans etc. Canned products are available in market but has several limitations such as metal imparts an undesirable taste to the product during storage, is an expensive method and prone to a high incidence of leakage through seams [2]. Similarly retort pouches also need an additional processing before storage that adds to the cost but has an advantage that can be stored at room temperature for several months. Hence in the present study polythene pouches were used for packaging the product that were of low cost and also maintained the quality of food at frozen storage without any damage to the packaging material that usually is seen with other polymer materials such as polypropylene. Anon [3] suggested that curry products can be frozen and marketed in polythene pouches. Changes in quality during chilled or frozen storage have been studied in cod and haddock fillets [3], muscle of volador [4], buffalo meat burgers [5], chicken nuggets [6,7] etc.

Kerala is known for its heavily spiced non vegetarian foods and pineapple chicken curry is one of the traditional recipes. A traditional recipe, pineapple chicken curry was prepared and packed in polyethylene bags and changes in its physico-chemical quality during frozen (−18°C ± 2°C) storage (for 6 months) were studied. An attempt was made in the present study to bring forth a standardized recipe for the pineapple chicken curry and also to cater the needs of working population and single
person staying alone. These days a number of RTE are already available in market but still there is a need of proper marketing and awareness among the consumer for judicial utilization of upcoming technology and improved products so that both the consumer and the manufacturer can be benefited. Changes in the physico-chemical quality of product were also determined in the course of technological processes of freezing and during storage of frozen products.

2. Materials and Methods

2.1. Preparation of RTE Pineapple Chicken Curry

Broiler chicken of 6 - 8 weeks age were procured from the local market, dressed conventionally and were brought to the FD-APT division lab, Mysore, India. On the day of preparation, the carcasses (1.10 to 1.20 kg) were washed under running tap water and deboned. Breast and leg muscles were cut into cubes of 2 - 3 cm and were marinated in curd for 1 - 2 hours. This was cooked for 10 - 15 min at 95°C - 100°C and cooled to 30°C - 40°C. The ingredient composition for the preparation of RTE pineapple chicken curry is given in Table 1. Cooking oil was heated in a stainless steel vessel to 110°C - 120°C, added clove, cinnamon and cardamom and roasted for 1 - 2 minutes. Sliced onion, ginger, garlic, coriander leaves and green chillies were added and sautéed till light golden colour. Then added tomato puree, red chilli powder, coriander powder, turmeric powder, salt and pineapple cubes and cooked for 4 min on low flame. To this gravy mix, added cooked chicken cubes along with cooked out juice and mixed well. This was further heated for 5 min at 85°C - 90°C. The product cooled to 30°C - 40°C for 40 - 50 min and separated the meat chunks from gravy.

The product was packaged in polythene pouches (300 gauge) with 100 g meat and 250 g gravy in each pouch. The sealed packets placed in wax coated carton and frozen in a plate freezer until the product temperature reached −45°C to −60°C (115 - 125 min). A digital temperature recorder with metallic probes (Aptec, Chennai, India) was used to ensure adequate temperature decline of the product. The frozen product was then stored at a freezer maintained at −18°C ± 2°C.

2.2. Quality Evaluation

Physical, chemical and microbiological studies were conducted for 6 months of frozen storage. The product was thawed at 26°C ± 2°C for 30 min and was subjected to the following analyses.

2.2.1. Physical and Chemical Parameters

Separated the meat chunks and gravy and homogenized in a mixer for sampling. Proximate composition and NaCl content in chicken chunks and gravy were determined [8]. FFA, pH and TBA of both chicken chunks and gravy were determined periodically during the storage period. Free fatty acid (FFA) [7] and thiobarbituric acid (TBA) were determined by aqueous extraction procedure [9] and pH by immersing a glass calomel electrode directly into the sample using a pH meter (Cyberscan 1000, Eutech Instruments, Singapore). Shear values were measured in a Lloyds Texturometer (LR5K, Lloyd Instruments Ltd., Hampshire, UK) in 100 kg load cell at a speed of 50 mm/min with a 1 mm thick blade using chicken chunks of 1 × 1 × 1.5 cm strips.

2.2.2. Free Fatty Acid Content (FFA)

The FFA content of the samples was estimated as per AOAC (1990) [8]. The FFA content of the samples was estimated as per AOAC (1990). A known quantity of fat extracted was taken in to a 100 ml flask and 50 ml of hot neutralised alcohol was added followed by 1 - 2 ml of phenolphthalein reagent. The flask was shaken vigorously to dissolve all the fat content and titrated against 0.25 N NaOH solutions to get a faint pink colour. From the titre value FFA content was calculated as follows:

\[
\text{Free Fatty Acid (\% as Oleic acid)} = \frac{\text{ml of alkali } \times N \text{ of alkali } \times 28.2}{\text{Weight of fat (g)}}
\]
2.2.3. Thiobarbituric Acid Reactive Substance (TBARS) Determination
TBARS values in meat/poultry samples were determined as per Taraldgis method [10]. Taraldgis method (1960) is one of the most widely used tests to evaluate the extent of lipid oxidation in meats. This is based on the reaction between important oxidation product malanaldehyde with TBA reagent to produce a colour complex. The chromogen results from the condensation of two molecules of TBA with one molecule of malonaldehyde.

20 g of blended sample was accurately weighed and transferred into a RB flask. To that 2.5 ml of conc. HCl was added along with 97.5 ml of distilled water. pH was adjusted to 1.5. Mixture was steam distilled and 50 ml distillate was collected in 10 min. From this 25 ml of distillate was transferred into stoppered glass tubes and 5 ml of TBA reagent was added. The test tubes were kept in boiling water bath for 35 min and it was cooled and OD was measured at 538 nm. The TBARS values were calculated using the standard curve.

2.2.4. Total Fatty Acid Analysis by Gas Chromatography
2.2.4.1. Esterification of Fatty Acids
The samples were esterified as per the procedure of Metcalfe et al. [11] with slight modifications.

About 150 mg of lipid was accurately weighed into a clean and dry stoppered test tube. 4 ml of 0.5 N alcoholic sodium hydroxide solutions was added and heated for 5 min over a water bath at 90°C. On cooling 5 ml of Boron trifluoride-methanol reagent (14%) was added and heated for 5 min at 90°C over a water bath, followed by addition of 10 ml of saturated sodium chloride solution. The samples were thoroughly cooled to room temperature and 5 ml of hexane was added to each tube. It was shaken well and kept undisturbed. The upper hexane layer was drawn out into clean dry conical flask and dried over anhydrous sodium sulphate to remove the traces of moisture if present. The samples were filtered and transferred to stoppered clean dry tubes for gas chromatographic analysis.

2.2.4.2. Quantification of Fatty Acid Analysis by Gas Chromatography
Analysis of total fatty acids was carried out by cere 800, Chemito model Gas chromatograph fitted with BPX 70 column (25 m, 0.32 mm ID) and flame ionisation detector. Temperature gradient programming was employed from 150°C to 220°C. Split ratio was adjusted to 1:25 and capillary flow of carrier 2 ml/min. Injector and detector port temperatures were adjusted as 230 and 240 respectively. For FID, Hydrogen and Oxygen was used and the flow was adjusted as 45 ml/min and 45 ml/min respectively. Along with samples standard esters of fatty acids were also injected and the fatty acids were detected by comparing the retention time of the standard esters of fatty acids. The quantification of the fatty acids was carried out by evaluating with the standard fatty acid esters area corresponding to each peak in the chromatogram. Iris 32 software is used to integrate and evaluate the chromatogram in the analysis.

2.2.5. Microbiological Quality
Chicken chunks in the curry were cut using a sterile knife and mixed with the gravy. Placed a 50 g sample of the mixture in a sterile stomacher bag containing 450 ml sterile saline (0.85% NaCl) solution and blended in a stomacher (Seward Stomacher 400, Seward Medical, London, UK). The blended samples were tested for standard plate counts (SPC), coliforms, yeast and mould (Y&M), staphylococci, salmonella and E. coli by pour plate method [12].

2.2.6. Sensory Quality
Packets of RTE chicken curry was thawed by holding them at 26°C ± 2°C and warmed the product in a hot pan maintained at 80°C - 90°C for 3 - 4 min. The coded samples were subjected to sensory evaluation by 10 in-house trained panelists using a 9-point hedonic scale [6,13] and recorded the mean score of each attribute (colour, flavor, mouthfeel, consistency of the gravy, meat texture and overall acceptability).

2.3. Statistical Analysis
Statistical analysis was performed with SPSS software (SPSS Inc., 1996) and used to test the significant effect of various parameters at 5% level of significance (P > 0.05). Each test was performed in replication of three.

3. Results and Discussion
3.1. Physical and Chemical Quality
In the present RTE recipe pineapple used were different from other regular chicken curry recipe. Pineapple had certain advantages and made the recipe much more acceptable. Pineapple has exceptional juiciness that added certain advantages and made the recipe much more acceptable. Pineapple had exceptional juiciness that added vibrant flavor with several health benefits. The enzyme bromelain present enhanced the texture of chicken by tenderizing and maintained it for longer also the highly acidic nature of fruit lowered the pH thus extending the shelf life. Chicken chunks were found to have less moisture, fat, ash and salt compared to gravy i.e. 69.40%, 11.08%, 1.80% and 1.58% respectively in chunks whereas in gravy a higher values were observed i.e. 74.5%, 13.43%, 1.9% and 1.8% respectively. The higher values in gravy can be contributed due to the added spices, salt and water during cooking of the chicken curry but since chicken is a good source of protein whereas in gravy no ingredient that has as high pro-
tein content in chunks (19.43%) thus curry had a significant lower value for protein i.e. 2.7%. The physio-chemical changes during frozen storage of pineapple chicken curry were depicted in Table 2. There was marginal fall in the pH (Table 2) of meat and gravy from 5.7 to 5.51 and 6.02 to 5.68 respectively during 6 months of frozen storage. FFA values (as % of oleic acid) were increased marginally from 0.28 to 0.453 in meat and 0.29 to 0.46 in gravy during frozen storage for 6 months (Table 2).

Lipase activity has been reported as the cause of increased FFA values in meat products during storage by many authors. But the increase in FFA did not increase the rancidity in pork sausages [14,15], fried chicken [16], buffalo meat burgers [6] and chicken nuggets [7]. Increased level of FFA does not cause any toxicological effects [17].

TBA is a measure of oxidative rancidity of the product. TBA values (mg of malonaldehyde/kg sample) fluctuated non-significantly between 1.68 and 2.64 for meat and 2.21 and 2.45 for gravy during the storage period (Table 2). This effect is attributed to the very low temperature storage and the antioxidant effect of the spices used [18-20].

A significant (P ≤ 0.05) increase in TBA value during frozen storage was reported in chicken nuggets [6,21], Iberian ham [22] (Martin et al., 2000), and fish fingers [23] and buffalo meat burgers [2003]. Wang et al. [24] has reported TBA values of around 2.0 in fresh chicken meat after vacuum packaging and storage for 7 weeks at 7°C.

### 3.2. Texture

The shear values for meat pieces observed a marginal decrease (17.64%) during 6 month frozen storage (Table 2), indicating that the freezing and storage in frozen condition has little effect on the textural qualities of meat in the curry. Initially, the samples required higher shear force i.e. 34 N but during storage the force decreased to 28 N. This decrease in shear force can be attributed to both cooking and freezing process. Combes et al. [25] suggested that with advancement in cooking process several changes occur due to heat application as well as mechanical properties are influenced. Similarly, freezing also leads to textural changes as during the process of freezing ice crystals are formed in between the fibres that causes stretching and ruptures of connective tissues thus inducing higher tenderization. Hence there is a decrease in shear force values during freezing. Also, Shanks et al. [26] have reported that tenderization in meat is highly dependent on freezing rate, storage temperature and frozen storage duration that affect the amount of intracellular crystal formation and physical disruption occurring in muscle.

### 3.3. Microbiological Quality

Various microbiological tests were carried out have shown that freshly prepared chicken curry (before freezing) had microbial counts of 1 × 10^2 and 2 × 10^1 (in log cfu/g) for SPC, yeasts and molds respectively (Table 3). Banwart [27] suggested that microbial specifications for cooked poultry products should be in the range of 4 - 5 log cfu/g for aerobic plate counts. During freezing microbes become dormant thus, there is limited or no microbial spoilage at low temperature [28]. Studies have revealed that beef frozen for up to 90 days did not spoiled due to microbial growth [29]. S. aureus, coliform, salmonella and E. coli could not be detected in these products.

After a period of 6 months a decrease in both SPC and yeasts & molds count were observed to <10 and 10 respectively that can be due to decrease in pH that was observed with an increase in storage period. Absence of S. aureus, coliform, salmonella and E. coli can be contributed by thermal processing initially followed by hy-

<table>
<thead>
<tr>
<th>Storage period (in months)</th>
<th>pH In Meat Sample</th>
<th>pH In Gravy</th>
<th>FFA (as percentage oleic acid) In Meat Sample</th>
<th>FFA (as percentage oleic acid) In Gravy</th>
<th>TBA (mg malonaldehyde per Kg fat) In Meat Sample</th>
<th>TBA (mg malonaldehyde per Kg fat) In Gravy</th>
<th>Shear force (N) In Meat Sample</th>
<th>Shear force (N) In Gravy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.73 ± 0.03</td>
<td>6.02 ± 0.11</td>
<td>0.28 ± 0.02</td>
<td>0.29 ± 0.05</td>
<td>1.68 ± 0.11</td>
<td>2.21 ± 0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>5.65 ± 0.05</td>
<td>5.90 ± 0.05</td>
<td>0.31 ± 0.02</td>
<td>0.35 ± 0.04</td>
<td>2.00 ± 0.23</td>
<td>2.32 ± 0.04</td>
<td>34 ± 1.52</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5.40 ± 0.03</td>
<td>5.60 ± 0.30</td>
<td>0.29 ± 0.07</td>
<td>0.42 ± 0.10</td>
<td>2.26 ± 0.12</td>
<td>2.36 ± 0.07</td>
<td>33 ± 1.00</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5.68 ± 0.09</td>
<td>5.70 ± 0.10</td>
<td>0.28 ± 0.04</td>
<td>0.37 ± 0.04</td>
<td>2.03 ± 0.25</td>
<td>2.25 ± 0.02</td>
<td>32 ± 1.20</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>5.60 ± 0.17</td>
<td>5.70 ± 0.10</td>
<td>0.34 ± 0.01</td>
<td>0.35 ± 0.04</td>
<td>2.08 ± 0.09</td>
<td>2.25 ± 0.22</td>
<td>31 ± 1.52</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>5.55 ± 0.03</td>
<td>5.69 ± 0.02</td>
<td>0.41 ± 0.05</td>
<td>0.44 ± 0.06</td>
<td>2.19 ± 0.20</td>
<td>2.30 ± 0.20</td>
<td>29 ± 1.23</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>5.51 ± 0.01</td>
<td>5.68 ± 0.09</td>
<td>0.45 ± 0.07</td>
<td>0.46 ± 0.04</td>
<td>2.34 ± 0.13</td>
<td>2.45 ± 0.08</td>
<td>28 ± 1.92</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean± SD, n = 3.
Physico-Chemical Changes in Ready to Eat Pineapple Chicken Curry during Frozen Storage

Table 3. Changes in microbiological quality (counts in log cfu/g) of pineapple chicken curry during frozen storage (−18°C ± 2°C).

<table>
<thead>
<tr>
<th>Samples</th>
<th>SPC</th>
<th>Coliform</th>
<th>Yeast &amp; Mold</th>
<th>S. aureus</th>
<th>Salmonella</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>1 × 10^2</td>
<td>Nil</td>
<td>2 × 10^1</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>2 Months</td>
<td>4 × 10^1</td>
<td>Nil</td>
<td>1 × 10^1</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>4 Months</td>
<td>2 × 10^1</td>
<td>Nil</td>
<td>1 × 10^1</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>6 Months</td>
<td>&lt;10</td>
<td>Nil</td>
<td>1 × 10^1</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

gienic packaging in addition to the antibacterial effect of spices [30,31]. Finally before storage, samples were subjected to low temperature that also had an inhibitory effect on the growth of undesirable microbes and preventing spoilage. Narasimha et al. [32] suggested that meat products are spoiled when an off odor, slime formation along with the microbial population of 8 log cfu/g on the surface is evident.

3.4. Fatty Acid Estimation by Gas Chromatography

The individual fatty acid composition of pineapple chicken curry were estimated to find out the effect of pineapple on fat oxidation during storage. The stability of various saturated, monounsaturated and polyunsaturated fatty acids during 6 months storage at −18°C were evaluated. The results of this study were depicted in Table 4. From the table it could be observed that the sample contains a mixture of fatty acids both saturated and unsaturated fatty acids. Unsatuated fatty acids constitute both monounsaturated (MUFA) and polyunsaturated (PUFA). Monounsaturated fatty acids are dominant and constitute approximately 52%. The oxidation of lipid is one of the primary causes of deterioration of meat during processing and storage leading to development of off-flavour, decrease in nutritive value, loss of colour, texture etc. So the degradation of unsaturated fatty acids have been investigated by GC.

From the data, it could be clear that, out of saturated fatty acids lauric, myristicand palmitic found in low quantities. Stearic contribute the major mono unsaturated fatty acids. The PUFA i.e., linoleic, linolenic and arachidonic acids were also present in small quantities. From the studies, it was observed that saturated fatty acids do not vary significantly during storage, whereas MUFA and PUFA showed a significant change during storage.

3.5. Sensory Quality

Mean sensory scores given by panel members for 0-day samples for overall acceptability were in the range of 8.34 ± 0.32 on a 9-point hedonic scale (Table 5). During the storage period of 6 months a gradual decrease in scores were observed from 8.34 to 7.46 on 0 day and after 6 months of frozen storage respectively. The scores given by panel members have shown that the samples

Table 4. Fatty acid profile of sample.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Gram percentage</th>
<th>Gram percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric (C12:0)</td>
<td>0.76 ± 0.03</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>Mysitic (C14:0)</td>
<td>5.40 ± 0.21</td>
<td>5.32 ± 0.19</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>17.61 ± 0.12</td>
<td>17.49 ± 0.15</td>
</tr>
<tr>
<td>Palmitoleic (C16:1)</td>
<td>3.02 ± 0.09</td>
<td>2.85 ± 0.07</td>
</tr>
<tr>
<td>Stearic (C18:0)</td>
<td>20.23 ± 0.24</td>
<td>19.91 ± 0.21</td>
</tr>
<tr>
<td>Oleic (C18:1)</td>
<td>32.34 ± 0.14</td>
<td>31.51 ± 0.17</td>
</tr>
<tr>
<td>Linoleic (C18:2)</td>
<td>11.4 ± 0.17</td>
<td>11.06 ± 0.20</td>
</tr>
<tr>
<td>Linolenic (C18:3)</td>
<td>1.5 ± 0.02</td>
<td>1.24 ± 0.04</td>
</tr>
<tr>
<td>Arachidonic(C20:4)</td>
<td>0.82 ± 0.12</td>
<td>0.64 ± 0.13</td>
</tr>
</tbody>
</table>

Mean ± SD, n = 3.

Table 5. Changes in sensory quality of pineapple chicken curry product during frozen (−18°C ± 2°C) storage.

<table>
<thead>
<tr>
<th>Storage period in months</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>8.6 ± 0.23</td>
<td>8.5 ± 0.23</td>
<td>8.3 ± 0.32</td>
<td>8.2 ± 0.21</td>
<td>8.0 ± 0.26</td>
<td>7.9 ± 0.35</td>
<td>7.8 ± 0.43</td>
</tr>
<tr>
<td>Flavour</td>
<td>8.3 ± 0.27</td>
<td>8.3 ± 0.27</td>
<td>8.1 ± 0.32</td>
<td>8.0 ± 0.29</td>
<td>7.8 ± 0.46</td>
<td>7.6 ± 0.36</td>
<td>7.4 ± 0.32</td>
</tr>
<tr>
<td>Mouth feel</td>
<td>8.4 ± 0.23</td>
<td>8.1 ± 0.32</td>
<td>8.0 ± 0.45</td>
<td>7.9 ± 0.42</td>
<td>7.7 ± 0.42</td>
<td>7.6 ± 0.24</td>
<td>7.5 ± 0.28</td>
</tr>
<tr>
<td>Consistency</td>
<td>8.2 ± 0.32</td>
<td>8.3 ± 0.42</td>
<td>8.0 ± 0.78</td>
<td>7.9 ± 0.64</td>
<td>7.7 ± 0.74</td>
<td>7.6 ± 0.74</td>
<td>7.5 ± 0.64</td>
</tr>
<tr>
<td>Texture</td>
<td>8.2 ± 0.54</td>
<td>8.4 ± 0.42</td>
<td>8.0 ± 0.68</td>
<td>7.7 ± 0.24</td>
<td>7.4 ± 0.32</td>
<td>7.3 ± 0.86</td>
<td>7.1 ± 0.32</td>
</tr>
<tr>
<td>OAA</td>
<td>8.34 ± 0.32</td>
<td>8.32 ± 0.33</td>
<td>8.08 ± 0.51</td>
<td>7.94 ± 0.36</td>
<td>7.72 ± 0.44</td>
<td>7.6 ± 0.51</td>
<td>7.46 ± 0.4</td>
</tr>
</tbody>
</table>

Mean ± SD.
maintained the quality even after the storage period of 6 months under frozen conditions since all the scores were observed above 7 that are in an acceptable range. Similarly a decrease in trend was observed for beef patties [33], chicken nuggets [7] and egg loaf [34] and chicken curry [1].

4. Conclusion

Consumer’s rising interest in RTE has motivated researchers to standardize the process of traditional products that are long being known as well as are highly consumed. In the present study recipe of pineapple products that are long being known as well as are highly quality for a period of 6 months at 2°C ± 2°C.

the combined effect of low temperature and anti-oxidant effect of spices used. Fatty acid profile has been established by GC and several fatty acids were quantified. Thus, the product quality was maintained and it can be concluded that it can be stored without marked loss in quality for a period of 6 months at −18°C ± 2°C.

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