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Effect of incubation and storage temperatures on Quality of set Yoghurt

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INTRODUCTION

Yoghurt is a popular fermented milk product consumed worldwide. Yoghurt is produced in different forms such as whole mild yoghurt, skim milk yoghurt, cream yoghurt, fruit yoghurt and liquid yoghurt (Ashton, 1963).

Milk for yoghurt production must have a low bacteria count, should not contain enzymes and chemical substances, which may slow down the development of the yoghurt culture and should not contain penicillin and bacteria phage (Deeth and Tamime, 1981).
Yoghurt can be spoiled after any contamination and growth of yeast and moulds. Besides type of milk, heat treatment, starter, incubation temperature and storage conditions can affect the quality of set yoghurt. In the Sudan, the manufacturing of yoghurt is inherently dependent on traditional methods but later some factories started to manufacture yoghurt using modern techniques that lead to improvement in yoghurt production methodology and consequently increased production quantitatively and qualitatively. In the Sudan, there are many different types of fermented dairy products such as robe and mish (Humphery and Maurean, 1969).

There are some defects occurring during the manufacture of set yoghurt from cow milk such as high acidity associated with harsh flavor and aroma, bitter-ness, bad taste, whey off and the defective texture and bodyness (Nikolov, 1964).

The objectives of this study were to try standardizing optimum incubation time and temperature for set yoghurt mix prepared as well as to investigate optimum storage temperature of such product.

MATERIALS AND METHODS

Materials:
All materials were obtained from Khartoum Dairy Production Company Ltd. (K.D.P.C). These include cow milk, skimmed milk powder and mixed starter culture (CH-1, B-3).

Methods:
The chemical analysis was carried out using the facilities of K.D.P.C laboratory.

Preparation of yoghurt mix:
A 100 Kg of low heat skimmed milk powder were reconstituted in 341 liters of distilled water added to 60 liters of fresh cow milk and blended for 20 minutes, pasteurized at 90°C for 20 minutes and cooled to 45°C. The starter culture was added and incubated at 45°C for four hours then cooled to less than 20°C.

The manufacture of modified yoghurt sample:
All the modified yoghurt mix with total solid (T.S) of 14.3%, fat of 2.5% were heated to 90°C for 20 minutes, homogenized (at 200 bar), cooled to 45°C, inoculated with 2% mother culture (type H1, B3) and incubated at different temperatures (41, 43, 45°C) for 4 hours. After incubation the modified yoghurt sample was transferred to refrigerator directly and stored at two temperatures of 6 and 10°C for 10 days. (Total acidity, whey volume, sensory quality and viscosity) were carried out at zero hour and after 1, 4, 6 and 10 days of storage.

The chemical analysis:
The chemical analysis was carried out for raw cow milk, toned milk and yoghurt mix. Tests carried out for raw milk include the followings:

Density:
According to AOAC the density was determined by calibrated lactometer. The lactometer readings were corrected. The density was calculated from the formula:

\[
\text{Density} = 1 + \left(\frac{C.L.R}{1000}\right)
\]

\[
C.L.R \text{ is Correct Lactometer Reading}
\]

Total solids (T.S):
T.S. of raw milk was determined according to AOAC 1990. First the correct lactometer reading and fat content were determined, and then the total solid was calculated from Richmond’s equation:

\[
\% \text{T.S} = (0.25 \times C.L.R) + (1.22 \times F) + (0.74)
\]

Where:

\[
C.L.R = \text{Correct Lactometer Reading}
\]

\[
F = \text{fat content}
\]

0.25, 0.74 and 1.22 = constants

Solid Non Fat (SNF):
SNF was determined according to AOAC (1990). First the fat content and total solids content were determined then
solid non fat was determined from the following equation:
\[
\% \text{S.N.F} = (\% \text{T.S} - \% \text{F})
\]
Where:
- S.N.F = Solid Non Fat
- T.S = Total Solids
- F = Fat

**Fat content:**
- It was determined by Gerber method according to (Bradly et al., 1992).

**Titratable acidity:**
- The acidity of milk was determined according to the AOAC method (1990).

**Chemical analysis of yoghurt:**

**Fat content:**
- The fat content of yoghurt was determined by Gerber method according to (Bradly et al., 1992).

**Total solids (T.S):**
- The total solids content was determined according to the modified method of AOAC (1990). The total solids content was calculated from the following equation:
\[
\text{T.S}\% = \frac{W_1}{W} \times 100
\]
Where:
- \(W_1\) = Weight of yoghurt sample after drying
- \(W\) = Original weight of yoghurt sample

**Solid Non Fat (S.N.F):**
- It was determined from the following equation:
\[
\% \text{S.N.F} = \text{T.S}\% - \% \text{F}
\]
Where \(F\) = Fat content.

**Titratable acidity:**
- The acidity of yoghurt was determined according to the AOAC (1990).

**Protein content:**
- The protein content was determined by kjeldahl method according to the AOAC (1990). The protein content was calculated from the following equation:
\[
\text{N}\% = \frac{T \times 0.1 \times 0.014 \times 100}{W}
\]
Where:
- \(T\) = Titration reading
- \(W\) = Weight of original sample
- \(\%\) protein content = \(\text{N}\% \times 6.38\)

**Lactose content:**
- It was determined by the Lane and Eynons method according to the AOAC method (1990). The lactose content was calculated from the sugar Table.

**Ash content:**
- It was determined according to the AOAC method (1990). The ash content was calculated by the following equation:
\[
\text{Ash} \% = \frac{W_1}{W} \times 100
\]
Where:
- \(W_1\) = Weight of ash.
- \(W\) = Original weight of yoghurt.

**pH:**
- It was determined by digital pH Meter.

**Viscosity:**
- The viscosity was determined by HAKK viscometer.
- Low speed: X sec.
- Fast speed: Y sec.
- Average = \((X+Y)/2 = Z\) sec.
- Viscosity = \(Z\) sec. \(\times 44.648\)
- \((\text{where} \ 44.648 = \text{viscosity factor})\).

**Wheying-off:**
- Was determined by sucking the water on surface of the curd and pouting in a graduated cylinder.

**Sensory evaluation of yoghurt:**
- The sensory evaluation was carried out by scoring method (panel test) according to (Madanlal, 1977) as follows:
  - Appearance and colour — 2 points;
  - Consistency — 3 points;
  - Flavor — 6 points;
  - Taste — 4 points;
  - Total score — 15 points
- A panel test was carried out by 10 trained technicians. Evaluation of test:
  - 13-15 points = very good / excellent.
  - 10-12 points = good.
  - 7-9 points = fair.
  - Less than 7 points = bad.

**Experimental design and data analysis:**
- The design used in this study was the 2 factor split-plot design and samples have been taken in triplicate. Mean separation has been done using least significant difference (L.S.D) at 5.00% probability, (SAS, 1988).
RESULTS AND DISCUSSION

Incubation temperature:

The results shown in Table (1) indicated that the best incubation temperature is 45°C which gave the best quality with regard to (total acidity (T.A), whey volume, viscosity and the average value of sensory quality) in comparison with incubation temperatures 41°C and 43°C (large amount of whey separated and decrease in consistency). The results are in agreement with that obtained by (Kosikowiski, 1982) who reported that warm mixture should be transferred into containers and then held at a temperature of 45°C in the incubator for 3-6 hours until a titratable acidity of 0.9-1.2% could be attained.

Table 1: Effect of incubation temperatures on the quality attributes of yoghurt.

<table>
<thead>
<tr>
<th>Incubation Temperature (°C)</th>
<th>Total acidity (%)</th>
<th>Separated whey (ml)</th>
<th>Viscosity (cP)</th>
<th>Sensory quality (total scores)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41°C</td>
<td>0.72c</td>
<td>0.50c</td>
<td>346.60c</td>
<td>14.37c</td>
</tr>
<tr>
<td>43°C</td>
<td>0.71b</td>
<td>0.40b</td>
<td>450.20b</td>
<td>14.50b</td>
</tr>
<tr>
<td>45°C</td>
<td>0.75a</td>
<td>0.31c</td>
<td>476.20b</td>
<td>14.67a</td>
</tr>
<tr>
<td>L.S.D (p&lt;0.05)</td>
<td>0.001924</td>
<td>0.001924</td>
<td>0.001924</td>
<td>0.001924</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within each column differ significantly (p<0.05).

Storage conditions:

The results in Table (2) indicated that the storage temperature of 6°C gave the best quality with regard to (total acidity, whey volume, viscosity and total score for sensory quality) compared to storage temperature of 10°C which gave large amount of separated whey and bitterness. The results are nearly in agreement with (Bertelosen, 1964) who showed that control of storage temperature at 4-5°C is most important and higher temperatures can lead to defects such as bitterness.

Table 2: Effect of storage period on the quality attributes of yoghurt.

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Total acidity (% lactic acid)</th>
<th>Separated whey (ml)</th>
<th>Viscosity (cP)</th>
<th>Sensory quality (total scores)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td>0.64c</td>
<td>0.00c</td>
<td>145.10c</td>
<td>14.23c</td>
</tr>
<tr>
<td>One day at 6°C</td>
<td>0.73b</td>
<td>0.06b</td>
<td>625.90a</td>
<td>14.80a</td>
</tr>
<tr>
<td>One day at 10°C</td>
<td>0.81c</td>
<td>1.14c</td>
<td>520.90b</td>
<td>14.50b</td>
</tr>
<tr>
<td>L.S.D (p&lt;0.05)</td>
<td>0.001924</td>
<td>0.001924</td>
<td>0.001924</td>
<td>0.001924</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within column differ significantly (p<0.05).

Effect of storage period temperature and incubation temperature on the yoghurt level of acidity (% lactic):

Table (3) shows that the development of acidity in yoghurt mix incubated at temperature of 45°C gave better results (optimum acidity without whey separated) compared with temperatures of 43°C and 41°C.

Table 3: The interaction between the storage period temperature and incubation temperature on the yoghurt level of acidity (% lactic).

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Incubation temperature</th>
<th>41°C</th>
<th>43°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td></td>
<td>0.65c</td>
<td>0.60b</td>
<td>0.68d</td>
</tr>
<tr>
<td>One day at 6°C</td>
<td></td>
<td>0.72c</td>
<td>0.72c</td>
<td>0.75d</td>
</tr>
<tr>
<td>One day at 10°C</td>
<td></td>
<td>0.80c</td>
<td>0.81b</td>
<td>0.84b</td>
</tr>
<tr>
<td>L.S.D (p&lt;0.05)</td>
<td></td>
<td>0.001031</td>
<td>0.001031</td>
<td>0.001031</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within columns and rows differ significantly (p<0.05).
Effect of wheying-off on quality of yoghurt:

Table (4) shows that the whey separated increased at storage temperature of 10°C more than at temperature of 6°C. That means the temperature of 6°C gave better quality compared to temperature of 10°C (good coagulum and texture). The results are in agreement with (Shukla et al., 1988) who found that the wheying-off is a major defect in yoghurt therefore stabilizers and additives of milk powder usually (0.5-2.5%) are used to check wheying-off in yoghurt.

Table 4: The interaction between the storage period temperature and incubation temperature on the yoghurt whey separated.

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41°C</td>
</tr>
<tr>
<td>Zero time</td>
<td>0.00a</td>
</tr>
<tr>
<td>One day at 6°C</td>
<td>0.20a</td>
</tr>
<tr>
<td>One day at 10°C</td>
<td>1.30a</td>
</tr>
<tr>
<td>L.S.D (p≤0.05)</td>
<td>0.001031</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within each column and row differ significantly (p≤0.05).

Effect of storage temperatures on the viscosity:

Table (5) shows a comparison between development of viscosity in yoghurt mix incubated at temperature of 45°C and stored at temperature of 6°C and 10°C. The viscosity of yoghurt held at temperature of 6°C gave better results than that held at temperature of 10°C, however, low temperature increased viscosity and improved product firmness and stability.

Table 5: The interaction between the storage period temperature and incubation temperature on the yoghurt viscosity (in cP).

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41°C</td>
</tr>
<tr>
<td>Zero time</td>
<td>133.90h</td>
</tr>
<tr>
<td>One day at 6°C</td>
<td>588.10c</td>
</tr>
<tr>
<td>One day at 10°C</td>
<td>401.80e</td>
</tr>
<tr>
<td>L.S.D (p≤0.05)</td>
<td>0.001031</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within columns and rows differ significantly (p≤0.05).

Effect of storage temperature on the acidity:

Table (6) shows a comparison between development of acidity in yoghurt mix incubated at temperature of 45°C and stored at temperature of 6°C and 10°C. The acidity of yoghurt held at temperature of 6°C was better than that held at temperature of 10°C. Higher temperature (temperature of 10°C) can lead to defects such as bitterness and low temperature (temperature of 6°C), can reduce the fermentation and allow the desired acidity to be reached.

Table 6: The interaction between the storage period and storage temperature on the yoghurt amount of acidity.

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Storage temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6°C</td>
</tr>
<tr>
<td>Zero time</td>
<td>0.68a</td>
</tr>
<tr>
<td>One day</td>
<td>0.75b</td>
</tr>
<tr>
<td>Four days</td>
<td>0.78d</td>
</tr>
<tr>
<td>Six days</td>
<td>0.85c</td>
</tr>
<tr>
<td>Ten days</td>
<td>1.10d</td>
</tr>
<tr>
<td>L.S.D (p≤0.05)</td>
<td>0.001012</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within columns and rows differ significantly (p≤0.05)
Effect of storage period temperature and incubation temperature on the yoghurt sensory quality:

The results in Table 7 indicated that the incubation temperature of 45°C and storage temperature of 6°C gave the best quality with regard to sensory evaluation in comparison with temperatures of 41°C and 43°C and storage temperature of 10°C. The results are in agreement with (Porter, 1975) who suggested that when the yoghurt was set firm it should be transferred to a cold store and stored at 5-8°C immediately.

Table 7: The interaction between the storage period temperature and incubation temperature on the yoghurt sensory quality.

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41°C</td>
</tr>
<tr>
<td>Zero time</td>
<td>14.00^b</td>
</tr>
<tr>
<td>One day at 6°C</td>
<td>14.70^c</td>
</tr>
<tr>
<td>One day at 10°C</td>
<td>14.40^f</td>
</tr>
<tr>
<td>L.S.D (p ≤ 0.05)</td>
<td>0.001031</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within columns and rows differ significantly (p ≤ 0.05).

Effect of the storage period on the quality attributes of yoghurt:

Table (8) shows that the amount of acidity increased at ten days compared to zero, one, four and six days. The results are in disagreement with that obtained by (Gaafer, 1992) who found that the decrease in acetaldehyde and acetone (aroma) and the increase in acetic acid were closely related to the rapid decrease in acceptability after 8-10 days.

Table (8) also shows that whey separated increased at ten days more than at zero, one, four and six days. The results are in agreement with (Pette and Smith, 1964) who noted that homogenization is essential for the production of a firm curd of yoghurt.

Table 8: The interaction between the storage period and the quality attributes of yoghurt

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Quality attributes of yoghurt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total acidity (% lactic)</td>
</tr>
<tr>
<td>Zero time</td>
<td>0.68^e</td>
</tr>
<tr>
<td>One day</td>
<td>0.79^d</td>
</tr>
<tr>
<td>Four days</td>
<td>0.95^c</td>
</tr>
<tr>
<td>Six days</td>
<td>1.15^b</td>
</tr>
<tr>
<td>Ten days</td>
<td>1.30^a</td>
</tr>
<tr>
<td>L.S.D</td>
<td>0.001242</td>
</tr>
</tbody>
</table>

Mean values having different superscript letters within each column differ significantly (p ≤ 0.05)

CONCLUSIONS
The study reached the following conclusions:

a. The incubation temperature of 45°C gave generally better quality compared to incubation temperatures 41°C and 43°C.

b. The storage temperature of 6°C gave generally better quality compared to storage temperature of 10°C.
REFERENCES