CHAPTER - 2

REVIEW OF LITERATURE

2.1 History -

Pickling is a relatively old method of food preservation. First pickles were produced over 4000 years ago using cucumbers native to India. Pickled cucumbers are known in Mesopotamia around 2030 B.C. when inhabitants from Northern India brought cucumber seeds to the Tigris Valley. Pickles are mentioned twice in Bible and were known to the ancient Egyptians like Cleopatra who attributed some of her beauty to fermented pickles while Aristotle praised the healing effects of pickled cucumbers. Other notable pickle lovers from history include Emperors like Julius Caesar and Tiberius, King John and Queen Elizabeth-I of England, Samuel Pepys, Amergio Vespucci, George Washington, Thomas Jefferson and Nepoleon Bonaparte (http://www.bbc.co.uk/dna/h2g2/A3/A310C168/2007). In America, pickles have always been popular. The first travelers to America kept pickles in large supply because they were nutritious and did not spoil during long journey. Amerigo Vespucci, America’s name sake was also a pickle salesman. He was the main pickle supplier to many ships. The first large scale commercial production of pickles did not take place until 1820, when Nicholas Appert began selling pickles in jars (http://www.bbc.madehow.com/Vol.4.Picklehtml).

2.2 Origin of word ‘Pickle’ -

The English word ‘Pickle’ is derived from ‘Pikel’ meaning “a spicy sauce or gravy served with meat.” This is different, but obviously related to the Dutch source, ‘pekel’, meaning a solution of spiced brine used for preserving and flavouring foods.

According to Webster’s dictionary, “Pickle is any brine, vinegar or spicy solution used to preserve or marinate food” while as per Universal dictionary, “Pickle is any edible product especially vegetables or fruit, that has been preserved and flavoured in a solution of brine or vinegar”.
2.3 Raw materials used for pickle making -

There are six basic types of ingredients used for pickle making. The main raw material is fruit, vegetable or any food using which the pickles are prepared. The additional ingredients include acids, flavouring agents, colorants, preservatives and stabilizers (Joshi and Pandey, 1999).

Vegetables and fruits selected for pickle production are with large water quantity and low sugar content. Slightly under ripe fruits and vegetables are used for pickle preparation. If ripened fruits or sweet fruits are used for pickle production, yeasts grow faster than lactic acid bacteria and produce alcohol instead of lactic acid. So the fruits with low sugar content or fruits harvested before ripening are used for pickle production by lactic acid fermentation.

Local availability of fruits and vegetables reflects in type of pickles in that locality. Pickles are prepared world wide. Raw Materials used in pickle production are different in different countries. The microbially fermented pickles show wide diversity with respect to raw materials used. The raw materials used are cucumber, durian fruit, beet roots, lemon, green mango, cabbage, green olives, jack fruit, radish, bambooshoot, red onion, ginger, papaya, carrots, turnips, banana, cauliflower stalks, aubergines, otaki-turnip, leafy vegetables and mustard leaf.

The common raw materials used for pickle production in India, especially in Western Maharashtra are listed in Table 2.1.
Table 2.1 Raw materials used for pickle production in Western Maharashtra  
(Sharma et al., 1996; Singh and Karthikeyan, 2000)

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Vernacular name</th>
<th>Common name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amba</td>
<td>Mango</td>
<td>Mangifera indica</td>
</tr>
<tr>
<td>2</td>
<td>Limboo/Kagadi Limboo</td>
<td>Lemon</td>
<td>Citrus medica vr. acida</td>
</tr>
<tr>
<td>3</td>
<td>Hiravi mirchi</td>
<td>Green chilli</td>
<td>Capsicum annum</td>
</tr>
<tr>
<td>4</td>
<td>Haldi</td>
<td>Turmeric</td>
<td>Curcuma domestica</td>
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<td>Awala</td>
<td>Amla</td>
<td>Emblica officinalis</td>
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<td>Aale</td>
<td>Ginger</td>
<td>Zinziber officinale</td>
</tr>
<tr>
<td>7</td>
<td>Chinch</td>
<td>Tamarind</td>
<td>Tamarindus indica</td>
</tr>
<tr>
<td>8</td>
<td>Bhokare/Bhokar</td>
<td>Cordia</td>
<td>Cordia myxa</td>
</tr>
<tr>
<td>9</td>
<td>Karavand</td>
<td>Karonda</td>
<td>Carrissa carandas</td>
</tr>
<tr>
<td>10</td>
<td>Bor</td>
<td>Jujube</td>
<td>Zinzypus jujube</td>
</tr>
<tr>
<td>11</td>
<td>Kanda</td>
<td>Onion</td>
<td>Allium cepa</td>
</tr>
<tr>
<td>12</td>
<td>Lasoon</td>
<td>Garlic</td>
<td>Allium sativum</td>
</tr>
<tr>
<td>13</td>
<td>Tomato</td>
<td>Tomato</td>
<td>Lycopersicon esculentum</td>
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<td>14</td>
<td>Karale</td>
<td>Bitter gourd</td>
<td>Momordica charantia</td>
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<td>15</td>
<td>Shevaga</td>
<td>Drumsticks</td>
<td>Moringa oleifera</td>
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<tr>
<td>16</td>
<td>Minemola</td>
<td>Minemola</td>
<td>Coleus barbatus</td>
</tr>
<tr>
<td>17</td>
<td>Phoolgobi/Kobi</td>
<td>Cabbage</td>
<td>Brassica oleracea var. rapitata</td>
</tr>
<tr>
<td>18</td>
<td>Phulwar</td>
<td>Cauliflower</td>
<td>Brassica olevacea var. bortrytis</td>
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<tr>
<td>19</td>
<td>Tondale</td>
<td>Kovai fruit</td>
<td>Coccinia cordifolia</td>
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<tr>
<td>20</td>
<td>Gajar</td>
<td>Carrot</td>
<td>Daucus carota</td>
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<td>21</td>
<td>Mula</td>
<td>Radish</td>
<td>Raphanus sativus</td>
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<tr>
<td>22</td>
<td>Methi</td>
<td>Fenugreek seeds</td>
<td>Trigonella foenum var. graecum</td>
</tr>
<tr>
<td>23</td>
<td>Govari</td>
<td>Cluster bean</td>
<td>Cymopsis tetragonoloba</td>
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<td>24</td>
<td>Bhendi</td>
<td>Ladies finger</td>
<td>Abelmoschus esculentus</td>
</tr>
<tr>
<td>25</td>
<td>Navalkol</td>
<td>Knoll-kohl</td>
<td>Brassica olevacea var. caulovapa</td>
</tr>
<tr>
<td>Sr No.</td>
<td>Vernacular name</td>
<td>Common name</td>
<td>Scientific name</td>
</tr>
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<td>----------------</td>
<td>--------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>26</td>
<td>Idlimbu</td>
<td>Bitter lemon</td>
<td><em>Citrus limon</em></td>
</tr>
<tr>
<td>27</td>
<td>Phanas</td>
<td>Jack-fruit</td>
<td><em>Artocaprus heterophyllus</em></td>
</tr>
<tr>
<td>28</td>
<td>Papaya/papai</td>
<td>Papaya</td>
<td><em>Carica papaya</em></td>
</tr>
<tr>
<td>29</td>
<td>Jawas</td>
<td>Linseed</td>
<td><em>Linum usitatissimum</em></td>
</tr>
</tbody>
</table>

In all above Indian pickles naturally occurring microbial starter microorganisms are used. The ingredients added during pickle production supply nutrients to lactic acid bacteria naturally present on the main raw materials. Lactic acid starter bacteria being fastidious need variety of nutrients supplemented extraneously. Hence favourable growth conditions provided to starter microorganisms are of paramount significance in the success of desired fermented pickle product. Hence selection of raw material and other ingredients is especially to be taken care of. The nutritive value of main raw materials and ingredients which supports the growth of Lactic Acid Bacteria (LAB) in microbially fermented pickle production is listed in Tables 2.2 and 2.3.

**Table 2.2 Nutritive value of main raw materials of pickles (per 100g)**

(Gopalan *et al.*, 1989)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mango</th>
<th>Lemon</th>
<th>Chilli</th>
<th>Amla</th>
<th>Carrot</th>
<th>Bitter gourd</th>
<th>Drumstick</th>
<th>Ginger</th>
<th>Tomato (Green)</th>
<th>Tomato (Ripe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kw)</td>
<td>65.00</td>
<td>11.10</td>
<td>29.00</td>
<td>13.70</td>
<td>48.00</td>
<td>25.00</td>
<td>26.00</td>
<td>67.00</td>
<td>23.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.5</td>
<td>1.0</td>
<td>2.9</td>
<td>0.5</td>
<td>0.9</td>
<td>1.6</td>
<td>2.5</td>
<td>2.3</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.3</td>
<td>0.9</td>
<td>0.6</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.9</td>
<td>0.1</td>
<td>0.2</td>
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<tr>
<td>Carbohydrates (g)</td>
<td>17.0</td>
<td>1.7</td>
<td>3.0</td>
<td>3.4</td>
<td>10.6</td>
<td>4.2</td>
<td>3.7</td>
<td>2.4</td>
<td>3.6</td>
<td>3.6</td>
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**Minerals (mg)**

<table>
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<tr>
<th>Element</th>
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<th>70.0</th>
<th>30.0</th>
<th>50.0</th>
<th>80.0</th>
<th>20.0</th>
<th>30.0</th>
<th>200.0</th>
<th>20.0</th>
<th>48.0</th>
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<tr>
<td>Calcium</td>
<td>0.13</td>
<td>00.26</td>
<td>4.40</td>
<td>1.20</td>
<td>1.03</td>
<td>0.61</td>
<td>0.18</td>
<td>3.50</td>
<td>1.80</td>
<td>0.64</td>
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<td>19.4</td>
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<td>-</td>
<td>17.2</td>
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<td>405.0</td>
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<tr>
<td>Magnesium</td>
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<td>-</td>
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<td>-</td>
<td>530.0</td>
<td>70.0</td>
<td>110.0</td>
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<td>36.0</td>
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<tr>
<td>Phosphorous</td>
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<td>225.0</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Potassium</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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</table>
### Table 2.3 Nutritive value of other ingredients of pickles (per 100g)

*(Gopalan et al., 1989)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Garlic</th>
<th>Turmeric</th>
<th>Mustard Seeds</th>
<th>Fenugreek Seeds</th>
<th>Asafoetida</th>
<th>Dry Chillies</th>
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</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>62.0</td>
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<td>8.5</td>
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<tr>
<td>Energy (Kw)</td>
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<td>349.0</td>
<td>541.0</td>
<td>333.0</td>
<td>297.0</td>
<td>246.0</td>
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<tr>
<td>Protein (g)</td>
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<td>6.3</td>
<td>20.0</td>
<td>26.2</td>
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<td>15.9</td>
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<tr>
<td>Fat (g)</td>
<td>0.1</td>
<td>5.1</td>
<td>39.7</td>
<td>9.8</td>
<td>1.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>29.8</td>
<td>69.4</td>
<td>23.8</td>
<td>44.1</td>
<td>67.8</td>
<td>31.6</td>
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</table>

### Minerals (mg)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Garlic</th>
<th>Turmeric</th>
<th>Mustard Seeds</th>
<th>Fenugreek Seeds</th>
<th>Asafoetida</th>
<th>Dry Chillies</th>
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<tbody>
<tr>
<td>Calcium</td>
<td>30.0</td>
<td>150.0</td>
<td>490.0</td>
<td>160.0</td>
<td>690.0</td>
<td>160.0</td>
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<tr>
<td>Iron</td>
<td>1.2</td>
<td>67.8</td>
<td>7.9</td>
<td>6.5</td>
<td>39.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Parameter</td>
<td>Garlic</td>
<td>Turmeric</td>
<td>Mustard Seeds</td>
<td>Fenugreek Seeds</td>
<td>Asafoetida</td>
<td>Dry Chillies</td>
</tr>
<tr>
<td>-----------------</td>
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<td>---------------</td>
<td>----------------</td>
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</tr>
<tr>
<td><strong>Minerals (mg)</strong></td>
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<td></td>
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<tr>
<td>Magnesium</td>
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<td>278.0</td>
<td>-</td>
<td>3.0</td>
<td>80.0</td>
<td>-</td>
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<td>370.0</td>
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<td>Zinc</td>
<td>1930.00</td>
<td>2720.00</td>
<td>4800.0</td>
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<td>Copper</td>
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<td>830.0</td>
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<td>Selenium</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>50.0</td>
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<td>Thiamine</td>
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<td>0.65</td>
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<td>211.0</td>
<td>1161.0</td>
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</tr>
</tbody>
</table>

### 2.4 Role of ingredients used in pickle fermentation

Ingredients used in pickle fermentation have three main functions –

a) Source of nutrients for fermentation microorganisms.

b) Restriction of unwanted microorganisms.

c) Development of flavour and taste in product.

#### 2.4.1 Salt

Lactic acid fermentation of fruits and vegetables is carried out in salted medium. The addition of salt is either by adding brine or by adding dry salt. There are two methods of adding brine 1) Low salt curing 2) High salt curing
1) **Low salt curing** - In this method brine which gives salinometer reading of $30^\circ$ (nearly 8% NaCl) is used initially and fruits are soaked in this brine after treatment to fruits. Then every week salt is added to rise the salinometer reading by $3^\circ$ until a final reading reaches to $60^\circ$(15.9% NaCl). By this method fermentation and curing occurs rapidly. But the chances of growth of spoilage causing organisms are also more.

2) **High salt curing** – In this method original brine used is of 10.6% salt ($40^\circ$ salinometer reading). The salt concentration of the brine is increased by $2^\circ$ per week till it reaches $50^\circ$ and then in further weeks salt concentration is added to increase $1^\circ$ salinometer reading to reach final $60^\circ$ salinometer reading. In this method, fermentation and curing are slower but risk of spoilage causing microorganisms is also less. Firmness of pickles is also more than low – salt curing method.

**Dry salted pickles** -

Dry salting is used for pickling many vegetables and fruits. For dry salt pickling any variety of common salt is suitable. But it should be of high quality. Impurities or additives cause problems -

1) Chemicals to reduce caking should not be used as they make the brine cloudy.

2) Lime impurities can reduce the acidity of the final product and reduce the shelf life of the product. So salt should contain less than 1% of sodium, calcium or magnesium carbonates or bicarbonates. These components will neutralise the acid produced by lactic acid bacteria and favour growth of proteolytic bacteria and make texture soft as undesired features (characters) of pickle.

3) Iron impurities can result in the blackening of the vegetables and also inhibition of starter microorganisms.

4) Magnesium impurities impart undesirable bitter taste.
5) Non iodised salt is recommended as iodine causes darkening of pickles (Khader, 2001).

Dry salted lime pickles are popular in India, Pakistan and North Africa. In preparing dry salted lime pickles, limes are placed in a layer of approximate height 2.5cm into a container. One kilogram of salt is added for every four kilogram of lime. The salt is sprinkled over the limes. Another layer of the lime and salt is added. This process is repeated until the container is three fourth full. A cloth is placed above lime layer and a weight added to compress lime. The brine is formed within 24 h. The container is then placed in sun for a week. Microbial fermentation starts as soon as brine formation and appearance of the bubbles of carbon dioxide takes place. Fermentation process completes between one to four weeks depending upon temperature which indicated by no release of gas bubbles (Ali, 2009).

The optimum salt concentration for lactic acid fermentation depends on the vegetable used for pickle production (Table 2.4).

**Table 2.4 Salt concentration during lactic fermentation of the most common vegetables**

(Montet et al., 1999)

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Salt concentration during fermentation (%)</th>
<th>Type of salting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sauerkraut</td>
<td>2 – 3</td>
<td>dry salt</td>
</tr>
<tr>
<td>Korean Kimchi</td>
<td>3</td>
<td>dry salt</td>
</tr>
<tr>
<td>Cucumbers</td>
<td>5 – 8</td>
<td>brine</td>
</tr>
<tr>
<td>Green olives</td>
<td>4 – 7</td>
<td>brine</td>
</tr>
<tr>
<td>Carrots</td>
<td>1.5 – 3</td>
<td>brine</td>
</tr>
</tbody>
</table>

(Salt concentration corresponds to NaCl content of brine)

**Effect of salt on fermenting organism and fermentation process** -

When vegetables or fruits are kept in salt, they lose water by exosmosis. Sugar, proteins, minerals and other substances from fruits and vegetables get dissolved in water. These dissolved substances are used as nutrients by lactic
acid bacteria and other microorganisms. Salt induces plasmolysis in plant cells. This creates anaerobic conditions around submerged plant product. At lowest concentration of salt (5%) lactic acid bacteria grow rapidly and produce acid. This production of acid decreases as there is increase in salt concentration (Table 2.5). But high salt concentration favours gas production. The acid formers grow best in 20° brine and poorest in 60° brine. The combination of acid and salt restrict yeast and other gas formers e.g. Enterobacter (Madeo et al., 2004).

Table 2.5 Effect of salt concentration on development of acid during pickling
(Pederson and Ward, 1949)

<table>
<thead>
<tr>
<th>Salt concentration %</th>
<th>Fermentation Time (day)</th>
<th>Total acid %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.91</td>
</tr>
<tr>
<td>2.25</td>
<td>1</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.78</td>
</tr>
<tr>
<td>3.5</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.91</td>
</tr>
</tbody>
</table>

Fabian and his associates studied effect of salt on bacterial activity in cucumber fermentation. They found proteolytic bacteria during first 24 h of fermentation, which go on decreasing as brine reaches upto 50° salinometer reading. The proteolytic bacteria were replaced by acid producers which died rapidly in 40° brine.
Salt not only prevents growth of spoilage causing and pathogenic bacteria but also favours growth of salt tolerant lactic acid bacteria and makes the pickle more firm and thus desired quality of pickle is achieved.

**Precautions during salt addition:** (USDA, 1995).

- Canning or pickling salt should be used, it contains no impurities.
- Too much salt will cause shrivelling.
- Do not use “Sour Salt” – It is citric acid and does not have the same inhibitory effect on microbes.
- Do not use reduced sodium salt in fermented pickle recipes. Reduced sodium pickles can be made using quick pickle recipes given in the USDA canning guidelines.
- Fresh pack pickles acidified with vinegar can be prepared with little salt but the flavour and texture will be affected.
- Concentration of salt should be increased gradually; as low concentration favours acid production and growth of lactic acid bacteria while high concentration inhibits lactic acid bacteria and other microorganism.

**2.4.2 Sugar -**

Addition of brown or white granulated sugar increases number of acid forming microorganisms, if added after 24 h. It also increases yeasts and amount of gas produced.

Lactic acid bacteria depend upon plant sugars for their growth. So addition of sugar promotes their growth and accelerates fermentation. In India “jaggery’ is added to some pickles. It is 1-2 g% of fruit or vegetable. It is found that addition of 1-2 g of sucrose or “gur” to 100 g hand – shredded turnip mixed with 6 g of salt and 6 g of mustard seed increases lactic acid production during fermentation. At higher concentration of sucrose lactic acid production is decreased due to inhibition of lactic acid flora (Anand and Das, 1971).
2.4.3 Vinegars -

Vinegar is used in quick pickles. It is used to produce acidity in low acid vegetables.

- The vinegar used should be 4-6% acetic acid (Khader, 2001).
- It should not be diluted except according to an approved recipe.
- White vinegar is preferred with light coloured fruits or vegetables.

2.4.4 Spices -

Spices or aromatic herbs are added to most lactic acid fruit and vegetable fermentations. It improves flavour of finished products. Fresh and whole spices packed in clean cloth bag are kept in fermentation container. Powdered spices cause darkening and clouding.

Spices have antimicrobial effect. They have selective role in the development of bacteria during fermentation and inhibit undesirable bacteria and fungi and favours starter microorganism like Lactic Acid Bacteria. Spices have antimicrobial activity due to compounds associated with it (Table 2.6).

Table 2.6 Antimicrobial compounds associated with common spices
(Thompsonsons and Singh, 2000)

<table>
<thead>
<tr>
<th>Spices</th>
<th>Antimicrobial Compound</th>
<th>Action</th>
<th>Microbial Inhibition Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>Allicin (2 – propenyl – 2 propenthiol sulfitne)</td>
<td>Bactericidal and antifungal</td>
<td>Bacillus cereus, Staphylococcus aureus and Salmonella sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Candida sp., Torulopsis sp. etc.</td>
</tr>
<tr>
<td>Onion</td>
<td>Allicin</td>
<td>Bactericidal</td>
<td>Escherichia coli, Shigella, Enterobacter and Pseudomonas sp.</td>
</tr>
<tr>
<td>Oregano</td>
<td>Thymol (5 methyl – 2 l – methyl ethyl phenol)</td>
<td>Inhibits growth, germination and toxin production</td>
<td>Salmonella, typhimurium, Aspergillus flavus Pediococcus sp.</td>
</tr>
<tr>
<td>Savory</td>
<td>Thymol</td>
<td>Antibacterial and Antifungal</td>
<td>Clostridium botulinum Aspergillus sp.</td>
</tr>
</tbody>
</table>
Spices | Antimicrobial Compound | Action | Microbial Inhibition Spectrum
---|---|---|---
Cinnamon | Cinnamic Aldehyde (3 – phenyl 2 – propenol) | Inhibits mould growth and mycotoxin production | Aspergillus parasiticus Staphylococcus and Bacillus sp etc.
Clove | Euganol (2- methoxy – 4 – 2 propenyl phenol) | Antibacterial and Antifungal | S. typhimurium, Bacillus subtilis Aspergillus and Staphylococcus sp.
Mustard Seed | Allyl isothiocyanate | Antibacterial, Antifungal and Inhibits yeast | S. typhimurium, Aspergillus sp. Saccharomyces cerevisiae

**Allicin** - *Allium sativum* (garlic) and *Allium cepa* (onion) contain a colourless pungent oil allicin. It inhibits several food spoilage and pathogenic bacteria including *Enterobacter, Escherichia, Salmonella, Shigella* and *Pseudomonas* (Durairaj *et al.*, 2009). Allicin inhibited food spoilage yeast at concentration as low as 25µg/mL. Allicin at concentration of 1500 µg inhibited toxin production by *Clostridium botulinum* (Naidu, 2000).

**Thymol** – Thymol is inhibitory to 25 bacterial genera. It inhibits growth and toxin production of mycotoxigenic molds. At the concentration of <0.4 µg/mL thymol caused complete inhibition of the growth of *Aspergillus flavus* and *Aspergillus versicolor*. The 2% concentration of oregano completely inhibited growth of seven mycotoxigenic moulds and eighteen pathogenic and non-pathogenic fungi. Thyme and Oregano have stimulatory effect on lactic acid production (Zaika *et al.*, 1983).

**Cinnamic aldehyde** – It is the major, antimicrobial compound in cinnamon. It is antibacterial and also inhibits mold growth and mycotoxin production. Ground cinnamon in 1-2% concentration; eliminate almost 99% of aflatoxin production (Vijayan and Tampuran, 2004).

**Eugenol** – It is most effective against *S. typhimurium, S. aureus* and *Vibrio parahaemolyticus* than thymol, anethol and menthol. Clove oil at
concentration of 250 µg/mL has been reported inhibitory to growth and toxin production by several molds. Ground clove inhibits growth of toxigenic Aspergilli in culture media. Clove powder (120µg/mL) and eugenol (200 µg/mL) adversely affect rate of germination of Bacillus subtilis (Jay et al., 2005).

**Allyl isothiocyanate** – It is a volatile aromatic compound with antibacterial and antifungal properties. It also inhibits yeast Saccharomyces cerevisiae. Finely ground mustard seeds or mustard oil are widely used in traditional fermented vegetable preparations in India. Mustard seed promotes lactic acid bacteria growth and selectively inhibits undesirable scum yeast which can break down lactic acid. This spice prolongs the shelf life of lactic acid fermented vegetables (Sethi, 1984)

2.4.5 **Chilli powder** –It contains a pungent principle capsaicin which inhibits spoilage causing bacteria.

2.4.6 **Asafoetida** – Flavour of asafoetida is due to volatile oil in it which has three sulphur compounds – 2 butyl propenyl disulphide, 1-1 propenyl disulphide and 2 – butyl disulphide. Sulphur compounds are toxic to molds and bacteria.

2.4.7 **Turmeric** - Curumin in turmeric inhibits spoilage causing and intestinal pathogenic bacteria.

2.4.8 **Buffers** – The vegetables and fruits with high fermentable sugar content needs addition of acetic acid/calcium acetate buffer to improve lactic acid fermentation process. This buffer slows down acid bacteria to consume all the sugar. The calcium ions released by the acetate can complex with pectins in the vegetables and protects pectin from enzymatic hydrolysis and enhance firmness of fermented vegetable (McDonald et al., 1991). Thus calcium ions from buffer or salts help to protect pickle pieces from pectinolytic microorganisms and avoid spoilage which is known as “Soft pickle formation”.
2.4.9 Oil – A layer of oil on top of any food prevents growth of molds and yeasts by creating anaerobic conditions. So pickles with a layer of oil on top can be preserved for longer periods. It provides anaerobic condition which favours growth of LAB.

2.4.10 Other ingredients favouring bacterial growth –

In olive fermentation, alkaline treatment is given to reduce bitterness of olives. Then olives are repeatedly washed to remove alkaline contents. This treatment reduces sugar content of olives, which is replaced by addition of 0.1-0.2% (w/w of product) glucose or sucrose prior to fermentation.

In traditional Indonesian fermented cabbage based dish “sajour asin”, cooked rice flour is added which supplies gelatinised starch required for lactic acid bacterial metabolism.

In Japan and Russia, rice bran and wheat bran are added as source and minerals which promote growth of lactic acid bacteria in fermentation of fruits and vegetables (Vitanova, 1971).

2.5 Generalised Maharashtrian recepies for microbiologically fermented pickles –

The following ingredients are added in variable quantities to main raw materials so as to suit local taste and favour the desired starter organism and inhibit spoilage causing microorganisms. For 1 kg of raw materials (fresh mature/unripe fruit/vegetable) ingredient quantity used and the possible role of ingredient is given in Table 2.7.
Table 2.7 Common ingredients used in fermented pickles
(Fellows, 1997; Battock and Ali, 1998; Morgan, 2006; Ali, 2009; Swamy, 2009)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredient</th>
<th>Quantity (range) (g)</th>
<th>Possible role in pickle</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Common Ingredients :</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Salt (NaCl)</td>
<td>150</td>
<td>Antimicrobial: inhibits spoilage causing and pathogenic microorganisms, extract nutrients from raw material useful for starter microorganism, improves flavour and taste</td>
</tr>
<tr>
<td>2.</td>
<td>Chilli powder</td>
<td>50</td>
<td>Antimicrobial: inhibits spoilage causing and pathogenic microorganisms, improves flavour, taste and appearance.</td>
</tr>
<tr>
<td>3.</td>
<td>Mustard seeds</td>
<td>40</td>
<td>Antimicrobial, enhance lactic acid bacteria</td>
</tr>
<tr>
<td>4.</td>
<td>Turmeric powder</td>
<td>10</td>
<td>Antimicrobial action inhibits spoilage causing and pathogenic microorganisms. Adds to aroma and flavour of pickle.</td>
</tr>
<tr>
<td>5.</td>
<td>Asafoetida</td>
<td>5</td>
<td>Antimicrobial action inhibits spoilage causing and pathogenic microorganisms</td>
</tr>
<tr>
<td>6.</td>
<td>Fenugreek seeds</td>
<td>15</td>
<td>Improves flavour</td>
</tr>
<tr>
<td>7.</td>
<td>Edible oil</td>
<td>150</td>
<td>Inhibits aerobic spoilage causing bacteria yeasts and molds. Enhance growth of LAB.</td>
</tr>
<tr>
<td>B) Optional Ingredients :</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Sugar</td>
<td>400 – 1000</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Coriander</td>
<td>40 – 50</td>
<td>Inhibit spoilage causing and pathogenic microorganisms,enhance starter microorganism, impart aroma, flavour and taste</td>
</tr>
<tr>
<td>3.</td>
<td>Cumin</td>
<td>40 – 50</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Cloves</td>
<td>3 – 4</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Cardamoms</td>
<td>3 – 4</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Cinnamon</td>
<td>3 – 4</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Garlic powder</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Ginger paste</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Lime / Lemon juice</td>
<td>100 – 150 mL</td>
<td>Especially added in low acid raw materials, inhibits spoilage causing and pathogenic microorganism.</td>
</tr>
<tr>
<td>10.</td>
<td>Vinegar</td>
<td>250 mL</td>
<td></td>
</tr>
</tbody>
</table>
2.6. Intentional food additives: Preservatives -

According to The Federal Food, Drug and Cosmetic Act, any chemical which when added to food tends to prevent or retard its deterioration is a chemical preservative (Sivasankar, 2002). Natural preservatives or condiments are excluded from chemical preservatives. Natural preservatives or condiments include common table salt, sugars, vinegars, spices and their oils. Presence of these materials in foods need not be mentioned on the label. Addition of specific chemicals in specific quantities is permitted.

Microorganisms, food enzymes or chemical reactions in the food may cause food spoilage. Preservatives inhibit microorganisms. At the same time it

1) should be non-toxic to human being or animals
2) should be economical
3) should have no adverse effects on flavour, taste or aroma of food.

The antimicrobial preservatives may be classified into two classes –

I) Naturally occurring preservatives such as organic acids (lactic, malic and citric acid and their salts), vinegar (acetic acid), sodium chloride, sugars, spices and their oils, wood smoke, carbon dioxide and nitrogen.

II) Substances generally recognised as safe (GRAS) for addition to foods such as propionic acid and its sodium and calcium salts, caprylic acid, sorbic acid and potassium, sodium and calcium sorbates, benzoic acid and benzoates and derivatives of benzoic acid such as methyl paraben and proply paraben, sodium diacetate, sulphur dioxide and sulphites, potassium and sodium metabisulphite and sodium nitrite.

Organic acids and their salts –

Lactic, acetic, propionic and citric acids and their salts are used as preservatives (Table 2.8).
Table 2.8 Organic acids and their salts as preservative (Jay *et al.*, 2005)

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Use</th>
<th>Type of Food Preserved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>Fruit flavour and preservation</td>
<td>Jellies, Syrups</td>
</tr>
<tr>
<td>Lactic acids &amp; acetic acid</td>
<td>Antibacterial</td>
<td>Brines of various kinds and Green Olives</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>Prevention of mold growth and ‘rope’ formation</td>
<td>Bread, Cheese, Cheese spread and other Bakery products</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>Antimicrobial (Conc. 0.05 to 0.1%) effectiveness more in acidic pH (2.5 to 4.0)</td>
<td>Jams, Fruit salads, Pickles, Fruit juices, Jellies etc.</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>Antifungal and also active against yeast (effective at higher pH)</td>
<td>Bakery products, Pickles, Soft drinks</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorbic acid salts of Calcium, Sodium, Potassium</td>
<td>Fungistatic agent (upto 0.3%) less effective against bacteria (effective at pH above 4.0 Maximum effective at 6.5)</td>
<td>Cheese, Cheese product, Syrup, Fruit juices, Pickles, Margarine</td>
</tr>
<tr>
<td>Acetic acid (Vinegar)</td>
<td>Effective against yeast and bacteria</td>
<td>Pickles, Mayonnaise, Pickled Sausages</td>
</tr>
<tr>
<td>Sodium diacetate</td>
<td>Antibacterial</td>
<td>Cheese spreads, Ketchup, Acid-pickled vegetables</td>
</tr>
</tbody>
</table>

III) **Other organic chemicals used as preservatives -**

1) **Sugars** - lower the water activity and tie water molecules to provide osmotic effect that kill spoilage causing microorganisms.

2) **Alcohols** - 70 – 95% concentration of alcohol is germicidal which is used in preservation of vanilla and lemon extract.

3) **Ethylene oxide** - Sterilization of packaging materials is carried out by ethylene oxide fumes. Whole spices are also exposed to ethylene oxide. Propylene oxide also can be used for the same purpose.
4) **Inorganic salts** - Sulphur dioxide and sulphites are used in wine industry. Lactic acid producing bacteria are suppressed by sulphur compounds. Nitrates and nitrites are used in preservation of meat. These inhibit growth and toxin production of *Clostridium botulinum*.

5) **Spices and condiments** - These help to inhibit bacteria, yeast, as they contain bacteriocidal or bacteriostatic agents like volatile oils, aldehydes etc.

6) **Salt** - Salt is used in brines or is applied directly to the food. Salt tie up moisture and inhibits the microorganisms. Small quantities of salt permit acid fermentation. Concentration of salt and temperature jointly provides preservative action of salt (Monroe, 1969; Sivasankar, 2002).

Salt has following functions -

1) Plasmolysis of microbial cells.
2) Dehydration of food.
3) Chloride ions have harmful effect on microorganisms.
4) Reduction of oxygen solubility in the moisture.
5) Sensitization of cells against CO$_2$.
6) Interference with the action of proteolytic enzymes.

2.7 **Spoilage of pickles** –

Decay or decomposition of food leading to undesirable nature and quality for human use is called spoilage (Jay *et al.*, 2005). Pickles are spoiled either due to growth and activity of microorganisms present in pickles or due to reasons other than microbial growth like addition of undesirable chemicals (Khader, 2001).

I) **Non-microbial spoilage of pickles** -

i. **Shrivelling** - When raw materials for pickle production are placed directly in a very strong solution of salt or sugar and even in very strong
v. Cloudiness - It is caused due to chemical action between vinegar and impurities in the used ingredients in pickle making such as calcium and magnesium in salt.

II) Microbial spoilage of pickles -

Pickles are subjected to a number of defects which are caused by microorganisms (Table 2.9). The activities of microorganisms which bring changes in the pickles depend largely on the composition of raw materials used for pickle production.

Pickles due to their low pH are spoiled by molds, yeasts and aciduric bacteria like *Acetobacter* and lactic acid bacteria (Brackett and Splittstoesser, 2001).

If low acidity is produced due to inadequate fermentation; pickles can be spoiled by coliforms and other gram-negative bacteria (Brackett and Splittstoesser, 2001).

1. Scum formation -

When pickles are kept for curing, a white scum is formed on the surface due to growth of wild yeast. This scum may be thin film or a thick wrinkled
layer which retards the formation of lactic acid (Khader, 2001). This action helps the growth of putrefactive bacteria which causes the pickles to become soft and slippery. It is essential to remove scum as soon as it is formed.

Possible reasons for scum formation are –

a) Vegetables or fruits are not submerged in brine.

b) Pickling container is not sealed. Presence of air favours growth of film yeast and molds.

2. Soft pickles –

Pickles are made soft by pectolytic enzymes produced by the bacteria and molds. Pectin is converted to pectic acid and uronides which are further hydrolysed to galacturonic acid. Pectin is cement like substance in cell wall of product, degradation of which leads to texture softening. Softening is favoured by –

a) an insufficient amount of salt

b) too high temperature

c) low acidity

d) little brine to immerse the pickle pieces

3) Slippery pickles –

When exposed to air, permitting the growth of encapsulated bacteria, pickles become slippery. Slipperiness also may be due to the broken scums of film yeasts which drop on the surface of pickles. Early stage of softening gives a slippery surface to pickles.

4) Bloaters -

Gas formation within the individual pickles leads to condition known as bloaters. Carbon dioxide is produced by heterofermentative bacteria. The gas produced is trapped in the pickle pieces by thick skin which does not allow gas to diffuse out.
Bloating is favoured by –

a) rapid gas production during fermentation
b) high initial amounts of salt
c) added sugar and salt

5) **Black pickles** –

Black colour to pickles is contributed by formation of hydrogen sulfide produced by bacteria or by growth of black pigmented bacteria.

Growth of these bacteria is favoured by –

a) presence of an available carbohydrate like glucose
b) neutral or slightly alkaline pH.

6) **Ropy pickles** –

Growth of gram negative, encapsulated rods causeropy pickle brine. It is favoured by low salt, low acid and high temperature.

**Table 2.9 Microorganisms causing spoilage of pickles**

*(Brackett and Splittstoesser, 2001)*

<table>
<thead>
<tr>
<th>Type of Spoilage</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloaters (Floaters)</td>
<td>Coliforms, lactic acid bacteria, <em>L. brevis</em> (hetero fermentative)</td>
</tr>
<tr>
<td>Soft pickles</td>
<td><em>Bacillus, Fusarium, Penicillium, Aspergillus, Alternaria, yeast</em></td>
</tr>
<tr>
<td>Black Pickles</td>
<td><em>Bacillus nigrificans, Aspergillus niger</em></td>
</tr>
</tbody>
</table>

Yeast species isolated from pickles are-*Candida etchellsii, C. magnoliae, C. sake, C. magnoliae, C. versatitis, Zygosaccharomyces bailii, Torulopsis* sp. *(Deak and Beuchat, 2004).*

**2.8 Principles of pickling -**

Pickling is an important food preservation system which combines salting to selectively control microorganisms and fermentation to stabilise the treated materials i.e. fruits and vegetable matter.
Fresh fruit vegetables get soften when placed into watery solution within 24 h. It is due to pectinolytic, proteolytic enzymes and putrefaction. It is necessary to suppress undesirable microbial activity and create a favourable environment for desired fermentation. The addition of salt permits the naturally present lactic acid bacteria to grow and produce sufficient acid from the carbohydrates present in raw materials (fruits and vegetables). During fermentation, the pH is lowered up to 4.0. This lowered pH and salt concentration inhibits enzymatic activities and bacterial activities and helps to preserve pickles. An important aspect of preservation is the removal of all fermentable carbohydrates from the pickles (Kordylas, 1990).

Natural microflora of the fruits and vegetables is variable and includes bacteria, yeasts and molds. The salt and reduced redox potential favours the growth of facultatively anaerobic organisms and inhibits molds (Fleming, 2006).

Fruits and vegetables which have naturally low sugar content or fruits harvested before ripening (mangoes and berries) are used for pickle formation (Montet et al., 1999).

2.9 General methods of preparation -  1) Domestic  2) Industrial

2.9.1 Domestic –

Raw mango, various varieties of limes, gooseberries (amla), ginger, turmeric, green chilies, etc. are used for pickling. The fruits are washed, pared, halved or quartered or sliced. The seeds are removed (if necessary) and then either dry salted or mixed with strong solution of brine. This is done in large wooden jars, earthen pots or glass jars or china clay pots. Spice mixtures and / or oil are then added and the fruit is allowed to ferment for a month or so.

Spoilage is prevented by covering the top with a layer of salt or oil and the tight lid. Some times lid additionally is covered by clean cloth. In both these conditions air is prevented from entering the system. Pickles are made in large
quantities and only at certain fixed intervals these are removed for household use. Main stock is stored at cool and dry place.

**Flow diagram of method of pickle preparation**

*(Mudambi and Rajagopan, 2003)*

1. Wash fruits
2. Dry with a clean cloth
3. Cut into pieces of suitable size with clean board and knife
4. Mix with salt and spices
5. Place in dry, sterilised fumigated (smoked) container
6. Cover with salt and/or oil
7. Close with a tight fitting lid and store.
Industrial production –

General flow chart

(Montet et al, 1999)

Raw Material -
- Fruits or Vegetable
  ↓
- Trimming, Washing
  ↓
- Shredding, Cutting
  ↓

Pre-treatments -
- Blanching
  ↓
- Alkali treatment
  (NaOH solution, 1 –2% w/v.)
  ↓
- Enzymatic treatment
  ↓
- Irradiation
  ↓
- Container filling and closing
  ← Salting, (dry salt / brine)
  ← Other ingredients
    - Sugars,
    - Acids, buffering system
    - Calcium salts
    - Spices, antiseptic agents
  ← Starter culture inoculation
  ↓
- Fermentation
  Temp – 15 - 25°C
  Duration – Several hours to few months
  NaCl -2 to 8% w/v
  ↓
- Stabilising treatment
  ↓
- Pasteurization
  ↓
- Salt addition
  brine concentration
  = 16% w/v.
  ↓
- Hermetic
  packaging
  ↓
- Sauerkraut
  Industrial Production
  ↓
- Cucumbers
  ↓
- Fermented fruits or Vegetables
  All household or small-scale production,
  - industrial productions
2.10 Starter microorganisms

2.10.1 Definition -

Starter cultures are selected strains of microorganisms deliberately added to food material to bring about specific changes in the appearance, body, texture and flavour characteristics of the desired final products (Yadav et al., 1993).

2.10.2 Historical background -

From ancient times man has used the microorganisms to preserve the food and to produce different types of foods. The well known example is ‘Dahi’ (Curd). Since the days of Aryans; conservation of milk by addition of previous day sour milk in it, is common practice. At that time the causative agents of souring were not known.

In 1890 use of pure “starter” was accepted (Yadav et al., 1993). Later on some pure cultures were isolated and used to prepare variety of fermented milks. The starter cultures impart pleasant flavour and aroma to the product giving longer shelf life.

The predominant bacteria isolated from the historical fermented food are used to prepare starter cultures. These bacteria include streptococci/ lactococci, leuconostoc, lactobacilli, pediococci, propionic acid bacteria; mainly which are used to prepare several types of fermented foods. These all bacterial genera produce lactic acid from the food in which they are added. Sourness of fermented food is due to lactic acid produced. Some raw materials are given pretreatment to reduce undesirable microorganism prior to the addition of starter culture for fermentation e.g. heating the milk, washing and blanching of fruits etc. The starter culture produce lactic acid and lactic acid decreases pH of the fermented food; by using such starters it is possible to preserve foods. These organisms make the food more nutritious and healthy.

2.10.3 Role of starters in fermented foods - (Yadav et al., 1993).

1) To develop acid in the product.
2) For expulsion of moisture, texture formation and initiation of flavour production.

3) To give pleasant acid taste.

4) To give protection against pathogens.

5) To increase the shelf life.

6) To speed up the fermentation.

7) To maintain consistency between different batches of product

2.10.4 Some common bacterial starters are used to produce fermented foods, are listed below (Table 2.10).

Table 2.10 Common bacterial starters
(Yadav et al., 1993)

<table>
<thead>
<tr>
<th>Starter culture</th>
<th>Role</th>
<th>Fermented dairy product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactococcus lactis</em></td>
<td>Acid production</td>
<td>Cultured butter milk, sour cream, cottage cheese, dahi etc.</td>
</tr>
<tr>
<td><em>Lactococcus cremoris</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactococcus lactis subsp diacetylactis</em></td>
<td>Acid and flavour production</td>
<td>Sour cream, ripened cream, butter, cheese, butter milk</td>
</tr>
<tr>
<td><em>Lactococcus thermophilus</em></td>
<td>Acid production</td>
<td>Cheddar, Italian cheese and Yoghurt</td>
</tr>
<tr>
<td><em>Lactococcus durans</em></td>
<td>Acid and flavour production</td>
<td>Soft Italian cheddar and some swiss cheese varieties.</td>
</tr>
<tr>
<td><em>Lactococcus faealis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leuconostoc citrovorum</em></td>
<td>Flavour production</td>
<td>Cultured butter milk, sour cream, cottage cheese, ripened cream, and butter</td>
</tr>
<tr>
<td><em>Leuconostoc dextranicum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus bulgaricus</em></td>
<td>Acid and Flavour production</td>
<td>Bulgarian butter milk, Yoghurt, Kefir, Kumiss, Swiss, Emmental of Italian cheese.</td>
</tr>
<tr>
<td><em>Lactobacillus lactis,</em> <em>Lactobacillus helveticus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>Acid Flavour and eye formation</td>
<td>Acido philus buttermilk Emmental and Swiss Cheese.</td>
</tr>
<tr>
<td><em>Propionibacterium shermanii</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.10.5 Types of starter -

1. **Single strain starters** -

   It is pure culture; which can give steady acid production and flavour production. It is economical. By phage attack or by any other reason if culture fails, the product is adversely affected. Pure cultures of *L. lactis* or *L.cremoris* are used as starters.

2. **Mixed strain starters** -

   Two or more species, two or more strains in combination constitute mixed strain starters. They show different properties like acid production, gas production and aroma production. Mixed strain starters are safe because if one strain fails to work due to phage attack, others continue to work. Mixed strain starters show tolerance to change in pH and temperature. These starters are difficult to maintain due to dominance of one strain after few transfers. This dominance is due to bacteriocin production by dominant organism. So when mixed strains are used, they are grown separately and mixed together just before the inoculation.

3. **Multiple strain starters** –

   Such type of culture is widely used in New Zealand and other European countries. These are mixtures of known, compatible, non-phage related strains. These consist of known number of single strains.

2.10.6 Characteristics of good starter culture: (Daeschel and Henry, 1984)

1. Rapid and massive establishment of selected strains.

2. It must produce sufficient lactic acid to run the fermentation process smoothly and acidify brine rapidly.

3. Acid production must be continued over the entire range of temperature used during fermentation.

4. Resistance to bacteriphages.
5. Lactic starter should be resistant to antibiotics, residual amounts of chemicals, sanitisers and detergents.

6. Rapid bacterial sedimentation to obtain clear brine.

7. A good starter should not produce bacteriocin inhibiting other strains in the mixed culture.

8. Preservation potential through drying, freezing or freeze drying.

9. Starter culture must not produce undesirable texture, flavour and aroma.

10. Total depletion of fermentable sugars.

11. In mixed cultures, the associative synergistic action must be quite stable and produce maximum aroma and flavour without inhibition of acid production.

12. Low biogenic amine production and higher production of L-lactic acid.

2.10.7 Classification of starter cultures –

Bacteria, yeasts and molds or their combinations are used as starter cultures. Among these bacteria are widely used as starters for dairy and pickle industries. *Streptococcus* (*Lactococcus*–Sandine, 1988), *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Propionibacterium* are the main genera used as starter culture. In pickle production genera involved are *Lactococcus*, *Pediococcus*, *Leuconostoc* and *Lactobacillus*. These genera produce lactic acid in food during their growth in the food. So they are designated as lactic acid bacteria (LAB).

Lactic acid bacteria are long or short rods or cocci, non motile, microaerophilic, gram positive, catalase negative, give poor growth on most ordinary laboratory culture media, with very little surface growth on any medium, need complex foods: various vitamins, an array of amino acids or certain peptides for nitrogenous food and a fermentable carbohydrate for energy. They ferment sugar to chiefly lactic acid (Homofermentative) plus
acetic acid, carbondioxide, alcohol (Heterofermentative) as products (Frazier, 1999; Ahmed and Basumatary, 2006).

The most important characteristic of lactic acid bacteria is their ability to ferment sugars to lactic acid and flavour and aroma compounds which is used in production of pickles. Lactic acid bacteria associated with pickle formation are mainly *Lactobacilli* and *Lactococci* which are either homofermentative or heterofermentative. Lactobacilli are either in short chain or single and grow at optimum temperature range of 30-35°C. Lactococci are in short to long chains or are in pairs or tetrads. Lactococci grow in wide range of temperature from 20-35°C. LAB found in fermented pickle are salt tolerant (Duk, 1970; Rahaman, 2006). Lactic acid bacteria being fastidious have diversified nutrient requirements like amino acids, peptides, vitamins, fatty acids and minerals which are not satisfied by plant origin. These nutrients are supplied through ingredients added during pickle production. During fermentation prototrophic bacteria first grow and their metabolic products also provide metabolites required for growth of lactic acid bacteria.

Growth of lactic acid bacteria utilises sugar from the plant material and produce lactic acid or lactic acid + acetic acid along with other products. It reduces pH of material. At acidic pH many Gram negative and Gram positive bacteria are inhibited which are mainly involved in spoilages.

During pickle fermentation 20-80g/L or more NaCl is added (Montet *et al.*, 1999). This high salt concentration causes plasmolysis of unwanted bacteria and plant cells creating anaerobic conditions (Montet *et al.*, 1999). Release of nutrients from plant cells promotes growth of salt tolerant lactic acid bacteria.

Bergey’s Manual of Determinative Bacteriology (*7*th edition) has placed all lactic acid bacteria under one family *Lactobacillaceae*. It was further divided into two tribes –Tribe I - *Streptococcaceae*, Tribe II – *Lactobacillae*.

In *8*th edition of Bergey’s Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) LAB were placed under two families as
Streptococcaceae and Lactobacillaceae. In 1981, Tamime has proposed an overall classification of lactic starter cultures. The genera that comprise the LAB are at its core Lactobacillus, Leuconostoc, Pediococcus, Lactococcus and Streptococcus as well as the more peripheral Aerococcus, Caronobacterium, Enterococcus, Oenococcus, Teragenococcus, Vagococcus and Weisella. These belong to the order Lactobacillales. Streptococcus reclassification: - In 1985, member of the diverse genus Streptococcus were reclassified into Lactococcus, Enterococcus, Vagococcus and Streptococcus.

2.10.8 Characteristics suitable for routine outline classification of lactic starters –

1. **Catalase and Benzidine test** -

   5.0 mL broth culture with durhams tube is added with 1.0 mL H₂O₂ solution, gas in durhams tube indicate catalase positive test. Lactics are catalase negative but if they are grown on low sugar media, may produce pseudocatalase, which is confirmed by benzidine test.

   Growth of organism is emulsified in one drop of benzidine solution and one drop of H₂O₂ solution is added. Development of blueish colour indicates positive test. Lactic cultures give benzidine test negative.

2. **Morphology** -

   Lactic cultures are separated on the basis of morphology observed in Gram staining. Lactococci and Leuconostoc are Gram positive cocci in chains while Pediococci forms tetrads or pairs.

   *Lactococcus lactis* has elongated cells, elongation is in the direction of chain; chains are short. *Lactococcus lactis* subsp. *cremoris* are spheres in long chain Leuconostoc also are spheres in long chain.

3. **Temperature range for growth** -

   Cultures inoculated either in litmus milk or MRS broth is incubated at 15, 33 and 45°C. The medium is pre-conditioned at 15°C or 45°C in the
incubator overnight and then inoculated. Growth at different temperature ranges is used for classification of starter organism.

4. **Growth types of starter organisms in different broths**

   Turbidity, nature of deposit in broth media varies as per the organism. *S. lactis* produces turbid medium and heavy deposit, while *L. lactis* subsp.*cremoris* produces slight deposit in yeast dextrose broth.

5. **Development of acidity in milk**

   This test separates fast growing starter from slow growing types. Fast growing starters require 24 h at 30°C to produce about 1% lactic acid while slow growers require 7-14 days to produce the 1% acidity.

6. **Sugar fermentation**

   Sugar fermentation tests for lactic acid bacteria cannot be used in rigid manner for classification purposes. Different media components like peptones, casein digest and yeast extract give variable results. In presence of peptone as a nitrogen source, *L. lactis* subsp. *cremoris* and *L. bulgaricus* may be unable to ferment even glucose. So, closely related species are separated by sugar fermentation or other test results are confirmed by sugar fermentation tests.

7. **Heat resistance**

   Heat resistance of starter culture organisms is tested by incubating the inoculated broth or litmus milk at 60 – 61°C/65 -70°C for 15 min in water bath and after cooling at 30°C, incubating at 30°C.

2.10.9 **Biochemical activities of lactic acid bacteria as starter organisms**

1. **Lactic acid fermentation**

   Pyruvate is reduced to lactate by NAD linked lactic dehydrogenase (pyruvate reductase).
The lactic fermentation is either homolactic or heterolactic. In homolactic fermentation lactic acid (lactate) is the only end product while in heterolactic fermentation half the product is lactic acid and remaining half is, of other products.

Homo-lactic fermentation
Lactose → glucose → Lactic acid

Hetero-lactic fermentation
Lactose → glucose → Lactic acid + acetyl methyl carbinol + diacetoin + diacetyl + acetic acid

2. **Citric acid fermentation** -

*Leuconostoc* sp. and *L. lactis* sub sp. *diacetylactis* utilise citrate and produce diacetyl, acetoin and 2, 3 – butylene glycol. These compounds contribute to flavour.

\[
\text{CH}_2\text{COOHOCO} \text{OOH CH}_2\text{COOH} \rightarrow 2\text{CH}_3\text{COCOOH} \\
\text{Citric acid} \quad \text{pyruvic acid}
\]

\[
\downarrow
\]

\[
\text{CH}_3\text{COHOHCH}_3 + 2\text{CO}_2
\]

Acetyl methyl carbinol + Carbon dioxide

\[
\text{Oxidized – 2H} \quad +2\text{H Reduced}
\]

\[
\text{CH}_3\text{CO COCH}_3 \quad \text{CH}_3 \text{CHOHCHOHCH}_3
\]

Diacetyl 2, 3 – Butylene glycol

3. **Proteolytic activity of LAB** -

Proteolytic activity of Lactococci is encoded on plasmid. Lactic acid production is closely related with proteolytic activity. Rapid acid production is
associated with proteolytic strains of LAB. It is mainly studied in milk starters. Fast acid producers are found to be casinolytic.

4. Lipolytic activity of LAB -

LAB possesses weak esterase and triglyceride splitting activities. These activities in cheese making produce fatty acid pool.

2.10.10.1 Genetics of lactose metabolism in LAB -

LAB ferments lactose to lactic acid. Firstly lactose is converted to glucose and galactose– 6 – phosphate by means of enzyme β–galactosidase (phospho– beta – galactosidase). Glucose is then fermented to lactic acid via hexose di phosphate pathway. Galactose is utilized via tagatoze pathway to produce additional lactic acid.

The regulation of enzyme synthesis in lactose utilization is governed by ‘lac’ operon. The lactose utilizing enzymes are encoded on plasmids present in LAB. These plasmids are unstable and may get lost during normal subculturing of these organisms, producing mutant deficient in lactose utilisation.

2.10.10.2 Genetics of citrate utilization -

Genes for citrate utilization are also located on plasmid in LAB. So by genetic manipulation of ‘cit’ plasmid, flavour and aroma production can be enhanced.
Pathway for diacetyl biosynthesis:

\[
\begin{align*}
\text{Citrate} & \quad \downarrow \\
\text{Citrate permease} & \quad \downarrow \\
\text{Citrate} & \quad \downarrow \text{Citritase} \\
\text{Acetic acid + oxaloacetic acid} & \quad \downarrow \text{oxaloacetate decarboxylase} \\
\text{CO}_2 + \text{Pyruvate} & \\
\text{Acetyl – Co A} & \quad \text{Acetaldehyde} \\
\text{Diacetyl} & \quad \alpha - \text{acetolactic acid} \\
\text{Acetoin} & 
\end{align*}
\]

Table 2.11 Correlation of flavour intensity with its diacetyl content in fermented food: Butter (Dey, 2006)

<table>
<thead>
<tr>
<th>Diacetyl content</th>
<th>Flavour intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Flavourless</td>
</tr>
<tr>
<td>0.2 to 0.6 ppm</td>
<td>Mild flavour</td>
</tr>
<tr>
<td>0.7 to 1.5 ppm</td>
<td>Full flavour</td>
</tr>
</tbody>
</table>
2.10.11 Factors affecting activity of starter culture -

Following factors affect growth and activity of lactic acid bacteria.

a) **Temperature** - 

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Optimum temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactococcus lactis, L lactis subsp. cremoris</em></td>
<td>27-32°C</td>
</tr>
<tr>
<td><em>L. thermophilus</em></td>
<td>37-42°C</td>
</tr>
<tr>
<td><em>Leuconostoc sp.</em></td>
<td>20-30°C</td>
</tr>
</tbody>
</table>

Depending upon the temperature provided for fermentation, the dominant microflora varies.

b) **pH** - Lactic streptococci produce lactic acid more than 10% of their weight per min. So pH of the medium drops down rapidly and when it reaches below 5.0, it adversely affects growth and activity of lactococci.

While preparing bulk starters, acid produced is immediately neutralised by using insoluble buffers.

c) **Strain compatibility** - Synergistic action of mixed strain starters produce desirable changes in food. If one strain is affected by phage, other continues or others continue the fermentation. Production of antibiotic like substance i.e. bacteriocin by one strain may inhibit other strains in mixed starter.

d) **Growth medium** - Lactic acid bacteria require complex growth medium. MRS, M-17, Lactic or Elliker’s medium are commonly used. M-17 contain beta-disodium glycerol phosphate as buffer which neutralises acid produced. Fast-slow differential agar differentiates slow and fast acid producer *Lactococci*. Fast lactic acid producer colonies (Lac⁺, Prt⁺) appear shiny white, convex and surrounded by a red zone against blue background and are 1-3 mm in diameter. Slow lactic acid producers are 0.2 – 0.5 mm in diameter, translucent and flat.
e) **Bacteriophages** - Bacteriophage infection of lactic acid bacteria causes decreased or no lactic acid production. Use of phage resistant single strain starter reduces the problem of phage infection to starter.

f) **Incubation period** - At optimum temperature LAB grow to its maximum within 16 – 24 h of incubation. Ripened starters should be stored at low temperature to maintain their activity. Activity is maintained at low temperature for 18 h. Prolonged incubation decreases the activity.

g) **Heat treatments** - Lactic starters are grown in milk medium. Heat treatement to milk improves the medium by

1. Removing dissolved oxygen.
2. Forming sulphydryl compounds which act as growth factor.
3. Destructing inhibitory substances.

*L. thermophilus* is favoured by heat while *L. lactis* subsp. *cremoris* is not.

h) **Degree of aeration** - LAB prefers reduced oxygen tension. Reduced oxygen tension initiates growth of LAB. Aeration, agitation and surface culture depress the activity of LAB.

i) **Effect of carbon dioxide** - For initiation of growth of LAB, 0.2 – 2.3% by volume of CO₂ is required. CO₂ lacking medium extends lag phase of growth in LAB. Use of 0.5% yeast extract decreases lag phase.

j) **Storage conditions** - Lactic starters are maintained in refrigerator at temperature range of 2-5°C in acid free media. Medium used is milk with calcium carbonate or stabs of selective media.
2.10.12 Starter defects -

1. **Insufficient acidity** - Good starter produces adequate quantity of acid at steady rate. Slow and inadequate production of lactic acid is defect in starter. Decreased acidity allows growth of harmful organisms in food. Genetic instability to lose ‘lac’ plasmid, use of unsuitable medium, unsuitable growth conditions (temp, pH and aeration) and bacteriophage attack are some of the reasons for slow starters.

2. **Insufficient flavour production** - Low pH favours the flavour – so slow starters reduce flavour.

3. **Sharp acid taste** - If starter is over-ripened, it produces sharp acid taste in food. By decreasing amount of inoculum, decreasing incubation temperature and reduction in incubation period can overcome this problem.

4. **Metallic flavour** - If starter is over ripened and kept in metal container ‘Metallic Flavour’ develops. It can be avoided by using glass or stainless steel containers and non over-ripened starters.

5. **Green or green apple flavour** - *Leuconostoc* cultures produce this defect.

6. **Ropiness** - Ropy strains of *Leuconostoc* and *L. lactis* var. *hollandicus* of starters or contaminants like *Alcaligenes* produce ropiness in starter culture.

7. **Gassiness** - During citric acid fermentation CO₂ is produced which forms gassiness in culture. This gassiness is produced by leuconostoc mainly. Coliform contaminants and lactose fermenting yeasts may also cause gassiness.

2.10.13 Starter preparation -

Quality of fermented product depends upon quality of the starter culture. Purity and activity of starter culture can be maintained by monitoring different factors –
1. **Choice of milk** -

Milk collected from diseased animal is unsuitable for starter propagation, as it has altered composition of milk. The abnormal milk has high bacterial count and so can contaminate the starter culture. Pasteurization cannot solve the problem. So the milk to be used for starter preparation must be collected from healthy cow. Milk with high total solids and low lipolytic activity should be used.

2. **Heat treatment to milk** -

Milk is heated at $71.1^\circ C$ for 30 min so as to kill contaminants and even normal flora of milk. Use of sterile milk is also recommended. Boiling of milk for 30-60 min. also is preferred. After heating, milk is immediately cooled to inoculation temperature rapidly so as to avoid physicochemical changes in milk.

3. **Utensils** -

Utensils made up of stainless steel, glass or aluminium enameled wares are the best for propagation of starter. The utensil should be cylindrical, with smooth surfaces, free from crevices, with at least double the height than diameter. Utensils should be sterilized by steaming at 100°C for 30 min. Detergents like 25% metasilicate; trisodium phosphate and sodium carbonate are used to clean utensils.

4. **Amount of inoculum** -

Amount of inoculum used to prepare starter depends upon time and temperature of incubation and individual characteristics of starter organisms. Standard inoculum size is 0.5 to 2%. In mixed starter culture, quantity of each organism depends upon desired quality of starter.

5. **Aseptic culture transfer** -

To avoid aerial contamination by bacteria, molds and bacteriophages, the transfer of starter culture must be done in strict aseptic conditions. The
inoculation chamber must be provided with laminar air flow, U.V –lamp, devices for proper spray of detergents and sanitizers. Any equipment used for transfer must be properly sterilized e.g. Platinum loop, glass tube, pipettes, etc.

6) **Incubation** -

After inoculation starter cultures should be incubated at 21.1°C to 30°C temperatures. Incubation time depends upon inoculation size. When inoculation is 1%, incubation period is 14-16 h.

7) **Cooling** -

After achieving desirable growth of starter, it is immediately refrigerated to stop further development.

8) **Method of propagation of starter** -

To maintain pure starter, milk is heated at 90°C for 1 h. This heat treatment produces nitrogenous substances and condensed breakdown products of sugar which are used as bacterial growth factors.

A sterilized bottle or jar is filled ¾th with milk and then heated to 90°C for 1 h, cooled to 20-25°C, inoculated aseptically with starter, incubated at 22-25°C till clotting of milk takes place and then it is refrigerated.

2.10.14 **Preservation of starter cultures** -

1. **Liquid starter** -

Lactic starters are maintained in antibiotic free, non-fat dry milk. The milk is inoculated with 1-2% starter organism and incubated at 22°C-30°C for 18 h.

Starting from 0.2 mL of stock culture, 100 L., bulk culture is obtained.

Stock culture → Mother culture → Working culture → Bulk culture

| 0.2 mL | 20.0 mL | 2.0 L | 100.0 L |
Bulk starters can be preserved in dried skim milk powder, decalcified milk and phosphate milk. Liquid cultures are stored in refrigerator and are reactivated once in three months.

2. **Dried cultures** -

Drying of cultures is achieved by – Freeze drying (lyophilization), Vacuum drying and Spray – drying

3. **Frozen starters** -

Deep-freezing of liquid cultures at 20 – 40°C in the medium containing 10% skim milk, 5% sucrose, 0.9% sodium chloride, 1.0% gelatin, 20% glycerine, sodium citrate or sodium-beta-glycerophosphate; maintains culture for few months. Such preserved cultures can be used directly to inoculate bulk starter. Cultures are preserved at -196°C, in liq. Nitrogen, by inoculating medium containing tween 80 and sodium oleate.

2.10.15 **Starter concentrates** –

Single strain or mixed starters are preserved by concentrating liquid culture into smaller volume liquid medium keeping at -196°C. Starter concentrates have high number of bacterial cells i.e. $10^{11}$ CFU/mL.

2.10.15.1 **Production of starter concentrates** -

Starter concentrates are produced by continuous culture, diffusion culture and batch culture. The biomass production depends on composition of growth medium, pH, temperature of incubation, time of fermentation and method of harvesting. Growth medium is selected depending on cost, ability to produce high yields of active cells, suitability of collecting cells by centrifugation, suitable to produce acid production and flavour production. pH within 6.0 to 6.5 gives maximum cell number of lactic acid starter. Mesophilic lactic starter cultures are grown at 20-30°C. Maximum yield of the biomass is obtained after 14-16 h with 1% inoculation at optimum temperature.
Cells are separated by centrifugation and concentrated starter is packaged aseptically in containers which are rapidly frozen.

Viability percentage in concentrated starter is calculated by the formula

\[
\text{Viability} \% = \frac{\text{Average chain length} \times \text{CFU/mL}}{\text{Total cells/mL}} \times 100
\]

2.10.15.2 Factors affecting viability and activity of cultures in starter concentrate –

1. Growth -
Starter concentrates are grown well by batch cultures than continuous cultures. Number of bacterial cells and metabolic activity of the bacterial cell must be equivalent to those of conventional starters.

2. Storage and transport -
Stability of concentrated cultures must be maintained during storage and transport. Storage at -196°C in liquid nitrogen is best means to preserve starter. Viability of cells during low temperature can be improved by addition of lactose, glycerol like cryoprotective agents.

3. Age of cells -
Better survival is shown by cells at late log phase or early stationary phase when kept at freezing temperature.

4. Nature of microorganisms -
Survival rates of lactic cocci is given in Table 2.12

### Table 2.12 Survival rate (Rate of viability) of Lactococci
(Yadav et al., 1993)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactococcus lactis</em> subsp. <em>diacetylactis</em></td>
<td>42.1%</td>
</tr>
<tr>
<td><em>L. lactis</em></td>
<td>96.2%</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> subsp. <em>cremoris</em></td>
<td>73.8%</td>
</tr>
<tr>
<td><em>Leuconostoc cremoris</em></td>
<td>52.9%</td>
</tr>
</tbody>
</table>
5. **Cryoprotective agents** -

Tween 80, 10% sucrose + 5% sodium citrate, 6% barley extract, glycerol are used when concentrated starter is stored at freezing temperature.

Survival of the starter concentrates during freeze-drying can be increased by addition of horse serum, 8% glucose, incorporation of gelatin, citrate, glutamate and sucrose (GCGS) in growth medium.

6. **Packaging materials** -

Glass ampoules are used to fill concentrated starter. Starter concentrates are packaged in glass vials with rubber stoppers, nylon sachets, aluminum foil sachets and metallised polyesters.

2.10.15.3 **Evaluation of the quality of starters** -

Tests used for quality control of starter culture are –

i) **Organoleptic tests** -

The taste should be clean acidic with typical flavour depending upon type of culture used. Texture of starter should be smooth and firm.

ii) **Chemical tests** -

Titrable acidity, volatile fatty acids, diacetyl, acetyl methyl carbinol, acetoin + diacetyl; content determines quality of starter.

iii) **Microbiological tests** -

Number of lactic acid bacteria in starter culture is determined by plate count, direct microscopic count and selective plating procedures.
iv) **Purity tests** -

Purity of lactic starters is checked periodically by catalase test and microscopic examination by Gram’s staining.

v) **Activity tests** -

1. **Titrable acidity** - Cultures which produce more than 0.8% lactic acid at 30°C within 16 h are considered as active fast acid producers.

2. **Horrell-Elliker Test** - The autoclaved reconstituted non-fat dry milk is inoculated with 3% starter and titrable acidity is determined after incubation at 37°C for 3-5 h. Production of 0.4% lactic acid indicates a good active culture for cheese making. For cottage cheese, test is carried out at 30°C.

3. **Resazurin reduction time test (RRT)** - Depending upon time required to reduce resazurin by culture inoculant, quality of starter is determined (Table 2.13).

   **Table 2.13 Resazurin reduction time**
   
   *(Yadav et al., 1993)*

<table>
<thead>
<tr>
<th>Reduction time</th>
<th>Quality of starter</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h &lt;</td>
<td>control (without added culture)</td>
</tr>
<tr>
<td>35 min</td>
<td>excellent</td>
</tr>
<tr>
<td>50 -60 min</td>
<td>Good</td>
</tr>
</tbody>
</table>

4. **Creatine test** -

Aroma producing cultures are detected by creatine test. Cultures which produce acetyl methyl carbinol and diacetyl form pink coloured complex with creatine.

2.10.16 **Starter failure** -

Failure in starter leads to poor quality of fermented products. Common factors causing starter failure are
A) **Intrinsic factors** -
   a. Physiological factors
   b. Genetic instability

B) **Extrinsic factors** -
   a. Manufacturing conditions
   b. Variations in milk composition e.g. due to mastitis, mineral content and period of lactation.
   c. Variations in levels of natural and added inhibitors e.g. bacteriophages, antibiotics, detergents and sanitizers etc.

**Intrinsic factors** -
Physiological factor and genetic instability affect starter culture activity.

**Physiological factors** -
LAB produces 10% LA of their weight per minute. Higher acid percent cause cell injury.

**Genetic instability** -
Spontaneous mutations in lactic acid bacteria produce (lac') lactose non-utilising strains. Such mutant strains are unable to produce lactic acid. Loss of plasmid during handling looses different properties of LAB like citrate utilisation, bacteriocin production, phage resistance and sugar utilisation.

**Extrinsic factors** -
Variations in processing conditions like temperature and salting rate, variation in culture media, variations in the amount of natural and added inhibitors affect the activity of starter culture.

**2.10.17 Genetic manipulation of lactic starter culture** -
Genetic engineering is used to improve microorganism to increase their ability to produce desired product. Different desirable properties of LAB are plasmid coded (Table 2.14).
Table 2.14 Plasmids and associated functions in LAB
(Yadav et al., 1993)

<table>
<thead>
<tr>
<th>Function</th>
<th>Plasmid mol.wt. (X 10^6 daltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose transport and hydrolysis</td>
<td>30-45</td>
</tr>
<tr>
<td>Citrate permease</td>
<td>5.5</td>
</tr>
<tr>
<td>Bacteriocin production</td>
<td>88</td>
</tr>
<tr>
<td>Restriction and modification</td>
<td>15</td>
</tr>
<tr>
<td>Phage resistance</td>
<td>40</td>
</tr>
<tr>
<td>Sucrose, glucose, mannose, xylose and galactose metabolism</td>
<td>Uncertain</td>
</tr>
<tr>
<td>Resistance to inorganic ions</td>
<td>Uncertain</td>
</tr>
<tr>
<td>Milk Proteolysis</td>
<td>30-45</td>
</tr>
</tbody>
</table>

By manipulating plasmids different properties can be added together to improve LAB as starter culture.

**Genetics of phage resistance** -

Bacteriophage attack is one of the causes of starter failure. The starter organism is made phage resistant due to presence of plasmid DNA. The activity of plasmid blocks the adsorption of phage, blocks replication of phage nucleic acid or cause modification – restriction of phage nucleic acid. But each of these mechanisms is controlled by individual phage. By using genetic engineering all these properties can be collected together in one plasmid.

**Gene cloning in lactic streptococci** -

The genes from LAB have been cloned in *E. coli* e.g. phospho-beta, galactosidase gene, citrate permease genes, proteinase genes, from different lactic streptococci, tegatose -1, 6-diphosphate aldolase gene from *L. lactis* and β-galactosidase gene from *L. thermophilus*. 
2.10.17 Significance of pickle starter microorganisms -

Starter microorganisms in pickle fermentation has probiotic properties which help to improve health of consumer and the antimicrobial substances produced by starter microorganism increase shelf life of pickle by a method of biopreservation.

A) Probiotic effect:

1) Pickles are fermented by lactic acid bacteria which lower serum cholesterol (Hepner et al., 1979).

2) Lactic acid bacteria in pickles help in preventing tumors by stimulating immune response (Perdigon et al., 1988).

3) Lactic acid bacteria reduce enzyme activity of fecal bacteria (Balsubramanyam and Varadaraj, 1995).

4) Lactic acid fermentation has probiotic effect as living organisms and their metabolites are carried into the intestinal tract via food products (Lee and Salminen, 1995).

5) Lactic acid bacteria colonise the wall of digestive tract and prevents pathogenic bacteria to establish (Montet et al., 1999).

6) Lactic acid fermented food products modify intestinal flora by production of inhibitory substances like H$_2$O$_2$, lactic acid, acetic acid by lowering pH and by breaking down certain enterotoxins (Montet et al., 1999).

7) Vitamin C preservation - Many vitamins are retained during lactic acid fermentation. Vit.C content of sauerkraut increases during fermentation as a result of microbial synthesis (Montet et al., 1999).

8) In human being L (+) lactic acid is completely and rapidly metabolised in glycogen synthesis. Lactic acid improves utilization of calcium, phosphorus and iron, stimulates secretion of gastric juice (Khader, 2001).
9) Mineral preservation - Assimilation of iron during meal which includes lactic acid fermented vegetables, is more than twice, than in a meal with equal proportion of fresh non fermented vegetable (Eriksson, 1991).

10) Denitrification - Plants require nitrates as source of nitrogen, for their growth. Increasing use of nitrogen fertilisers, accumulate high quantity of nitrates in food materials which can be reduced to nitrites and in turn can be involved in the formation of nitrosoamines which are highly carcinogenic.

During lactic acid fermentation nitrites are transformed into nitrogen dioxide and eliminated as gas. Spontaneous fermentation of sauerkraut does not reduce nitrates but 90% nitrate is reduced in the fermentation carried out in presence of \textit{Lactobacillus plantarum} (Anderson, 1985).

11) Improved digestibility - Sulphur compound in garlic or onion are indigestible which can be broken down by lactic acid fermentation (Montet \textit{et al.}, 1999).

12) Glycoproteins produced by \textit{L. plantarum} KLAB 21 are found to be antimutagenic (Rhee and Park, 2001).

\textbf{B) Antimicrobial compounds produced by lactic starter –}

From long time food was preserved by using process of canning and by using chemical preservatives. Chemical preservatives inhibit growth of spoilage bacteria but are deterrent to the health. So the present day customers demand for the product without chemical preservatives, free from additives but natural with safety and better shelf-life. So necessity for natural substances from plants, animals and microorganisms rose up, which can be used as natural biopreservatives.

Natural biopreservatives can be obtained from plants, biological fluids and microorganisms. Microorganisms are able to produce substances which act as preservatives. Being natural, they are stable under processing conditions and so are ideal biopreservatives for foods (CoN and Karasu, 2009).
Microorganisms produce bacteriocins that inhibit their own species and other microorganisms. Bacteriocins are low mol. wt. proteins produced by many strains of starter bacteria which are bacteriocidal to closely related strains and species.

Genera of starter bacteria – *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, etc. are bacteriocin producing. Bacteriocins are “any proteinaceous compound (usually peptide) that has bactericidal action against a limited range of organisms, which are usually closely related to the producer organism.” Bacteriocins are added in food either as a pure compound or in case of fermented products by use of lactic acid bacteria (LAB) which secrete bacteriocins. Bacteriocins produced by LAB may be considered as biopreservatives which are GRAS (Singhal, 1999). Different bacteriocins are produced by lactic acid bacteria (Table 2.15).

**Table 2.15 Lactic acid bacteria and their antibacterial compounds**

(Singhal and Kulkarni, 1999)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Bacteriocin type</th>
<th>Antibacterial spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Bifidobacterium</td>
<td>Bifidin</td>
<td><em>Bifidobacteria, E. coli</em>, other pathogens</td>
</tr>
<tr>
<td>2) <em>Lactococcus lactis</em> subsp. <em>lactis</em></td>
<td>Nisin</td>
<td><em>Micrococcus, Listeria, Staphylococci, Bacillus sp.</em></td>
</tr>
<tr>
<td>3) <em>Lactococcus lactis</em> lactis CNZR</td>
<td>Lacticin – 481</td>
<td><em>Staphylococci, Bacillus sp. Clostridium ,etc.</em></td>
</tr>
<tr>
<td>4) <em>Lactococcus lactis</em> subsp. <em>cremoris</em></td>
<td>Diplococcin</td>
<td>Wide range of gram positive bacteria</td>
</tr>
<tr>
<td>5) <em>Lactobacillus brevis</em></td>
<td>Lactobacillin / Lactobracin</td>
<td><em>L. brevis</em> and other related strains of LAB</td>
</tr>
<tr>
<td>6) <em>Lactobacillus sake</em></td>
<td>Sakacin A</td>
<td>Some lactic acid bacteria, <em>Listeria</em></td>
</tr>
<tr>
<td>7) <em>Lactobacillus plantarum</em></td>
<td>Lactolin / Plantericin</td>
<td>*L. brevis, L. acidophilus, L. viridescens, L. xyloses, Enterococcus fecalis, etc.</td>
</tr>
<tr>
<td>8) <em>Lactobacillus acidophilus</em></td>
<td>Lactocidin / Acidolin / Acidophilin / Lactacin</td>
<td>Narrow spectrum antibiotic – <em>L. leichmanni, E. coli, L. helveticus</em>, etc.</td>
</tr>
<tr>
<td>Microorganism</td>
<td>Bacteriocin type</td>
<td>Antibacterial spectrum</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9) Lactobacillus helveticus</td>
<td>Helveticin</td>
<td>Lactobacillus sp. of closely related strains.</td>
</tr>
<tr>
<td>10) Leuconostoc mesenteriodes UL5</td>
<td>Mesenterocin 5</td>
<td>Enterococcus, Micrococcus, Listeria, Staphylococcus sp. etc.</td>
</tr>
<tr>
<td>11) Pediococcus acidolactis</td>
<td>Pediocin A / AcH PA1</td>
<td>B. cereus, Listeria sp., S. aureus, Clostridium perfringes</td>
</tr>
<tr>
<td>12) Pediococcus pentosaceus FBB61</td>
<td>Pediocin A</td>
<td>Cl. botulinum, Pediococcus, Leuconostoc, etc.</td>
</tr>
<tr>
<td>13) Propionibacterium jensenii</td>
<td>Jensenin G</td>
<td>Limited inhibitory effect Propionibacterium and related strains, other gram negative bacteria, yeasts and molds</td>
</tr>
<tr>
<td>14) Propionibacterium shermanii</td>
<td>Microgard</td>
<td>Gram negative bacteria, yeasts and molds.</td>
</tr>
</tbody>
</table>

These bacteriocins are produced by food grade bacteria, they are stable under processing conditions and degradation by proteolytic enzymes of human gastrointestinal tract. Bacteriocidal activity of bacteriocins is maintained at wide range of pH and temperature used in food processing and it is maintained for long periods under storage conditions.

**Lantibiotics -**

These are substances which contain lanthionine and $\beta$–methyl-anthionine (sulfur containing amino acids) in their structure.

i) **Nisin -**

It is made up of 34 amino acids. It has molecular wt. of 3510 Da. It exists in dimeric or tetrameric form. It can form ring structures with lanthionine and $\beta$- methylanthionine. It is the first bacteriocin produced by LAB of the genus *Lactococcus*. It is approved biopreservative for dairy products. Amount of nisin produced varies from strain to strain. The active molecules are present on cell surface and in the medium. Nisin is effective against Gram positive bacteria and also Gram negative bacteria. Nisin attacks the cytoplasmic membrane. It also inhibits the synthesis of the cell wall polymer murein. It also inhibits germination of spores of *Bacillus* and *Clostridium*. Nisin has low solubility and
it is heat sensitive at neutral pH, so it is used only in acid foods. Its activity is also reduced by interactions with phospholipid components so it is not used in foods containing emulsifiers. During storage of food nisin activity is lost. Nisin is used to prevent late blowing of processed pasteurised cheese. It may be added to low acid canned foods such as vegetables. It is also used to prevent spoilage of beer and wines by lactic acid bacteria. The use of nisin in the fermentation mash for fruit brandies can increase alcohol content about 10%. Nisin can not be used for meat preservation as it is less soluble at the pH of meat and is inactive against spoilage or pathogenic organisms associated with meat (Delves, 1990).

Nisin and pediocins control various pathogens by synergistic action.

ii) **Reuterin -**

*Lactobacillus reuterii* is a heterofermentative organism. It produces a low molecular weight, non proteinaceous compound. Reuterin is highly soluble at neutral pH and acts against gram positive and gram negative bacteria, yeasts, fungi and protozoa. Human and animal food stuffs are preserved by reducing pathogenic and spoilage microorganisms by action of reuterin.

iii) **Pediocins** - These are bacteriocins produced from *Pediococcus* sp. *P. pentosaceus* and *P. acidilactici* are the two main strains producing pediocins. Pediocin A, Pediocin Al and Pediocin AcH are well studied. Pediocin A is secreted by *P. pentosaceus*. It is used as food additive. But it is unstable and organisms loose ability to produce pediocin A on subculturing and freeze drying. Pediocin has wider host range and it inhibits *Clostridia, Lactobacillus, Staphylococcus* and *Listeria*. *Pediococcus acidilactici* produces large amounts of pediocin A1. MRS broth + 2% yeast extract provides suitable growth medium for production of higher quantities of pediocin A1. This bacteriocin is bacteriocidal at wide range of temperature from 4 – 32°C. The antibacterial activity is retained even after heating at 100°C. Pediocin A1 reduces the number of *Listeria monocytogenes* in fresh beef. The reduction is directly proportional
to the concentration of pediocin. Pediocin AcH is produced by *Pediococcus acidilactici* H. It has mol wt. of 2700 Da. It is sensitive to proteolytic enzymes, resistant to heat upto 121°C and organic solvents. It is stable at pH range of 2.5-9.0. Pediocin AcH is inhibitory to Gram Positive bacterial strains and their spores from the genera *Bacillus*, *Clostridium* and *Listeria*, along with sp. of *Leuconostoc*, *Micrococcus*, *Enterococcus*, *Pediococcus*, and *Lactobacillus*. The effectiveness of pediocin AcH has been studied in sterile and non sterile food systems against Gram positive spoilage causing and pathogenic bacteria. There is rapid drop in viable counts of *Listeria monocytogenes* in all types of foods (milk, cheese, ground meat, sausages etc.) in which Pediocin AcH is added.

Pediocin AcH binds to the cell surface at the lipoteichoic acid sites of Gram Positive bacteria (Ruechert, 1979).

iv) **Lactacin/Lactococin/Lacticin** -

*Lactobacillus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus acidophilus* produce these bacteriocins. Lactococcin A is found to inhibit strains of the same genus. It also inhibits nisin and diplococcin producing strains. Lacticin 481 was found to inhibit the strains of *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Clostridium*. Lactacin A produced from *L. delbrueckii* subsp.*lactis* looses antibacterial activity upon heating to 60°C for 10 min. While Lactocin B produced by *L. acidophilus* is found to be heat resistant bactericidal protein. Lactocin 27 produced by *Lactobacillus helvaticus* remained stable even after heating at 100°C for 1 h (Piard *et al.*, 1992).

All these bacteriocins increase cell membrane permeability of the target organism.

*Leuconostoc* sp. also produces bacteriocin like substances. The bacteriocin from *Leuconostoc gelidium* is bacteriostatic rather than bacteriocidal. It has molecular wt. of about 1,000 Da. It is active against three strains of *Listeria monocytogenes* and other lactic acid bacteria. It does not
inhibit gram negative and spore forming gram positive bacteria. Heating at 100°C for 1 h does not inactivate this bacteriocin (Stiles and Hastings, 1991).

The bacteriocin producing cultures that are used as starter cultures in fermented foods can be regarded as processing aids or ingredients rather than additives. Addition of pediocin AcH and nisin in vacuum packaged beef inhibited *L. mesenteroides*, bacteriocin of *Lactococcus lactis* inhibited *L. monocytogenes* in Mozzarella cheese and Pediocin M inhibited *L. monocytogenes* in Kimchi.

v) **Entrocin**

These are the bacteriocins produced by *Enterococcus* sp.

Efficiency of bacteriocins in foods is affected by –

1) Presence of bacteriocin resistant pathogens or spoilage causing microorganisms.
2) Inactivation by other additives.
3) pH of the food.
4) Solubility of bacteriocin.
5) Uneven distribution of bacteriocin in food matrix.
6) Destabilisation by proteases.
7) Binding to food components like fat particles.

C) **Antifungal lactic acid bacteria as biopreservative**

Antifungal effects of lactic acid bacteria are shown by Johan Schnurer and Jesper Magnusson. Cyclic dipeptides, phenyllactic acid, proteinaceous compounds and 3 – hydroxylated fatty acids have been isolated from lactic acid bacteria. It suggests the potential application of antifungal lactic acid bacteria in the preservation of food and feeds (Johan and Magnusson, 2005).

D) **Negative dietary effects**

i) **Biogenic amine synthesis**

Decarboxylation of amino acids produces biogenic amines. These amines impart unpleasant flavour to the final product or even can be toxic. In human,
ingestion of certain amines can cause headache, fever and vomiting (Kuensch et al., 1990, Landete et al., 2007). Different biogenic amines are produced in lactic acid fermented vegetables (Table 2.16).

**Table 2.16 Biogenic amines in lactic acid fermented vegetables**

(Anderson, 1988)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Biogenic amine (mg/kg)</th>
<th>Sauerkraut</th>
<th>Carrot</th>
<th>Red beet, vegetable mixture (carrot, turnip, cabbage, pepper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Histamine</td>
<td>29</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ornithine</td>
<td>Tyramine</td>
<td>43</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Pustersine</td>
<td>174</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Lysine</td>
<td>Cadaverine</td>
<td>28</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

Consumption of 0.07 to 1 g of histamine/meal is supposed to elicit histamine toxicity (Arnold and Broan, 1978). Lactic acid bacteria like *Leuconostoc mesenteroides* contain aminoacid decarboxylase enzyme which induces biogenic amine formation. *Lactobacillus plantarum*, commonly found in lactic acid fermentation can limit biogenic amine concentrations in lactic acid fermented vegetables (Anderson, 1988).

Quality of fermented vegetables can be improved by selecting starter bacteria which do not produce amino acid decarboxylases.

**ii) Synthesis of D (-) lactic acid -**

During lactic acid fermentation, two different forms of lactic acid can be produced viz. L (+) isomer or D (-) isomer.

L-lactic acid is absorbed by intestinal mucosa and utilised as an energy substrate. D-form cannot be assimilated but is eliminated by the kidneys in the form of salts of calcium and magnesium, leading to the loss of calcium and magnesium. WHO recommends D-lactic acid to be avoided in childrens’s diet and adult consumption must be restricted to 100 mg/d/kg of body weight.
Both lactic acid forms are present in the homemade or small scale fermented vegetable preparations. So the bacterial strains producing L (+) forms are preferred for starter culture (Buckenhuskes et al., 1991).

2.11 Quality of pickles –

Parameters determining quality of pickles are listed in Table 2.17

Table 2.17 Parameters determining quality of pickles
(Frazier, 1999; Jay et al., 2005; Ranganna, 2008)

<table>
<thead>
<tr>
<th>1) Analytical</th>
<th>2) Flavour and odour</th>
<th>3) Colour</th>
<th>4) Uniformity of size</th>
<th>5) Texture</th>
<th>6) Defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii) Total acidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid/100 ml.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid g/100 ml.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iii) Chlorides expressed as NaCl/100 ml</td>
<td>Below 4.0</td>
<td>4 to 6</td>
<td>2.75 – 5.0</td>
<td>Pleasant sour</td>
<td></td>
</tr>
<tr>
<td>2) Flavour and odour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Colour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh Red, Green, Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Uniformity of size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small pieces (5.0 g or less)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crunchy, Hard and Crackling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) Defects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spoilage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.12 Pickles made by using starters -

The traditional fermentation of pickles is dependent upon growth of naturally occurring lactic acid bacteria to metabolise sugars present in the raw materials, together with added salt, results in preservation.

However, recent efforts to improve pickle fermentation and to develop controlled fermentation methods for pickle production resulted in an increased interest in developing cultures suitable for application. Rapid and dominant growth, type and extent of acid production, salt tolerance, temperature range, CO₂ production, cell sedimentation, bacteriophage resistance, nutritional value and ability to survive as concentrated cultures are factors to be considered in
developing lactic acid bacterial cultures for use in controlled fermentation of vegetables (Daeschel and Henry, 1984). It is also seen that controlled fermentation process of cucumbers by using pure cultures of *Pediococcus cerevisiae* and *Lactobacillus plantarum* and mixtures of these two species, reduce the time of pickling, eliminate undesirable organisms and maintains uniformly high quality as compared to natural fermentation process of cucumbers (Etchell et al., 1966).

An evaluation of various lactic acid bacteria for fermentation of cabbage and carrot was done and it was observed that there were greater differences between the pure cultures than mixed ones. The mixed starter used for cabbage fermentation consists of *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and *Pediococcus acidilactici* (Gardner et al., 2001).

Lactic starter cultures are developed for hyposalt pickles. A mixed starter culture consisting of strains of lactic acid bacteria – *Leuconostoc* sp. D–133 and *Lactobacillus casei* L–14 was used for the preparation of Akakabutsuke or the traditional Japanese red turnip pickles. The starter culture prevented growth of undesirable organisms though no sodium chloride was added. The hyposalt pickles can be prepared easily by the use of these psychrotrophic lactic starter cultures (Ishikawa et al., 2003).

Almagro egg plants were fermented by using starter cultures. The lactic acid bacterial strains used in the starters had previously been isolated from spontaneous fermentation. The starter used here consisted of *L. plantarum* and *L. brevis* (Seseria et al., 2001).

Sweet potato roots were pickled by lactic fermentation by using strain of *Lactobacillus plantarum* MTCC 1407 for 28 days. The final product had a pH of 2.9 – 3.0, titrable acidity of 2.9 – 3.7 g/kg (Panda et al., 2007).

*Leuconostoc mesenteroides* starter culture was used for fermentation of cabbage with reduced salt concentrations. The addition of an appropriate starter culture to shredded cabbage yields sauerkraut with increased uniformity and
quality (i.e. firm, crunchy texture and reduced off-flavour) even under reduced salt conditions (1% NaCl) (Johanningsmeier et al., 2007).

Two of the lactic acid bacteria strains, namely, *Lactobacillus pentosus* and *Leuconostoc mesenteroides* were used as starter cultures for vegetable fermentation i.e. paocai fermentation. During spontaneous paocai fermentation, nitrite content of cabbage rose, reached the nitrite peak and decreased thereafter, while only small fluctuation in nitrite concentration were observed for cabbages inoculated with starter cultures of *L. pentosus* and *L. mesenteroides*. The numbers of some nitrate reducing bacteria, such as enterobacteria were significantly lower than those in spontaneous fermentations. Hence the abilities of LAB to deplete nitrite and to inhibit growth of nitrate reducing bacteria could be the reasons that limit the formation of nitrite during paocai fermentation (Ping-Mei Yan et al., 2008). *L. brevis* sp. *lindneri* 2103, *L. viridescens* 241, 242, *Pediococcus* sp. E5 and *L. delbruckii* F5 were used as starter culture for sourdough process. These bacteria are used for souring dough in bakeries as these have antimicrobial properties and production of diacetyl (Simsek et al., 2006). Mixed lactic cultures of *L. plantarum*, *L. casei* and *Lactococcus lactis* were used to stabilise meat pickles. The combined lactic cultures were used to enhance fermentation process and preserve fresh meat for extended periods at high temperature (Sakhare and Rao, 2003).

2.13 Commercial bacterial starter cultures for fermented foods of the future -

Starter cultures for fermented foods are today developed mainly by design rather than by screening. The design principles are based on knowledge of bacterial metabolism and physiology as well as on the interaction with the product. In the genomics era, we will obtain a wealth of data making design on a rational basis even simpler. The design tools available are food grade tools for genetic, metabolic and protein engineering and an increased use of laboratory automation and high screening methods.
The large body of new data will influence the future patterns of regulation. It is currently difficult to predict in what direction the future regulatory requirements will influence innovation in the food industry. It can either become a promoting force for the practical use of biotechnology to make better and safer products, or it can be limiting the use of starter cultures to a few strains with official approval. Successful cultures based on modern technology is expected to be launched in the areas of probiotics, bioprotection, general improvement of yield and performance for the existing culture market and probably the introduction of cultures for fermenting other food products. A scientific basis for dramatic innovations that could transform the culture industry is currently being established (Hansen, 2002).

In the controlled fermentation use of starter cultures from extraneous cultures is practiced in pickles produced in the Western World especially in pickles of cucumber, cabbage, olives, etc. Use of pure and mixed promising starter cultures for pickle making is not commonly practiced in India. Hence very scanty literature is available regarding the application of controlled fermentation and promising type of microorganisms for pickle fermentation.