DEVELOPMENT OF FERMENTED RABBIT SAUSAGE PRODUCT

PRESENTED BY: CHIAMBI A MERCY

A24/1868/2010

SUPERVISOR: CATHERINE KUNYANGA

SIGNATURE:

UNIVERSITY OF NAIROBI

COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

DEPARTMENT OF FOOD SCIENCE NUTRITION AND TECHNOLOGY

A PROJECT REPORT SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN FOOD SCIENCE AND TECHNOLOGY
1. INTRODUCTION

1.1 Background information

By all appearance, rabbit could be the meat of the future (Michael Pollan.....2004). Being the best white meat, demand for rabbit meat is increasing in the local market. Most of the five-star hotels in Kenya are willing to introduce rabbit meat in their menu but their challenge is a constant source which could sustain demand versus supply chains. The Norfolk for example is serving rabbit meat on plate (aqua farm consultants.kbo.co.ke/)

Apart from Kenya, Rwanda and Congo have grown a bigger appetite for rabbit meat. Therefore all we need to do is to put value addition strategies such as processing rabbit meat into sausages, pies, kebabs e.t.c. in Kenya, there is only one company which processes rabbit meat to the market (Keraya rabbit processing limited). It is situated along Thika Road near Kenyatta University. Its major products are: rabbit felts, sausages and lean meat which is packaged fresh, frozen or chilled for the sake of transport to prevent perishability (Daily nation news paper, Jan, 2014).

An association of rabbit breeders has been established following the introduction of the new rabbit industry. It is named Rabbit Breeders Association of Kenya (RABAK). Its services are to promote the rearing of domestic rabbit as well as those for commercial purposes (rabak.or.ke).

Rabbit meat is known to have all white meat, low sodium content, low fat content, almost cholesterol free, high protein content and easy digestibility.

1.2 Problem statement

As seen earlier in the introduction, there is lack of variety in the rabbit meat products in the Kenyan market. There are found fresh sausages and lean meat (fresh frozen or chilled). There is no fermented rabbit sausages in the Kenyan market (Keraya.or.ke).

Red meats are known to have high cholesterol levels. Excess cholesterol is a threat to health as it causes cardiovascular diseases and stroke.

Most of the processed meat products in the market have very short shelf life including the rabbit meat products mentioned earlier. This is due to minimum processing conditions and treatments they are subjected to. As a result they are mostly consumed by people with appropriate preservation methods e.g. freezers. The products cannot stay for long at room temperature hence distribution to remote areas is a problem due to spoilage. As a result they are mostly consumed in towns.
1.3 Justification

By developing this product, it is filling the gap that exist in the kenyan market in the space of fermented rabbit sausage. Rabbit meat has been used and is suitable for special diets for heart disease patients, the aged, low sodium diet, weight reduction diets e.t.c (www. Rabbit production.co.ke).

By fermenting rabbit meat it afforded the product a longer shelf life than what exists in the market. This is because during fermentation, there is accumulation of lactic acid which lowers the ph hence preventing growth of spoilge microorganisms.

The starter cultures used also exhibit catalase activity which counteracts the formation of hydrogen peroxide and hence helps to prevent colour defects and rancidity.

1.4 Main objective

To develop an acceptable shelf stable fermented rabbit meat sausage

1.5 Sub-objectives

1. To carry out base-line survey on rabbit meat products for needs assessment.
2. To formulate fermented sausages from rabbit meat.
3. To determine the chemical composition of the product.
4. To do microbial analysis of the product.
5. To determine the shelf life of the product.
6. To determine the acceptability of the product.

1.6 Hypothesis

An acceptable fermented rabbit sausage can be developed from rabbit meat.

2. LITERATURE REVIEW

2.1 Abstract

The traditional aim of the fermentation is to transform the highly perishable substrate meat in to a shelf stable and safe product ensuring an optimum nutritive value and sensory quality. Today various starter cultures are used. The most widely recognized are the lactobacillus and micrococcus(cilla et al.,2005).
2.2 Nutritional composition of rabbit meat

Nutritional facts of rabbit meat (per 100g) (www.rabbitcomposition.com).

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Content (g)</th>
<th>% Daily value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.082</td>
<td>27</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.047</td>
<td>1</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>0.005</td>
<td>0.5</td>
</tr>
<tr>
<td>Protein</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>Iron</td>
<td>-</td>
<td>12</td>
</tr>
</tbody>
</table>

2.3 Starter cultures

Bacterial starter cultures have a variety of functions including (siret and issanchu., 2000):

- Boosting acidity
- Intensify the curing colour
- Counteract rancidity of fats
- Development of flavour and taste
- Texture improvement of ripened products.

Depending on the desired taste, texture and appearance of the product, specific cultures are selected (sheard et al., 2001). The use of lactobacillus result in fast acidification, the use of pediococcus leads to slower and milder acidification. Staphylococcus cause a speedy reduction of nitrate, stable curing colour and reduced risk of rancidity. A combination of lactobacillus and staphylococcus have been found to produce the best results (hope., 2007).

2.4 Bio-chemical process in the manufacture

2.4.1 Natural fat alterations (rancidity).

This occurs and leads to the production of strong flavours. This process can be substancially slowed down by selecting suitable raw materials (preferably pork back fat) and applying relatively low ripening and climatization parameters (e.g., 20°C and 70-75%RH) (valencia et al., 2000).

2.4.2 Low moisture contents.

This is caused by long ripening periods. This leads to concentrated flavor component and a firmer sausage texture (fernandez et al., 2001). The targeted moisture content here is around 30-35% or below. This corresponds to $a_w$ of 0.90 and below and makes the product shelf stable.
2.4.3 Acidity.

The targeted acidity here is final pH of 4.0-5.0. Acidity is developed by the lactic acid bacteria as seen earlier. This prevents growth of spoilage microorganisms.

2.4.4 Variables in sausage production.

The many types of fermented sausages are as a result of a great variety of process conditions (Banon et al., 2003). Variables include:

- The particle size of the comminuted meat and fatty tissue.
- The selection of additives.
- The temperature and humidity conditions prevailing in the course of fermentation until ripening.
- Diameter of the sausages.
- The nature of the casings.
- Smoking.
- Heating after fermentation.

3.0 RESEARCH DESIGN
4.0 EXPERIMENTAL DESIGN

<table>
<thead>
<tr>
<th>Raw materials and ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample A</td>
</tr>
<tr>
<td>Rabbit lean meat</td>
<td>70%</td>
</tr>
<tr>
<td>Pork back fat</td>
<td>30%</td>
</tr>
<tr>
<td><strong>Total mass-1.72kg</strong></td>
<td>100%</td>
</tr>
<tr>
<td>NPS</td>
<td>48g</td>
</tr>
<tr>
<td>Na-glutamate</td>
<td>1.72g</td>
</tr>
<tr>
<td>White pepper</td>
<td>8.6g</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.86g</td>
</tr>
<tr>
<td>Sugar</td>
<td>3.4g</td>
</tr>
<tr>
<td>Culture (L. bulgaricus)</td>
<td>2ml</td>
</tr>
<tr>
<td>Culture (S. lactis)</td>
<td>_</td>
</tr>
</tbody>
</table>

Summary

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment (culture)</th>
<th>Quantity (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>L. bulgaricus</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>S. lactis</td>
<td>2</td>
</tr>
</tbody>
</table>
5.0 Flow diagram of the process

Rabbit meat and fat

Freezing

Mincing

Ingredients

Weighing

Mixing

Chopping /grinding(temp. not exceeding 2°C)

Mixing

Stuffing

Linking

Ripening (for two days at chilling room 4°C)

Ripening (ripening room at 10°C and 85% RH -2 weeks)

Ripening (room temperature at 70-75% RH -2 weeks)

Processes flow diagram.
6.0 METHODOLOGIES

6.1 Base line survey

I visited four supermarkets in Nairobi town and inquired about the available rabbit meat products in the market. I recorded their responses and later tabulated them for analysis.

6.2 Formulation of fermented sausages

I adopted the procedure as outlined in the practical manual-meat science and technology in the pilot plant. I replaced beef lean meat with rabbit lean meat and calculated the rest of the ingredients basing on my rabbit meat quantity. I came up with my ratios based on the quantity of meat I had and came up with fermented rabbit sausages as outlined in the process flow diagram in the previous page.

6.3 Determination of the proximate composition of the formulated product

6.3.1 Moisture content

I determined moisture content according to the AOAC 2000 method of analysis.

6.3.2 Protein content

I determined the protein content of my product by kjeldal method according to the AOAC 2000.

6.3.3 Fat content

Also according to the AOAC 2000 procedures.

6.3.4 Ash content

Was determined according to AOAC 2000 techniques of analysis.

6.3.5 Crude fibre content

Was also determined according to AOAC 2000 techniques of analysis.

6.3.6 Carbohydrates

Was calculated by difference of the above.
6.4 Other chemical analysis

6.4.1 Titratable acidity

According to AOAC, 2000.

6.4.2 Free fatty acids

To 2g of sample in a conical flask, I added 25ml of the mixture, Ethanol:diethyl ether (1:1). I then titrated with 0.1N NaOH using phenolphthalein as an indicator. I then recorded the titre value and calculated using the formulae:

\[
2.82 \times \text{titre} \times \frac{\text{weight of sample}}{2}
\]

6.4.3 Peroxide value


6.5 Microbial analysis

6.5.1 Yeast and moulds

Pour plate technique according to the laboratory manual.

6.5.2 Enterobactericeae

Pour plate technique according to the laboratory practical manual.

6.6 Shelf life determination

I achieved this by accelerated temperature techniques of shelf life determination. I took five samples from each product and put them in an oven at 54°C. Each day, I tested them for fat rancidity through peroxide value determination and monitored changes in colour and aroma. I equated one day to one month.
6.7 Acceptability determination

By sensory analysis which was done by a panel of 10 people using a 7-point hedonic scale to give scores for each sample based on various attributes. The attributes I was observing in this study were: appearance, colour, flavour, aroma, taste and texture.

**Hedonic scale**

1-Dislike very much

2-Dislike

3-Dislike slightly

4-Fair

5-Like slightly

6-Like

7-Like very much

6.7.1 Statistical analysis of data

I analysed my data using mean and standard deviation to determine acceptability.
7.0 RESULTS

7.1 Needs assessment in the Kenyan market based on rabbit meat products

<table>
<thead>
<tr>
<th>SUPERMARKETS</th>
<th>PRODUCTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>frozen lean meat</td>
</tr>
<tr>
<td>Uchumi</td>
<td>✓</td>
</tr>
<tr>
<td>Nakumatt</td>
<td>✓</td>
</tr>
<tr>
<td>Tuskys</td>
<td>✓</td>
</tr>
<tr>
<td>Naivas</td>
<td>✓</td>
</tr>
</tbody>
</table>

Explanation

The results above is a clear indication of non-existence of fermented rabbit sausages in the market. There exist few other varieties of rabbit products as shown in the table. This therefore implies that rabbit meat is underutilized in Kenya due to the existence of the few rabbit products in the market.

7.2 Formulation of a fermented rabbit sausage

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TREATMENT (culture)</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>L. bulgaricus</td>
<td>Fermented rabbit sausage</td>
</tr>
<tr>
<td>B</td>
<td>S. lactis</td>
<td>Fermented rabbit sausage</td>
</tr>
</tbody>
</table>

Explanation

Fermented sausages can be made using both cultures. Though there is a variation in the end products in terms of quality as we shall see later.
7.3 Proximate composition

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUTRIENT</td>
<td>%/100G WWB</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>6.52</td>
<td>7.58</td>
</tr>
<tr>
<td>Protein</td>
<td>10.6</td>
<td>16.8</td>
</tr>
<tr>
<td>Fat</td>
<td>21.8</td>
<td>25.1</td>
</tr>
<tr>
<td>Ash</td>
<td>4.45</td>
<td>3.8</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>14.86</td>
<td>20.1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>41.77</td>
<td>26.62</td>
</tr>
</tbody>
</table>

Explanation

From the above table, it is clear that the treatments do not bring about a big variation in the chemical composition of the product. There is only one component whose content is statistically different in the two samples. That is the carbohydrates. These are because of the reasons that will be seen later in the discussion. By fermenting the rabbit meat the moisture content reduces and also the protein content reduces but not so much.

7.4 Other chemical analysis

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titratable acidity</td>
<td>11.7%</td>
<td>6.3%</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>8.43%</td>
<td>8.46%</td>
</tr>
<tr>
<td>Peroxide value</td>
<td>Negative results</td>
<td>Negative results</td>
</tr>
</tbody>
</table>

Explanation

Sample A is less acidic than B. This indicates that treatments have different impact on the final acidity of the product. L. Bulgaricus produces a product with less acid content than S. Lactis.
Free fatty acids is at the same level and peroxide value tested negative. This shows that the fat used did not develop rancidity during the ripening period and therefore it is a high quality fat.

### 7.5 Microbial analysis

<table>
<thead>
<tr>
<th>MICROBES</th>
<th>SAMPLE A</th>
<th>SAMPLE B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast and moulds</td>
<td>$43 \times 10^2$</td>
<td>$48 \times 10^2$</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>$31 \times 10^2$</td>
<td>$38 \times 10^2$</td>
</tr>
</tbody>
</table>

**Explanation**

The microbial counts in both products were a bit high and this can be attributed to the poor conditions of the ripening rooms. Microbial load increases with decrease in acidity. Acidity inhibits microbial growth (Dhameer, 2007).

### 7.6 Shelf life determination

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>SHELF LIFE IN MONTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
</tr>
</tbody>
</table>

**Explanation**

L. bulgaricus yield a product with a shorter shelf life than S. Lactis. This can be attributed to fact that high acidity accelerates the onset of fat oxidation and hence spoilage of the product.
7.7 Acceptability of the product

<table>
<thead>
<tr>
<th>ATTRIBUTES</th>
<th>SAMPLE A</th>
<th>SAMPLE B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean score</td>
<td>standard deviation</td>
</tr>
<tr>
<td>Appearance</td>
<td>6.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Colour</td>
<td>6.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Flavor/aroma</td>
<td>6.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Taste</td>
<td>5.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Texture</td>
<td>5.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Explanation**

Sample A was the most liked with the highest mean score and standard deviation. It was the most liked in terms of all attributes as shown above.

8.0 DISCUSSIONS

8.1 Formulation and proximate composition

I managed to formulate a fermented rabbit sausage which, by the results of my base line study, seems not to exist in the market. This product therefore has filled the gap.

Discussion of the proximate composition of this product cannot be complete without comparison with the normal fresh sausages that exist in the market. Therefore the following table shows a comparison between the developed product and the conventional sausages in the market in terms of proximate composition(pak.j.Nutr., 8(4):332-334, 2009).
<table>
<thead>
<tr>
<th>Nutrients (%/100g on wwb)</th>
<th>Conventional sausage</th>
<th>Developed sausage(A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>63.9</td>
<td>6.52</td>
</tr>
<tr>
<td>Protein</td>
<td>12.8</td>
<td>10.6</td>
</tr>
<tr>
<td>Fat</td>
<td>16.7</td>
<td>21.8</td>
</tr>
<tr>
<td>Ash</td>
<td>2.3</td>
<td>4.45</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>6.3</td>
<td>14.86</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4.3</td>
<td>41.77</td>
</tr>
</tbody>
</table>

From the table above it’s clear that there are no big variation in the proximate composition of the two except for the moisture content and carbohydrate content. The developed product has a lower moisture content which gives it an upper hand in terms of shelf stability. Low water content translates to a lower water activity hence inhibition of microbial spoilage. The developed product can therefore stay for a longer period without spoiling.

The higher carbohydrates content can be attributed to difference in ingredients in the product. For the fermented sausage, sugar is added as food for the culture.

8.2 Other chemical analysis

Sample A had the highest acidity. L. Bulgaricus is associated with rapid acidification than S. Lactis (Deva and Narayan., 1989). This high level is attributed to lactic acid formation which contribute to the development of a characteristic taste of the product and hence acceptability. This also contribute to the prolonged shelf life.

Negative results of peroxide determination shows that the fat was of good quality and therefore it had not gone rancidity.

Free fatty acid content is high though it is not dangerous in this case because first; the type of fat used has higher percentage of saturated fatty acids. Second, their oxidation is prevented by the enzyme catalase released by the culture used in the experiment (Edward R. Fanworth., Hand book of fermented foods :265).
8.3 Microbial count

The higher the acidity the lesser the microbial load (Dharmaveer et al., 2007). This is also illustrated by the results we saw earlier. The most spoilage organisms likely to occur under these conditions are the yeast and moulds. Their high numbers is attributed to low moisture contents of the product and poor ripening room conditions. Product A has the lowest microbial counts due to its high acidity.

Enterobactericeae is a group of indicator microorganisms for unhygienic handling.

8.4 Acceptability

The most accepted product was A. L. bulgaricus is associated with production of fine textured and coloured products (Deva and Narayan, 1989). Product A had an appealing appearance. Colour, texture and taste compared to its counterpart. It was therefore the most accepted.

In the course of ripening, peptides and amino acids accumulate up to 1%. (Fidel Toldra, Handbook of processed meat and poultry analysis). Peptides and amino acids themselves may contribute to the characteristic taste of the product and act as flavour enhancers. Furthermore amino acids and peptides are used by microorganisms for conversion into flavour compounds (Edwardmented R. Fanworth, 2002. Handbook of fermented functional foods: 263).

9.0 GENERAL DISCUSSION OF THE CHANGES THAT TAKE PLACE DURING MEAT FERMENTATION

The, biochemical physical and microbial changes during sausage fermentation are summarized as follows. Growth of LAB and concomitant acidification of the product, reduction of nitrites to nitromyoglobin solubilization and gelification of myofibrillar and sarcoplasmic proteins, degradation of the proteins and lipids and dehydration.

9.1 Acidification, dehydration and microbial antagonism
Rapid acidification and consequent drying are of paramount importance for inhibition of the growth of pathogens and their inactivation during ripening. Growth and metabolism of LAB result in fast pH drop during the first days of sausage fermentation followed by a slight increase during the ripening period. Lactic and acetic acids are the major fermentation products.

9.2 Proteolysis and lipolytic degradation during fermentation

The hydrolysis of muscular proteins to peptides is mainly achieved by endogenous enzymes. The edopeptidases were found to remain active during fermentation. Furthermore muscle exopeptidases contribute to the conversion of peptides to amino acids (Edward Fanworth, 2002. Handbook of fermented functional foods).

During fermentation, long chain fatty acids are released from tri-glycerides and phospholipids. Typically, an increase in the level of free fatty cids up to approximately 5% of the total fatty acids has been found. The specific release of poly-unsaturated fatty acids is higher than that of mono-unsaturated fatty acids or saturated fatty acids. The lipolysis is attributed to meat endogenous enzymes (E. Fanworth, 2002. Handbook of fermented functional books: 263-264).

9.3 Generation of flavour volatiles

Flavour compounds are generated during sausage fermentation by the following general routes (E. Fanworth, 2002. Handbook of functional foods: 264):

1. Flavour volatiles are generated by lipolysis and hydrolysis of phospholipids, followed by oxidation of free fatty acids.

2. Microorganisms produce organic acids; convert amino acids and peptides to flavour active alcohols, aldehydes and acids; and modify products of lipid oxidation.

3. Addition of spices and smoking.
10.0 CONCLUSION

Base-line survey carried out ws successful since it showed the gap in the market. This lead to the accomplishment of my second objective of this study which was to formulate a fermented rabbit sausage. All analysis pertaining the developed product was achieved successfully. The outcome of all these was an acceptable developed fermented rabbit sausage.

11.0 RECOMMENDATIONS

- If fermented sausages are to be produced in the future in the pilot plant, then conducive ripening rooms for the products should be put in place.
- Some chemicals that can be used to retard mould growth in the product should be incorporated as one of the additives.
- The production of this product is such an expensive thing that it requires huge capital investments.
- Suitable casings should be used inorder to attain satisfactory results.
12.0 REFERENCES

5. AOAC (association of official analytical chemists), 2000