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NUTRITIONAL STUDIES WITH SPECIFIC
REFERENCE TO THIAMINE ON KG. C.
A GHANAIAIN CEREAL PRODUCT
A THESIS

Submitted to

THE UNIVERSITY OF LEEDS

in partial fulfilment of the

requirement for the award of the

degree of

Master of Philosophy

Work Supervised by H. G. Muller Esq., FSc., Ph.D., F.I.F.S.T.

Procter Department of Food and Leather Science

The University

To
my Husband
and
my Daughter
INTEGRi PROCEDAMUS
SUMMARY

'Koko' was prepared in the laboratory from Ghanaian maize. The effect of grinding and sieving on the protein content was determined. Protein losses were highest with the coarsest grinding. Starters were used in the fermentation and were found to increase the rate of acid production.

Preservation of 'Koko' was carried out by Roller-drying, Spray-drying, Freeze-drying and Canning. The volatile constituents of the flavours were lost during Roller-drying and Spray-drying. Freeze-drying was very successful but it would be too expensive for commercial processing of 'Koko'. It could, however, be used in the laboratory for research work. Canning of 'Koko' was also successful, though more work on its microbiological aspect is needed to give conclusive results.

Thiamine and Riboflavin contents were determined at various stages in the preparation of 'Koko'. Slight losses of these two vitamins occurred during steeping of maize. Fermentation increased Thiamine considerably although only very slight increases were recorded for Riboflavin. The significance of these results are discussed.
ACKNOWLEDGEMENTS

This work was carried out under the supervision of Dr. H.G. Muller, to whom the author is greatly indebted.

She also wishes to thank Professor A.G. Ward, O.B.E. for allowing her to carry out this research in the Procter Department and all members of that Department particularly Mr G. Glew and Miss J. Millross who have helped in various ways.

Last but not the least, the award of a Postgraduate Scholarship by the Ministry of Overseas Development under the Technical Assistance Schemes is gratefully acknowledged, without which this study would not have been possible.
LIST OF CONTENT

ACKNOWLEDGEMENTS

1. INTRODUCTION

1.1. FOOD CONSUMPTION IN GHANA
1.1.1. Food Production and Consumption in Ghana
1.1.2. The Nutrition of the Ghanaian Population
1.1.3. The Role of 'Koko' in Ghana with particular reference to Protein and Vitamin Contents

1.2. TRADITIONAL PREPARATION OF KOKO

1.3. PRESERVATION
1.3.1. General Principles of Roller drying
1.3.2. Spray-drying
1.3.3. Freeze-drying
1.3.4. Canning

2. EXPERIMENTAL

2.1. LABORATORY PREPARATION OF KOKO
2.1.1. Materials
2.1.2. Methods
2.1.3. Effect of Grinding on Particle Size Distribution
2.1.4. Effect of Grinding on Protein Content
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. 1.5. General Preparation of 'Koko' in the Laboratory</td>
<td>35</td>
</tr>
<tr>
<td>2. 1.6. Proximate Analysis</td>
<td>36</td>
</tr>
<tr>
<td>2. 1.7. Yield of material in the laboratory</td>
<td>38</td>
</tr>
<tr>
<td>2. 1.8. Total Protein Yield in the laboratory</td>
<td>40</td>
</tr>
<tr>
<td>2. 1.9. The Use of Starters</td>
<td>42</td>
</tr>
<tr>
<td>2. 2. PRESERVATION OF KOKO</td>
<td>46</td>
</tr>
<tr>
<td>2. 2.1. Equipment</td>
<td>46</td>
</tr>
<tr>
<td>2. 2.2. Roller-drying</td>
<td>47</td>
</tr>
<tr>
<td>2. 2.3. Spray-drying</td>
<td>49</td>
</tr>
<tr>
<td>2. 2.4. Freeze-drying</td>
<td>51</td>
</tr>
<tr>
<td>2. 2.5. Canning</td>
<td>53</td>
</tr>
<tr>
<td>2. 3. FATE OF THIAMINE AND RIBOFLAVIN DURING MANUFACTURE AND PROCESSING OF KOKO</td>
<td>56</td>
</tr>
<tr>
<td>2. 3.1. The Chemistry of Thiamine and Riboflavin and Methods Available for their Analysis</td>
<td>56</td>
</tr>
<tr>
<td>2. 3.2. Materials and Methods</td>
<td>58</td>
</tr>
<tr>
<td>2. 3.3. Thiamine Content in maize steeped over a period of 1-4 days</td>
<td>61</td>
</tr>
<tr>
<td>2. 3.4. Thiamine Content at Different Stages of 'Koko' Preparation</td>
<td>62</td>
</tr>
<tr>
<td>2. 3.5. Riboflavin Content at Different Stages of 'Koko' Preparation</td>
<td>64</td>
</tr>
</tbody>
</table>
3. GENERAL DISCUSSION

4. REFERENCES
1.1 FOOD CONSUMPTION IN GHANA

1.1.1 Food Production and Consumption in Ghana.

The southern coastal boundary of Ghana almost runs along latitude 5°N. The country extends inland for over 450 miles to the latitude of 11°N and has an area of about 92,000 sq. miles (Platt and Mayer, 1959).

The 1960 Population Census gave a population size of about 7 million and a population density of 73 persons per square mile. These figures indicate that Ghana is a thinly populated country (Birmingham, Nenstadt and Omaboe, 1967).

Ghana can be divided into three main vegetational zones namely the Coastal Savanna Plains, the Forest Zone and the Northern Savanna Zone.

The country is relatively cooler than most other countries which lie in the same latitudes. The annual mean temperatures for the whole country range from 26.1°C (79°F) to 28.9°C (84°F) with the lowest figures in the south and the highest occurring inland in the north (Boateng 1959).

The relative humidity is very high, ranging from 65% to 95% for most part of the year. The forest regions, especially the south western part tend to have the highest humidity whilst the north tends to be less humid. The mean annual rainfall is 76.2 cm (30 ins) to 152.4 cm (60 ins) in the savanna regions and 152.4 cm (60 ins) to 254.0 cm (100 ins) in the forest regions (Boateng, 1959; Varley and White, 1958).

The vegetation in the coastal Savanna Plains consists of comparatively
small trees and scrub with grass as undergrowth. There is a park-like vegetation in this region.

The forest region has large trees, often a hundred feet or more in height, with a dense undergrowth of herbs and climbing plants. The tops of the tall trees touch one another and form a continuous layer or "canopy".

In the Northern Savanna Zone there is a long dry season in the year. The few scattered trees are often small and tall grass is found everywhere.

There seem to be no reliable figures for food production in Ghana since the various estimates differ considerably (Whitby, 1969). However, a tentative Food Balance Sheet has been constructed based on the estimates of "Crop Area, Yield and Production : Ghana 1963 and 1964". This is a mimeographed sheet produced by the Division of Economics and Statistics of the Ministry of Agriculture. These estimates help to give an overall picture of annual food production but may be misleading in detail.

The National Nutrition Surveys of 1960–62 covered the whole country and give quantitative information on food consumption.

Table 1 gives figures for food production and consumption. Column 4 gives estimates of consumption per head in grams of edible portion per day i.e. the weight of the edible portion of each food item that is eaten by an individual in a day, is given in grams.
Table 1: Food Production and Consumption Estimates for Ghana. (After Whitby 1969)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Food Production Estimate 1963 Approx. wts. 1000 metric tons</th>
<th>Food Consumption 1960-1962 Survey, Approx. wts. 1000 metric tons</th>
<th>Consumption per head (g. edible portion per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize (Zea mays)</td>
<td>183</td>
<td>224</td>
<td>80</td>
</tr>
<tr>
<td>Rice (Oryza sativa)</td>
<td>22</td>
<td>47</td>
<td>17</td>
</tr>
<tr>
<td>Millet (Pennisetum sp.)</td>
<td>68</td>
<td>71</td>
<td>25</td>
</tr>
<tr>
<td>Sorghum (Sorghum sp.)</td>
<td>109</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>Cassava (Manihot utilissima)</td>
<td>1194</td>
<td>980</td>
<td>307</td>
</tr>
<tr>
<td>Plantain (Musa paradisiaca)</td>
<td>1220</td>
<td>807</td>
<td>183</td>
</tr>
<tr>
<td>Yam (Dioscorea sp.)</td>
<td>1099</td>
<td>194</td>
<td>77</td>
</tr>
<tr>
<td>Cocoyam (Xanthosomas mafaffa)</td>
<td>340</td>
<td>245</td>
<td>65</td>
</tr>
<tr>
<td>Cowpeas (Vigna unguiculata)</td>
<td>2.6</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Lima beans (Phaseolus lunatus)</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bambara beans (Voandzeia subterranea)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groundnuts (Arachis hypogea)</td>
<td>28</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Category</td>
<td>Unit 1</td>
<td>Unit 2</td>
<td>Unit 3</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Palmnuts (Elaeis guineensis)</td>
<td>106</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td>15</td>
<td>43</td>
<td>18</td>
</tr>
<tr>
<td>Garden eggs (Solanum melongena)</td>
<td>40</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Okro (Hibiscus esculentus)</td>
<td>33</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Leafy vegetables</td>
<td>42</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Pepper (Capsicum sp.)</td>
<td>34</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Onions (Allium sp.)</td>
<td>8</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Meat, butcher's</td>
<td>31</td>
<td>47</td>
<td>23</td>
</tr>
<tr>
<td>Bushmeat</td>
<td>17</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Fowl</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish (fresh)</td>
<td>139</td>
<td>160</td>
<td>55</td>
</tr>
<tr>
<td>Banana (Musa sapientium)</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pawpaw (Carica papaya)</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple (Ananas sativus)</td>
<td>232</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange (Citrus sinensis)</td>
<td>257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango (Mangifera indica)</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Some wheat is eaten in Ghana mainly in the form of bread and snacks. Since the climatic conditions do not permit the cultivation of wheat, all the wheat used in the country - some 15 thousand metric tons per annum - is imported.

Fruit does not form part of the diet taken at meals time though considerable amount is eaten as refreshment.

Meat is obtained from cattle, sheep, goats and pigs. Since the forest zone is infested with tsetse fly which transmit trypanosomiasis, the few cattle kept in the country are in the northern savanna zone and on the Coastal plains. Much cattle is imported from Upper Volta and the neighbouring countries to meet the demand for meat which is higher than the local supply.

Sheep and goats are kept in all regions in the country mainly for meat and hides. Some local breeds of black pigs are also kept in some places in the country.

Most of the men in rural areas hunt for wild animals. This provides "bush-meat" which is popular in small scale catering places and markets.

Very little fresh milk is obtained from cows, under very unhygienic conditions. Most of the milk consumed in Ghana is in processed form and imported. Evaporated milk is the most popular though some sweetened condensed milk and dried milk are also used.

Most of the fish is caught in small canoes or large trawlers in the sea. Fresh-water fish is also caught in rivers. There are some cold stores for fish storage and these are private or Government owned.
The supply of fish to the inland is sent in frozen form in refrigerated vans and in smoked and dried forms too.

Dietary patterns differ in the various regions and depend to a large extent on what is grown in each region. The staple foods are cereals, plantains, starchy roots and tubers. Table 2 shows staple foods eaten in the various regions.

<table>
<thead>
<tr>
<th>REGIONS</th>
<th>STAPLE FOODS</th>
<th>SUBSIDIARY FOODS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal Savanna</td>
<td>Maize</td>
<td>Yam</td>
</tr>
<tr>
<td>Plains</td>
<td>Cassava</td>
<td>Plantain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cocoyam</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rice</td>
</tr>
<tr>
<td>Forest Regions</td>
<td>Plantain</td>
<td>Yam</td>
</tr>
<tr>
<td></td>
<td>Cocoyam</td>
<td>Maize</td>
</tr>
<tr>
<td></td>
<td>Cassava</td>
<td>Rice</td>
</tr>
<tr>
<td>Northern Savanna Region</td>
<td>Millet</td>
<td>Maize</td>
</tr>
<tr>
<td></td>
<td>Sorghum</td>
<td>Rice</td>
</tr>
<tr>
<td></td>
<td>Yam</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 gives a map of Ghana showing the three main vegetational zones with their staple foods.
FIGURE 1: STAPLE FOODS IN THE 3 MAIN VEGETATIONAL ZONES OF GHANA.
1. 1:2 The Nutrition of the Ghanaian Population

The overall availability of nutrients in Ghana was calculated by Whitby (1969) from FAO Recommended Dietary Allowances Tables and data from various food consumption surveys. An extract is shown in Table 3.

Table 3: Percentage of Protein and Vitamin requirements fulfilled in the diet of various classes of the Ghanaian Community (Total requirement 100%; intakes of less than 75% underlined (After Whitby 1969).

<table>
<thead>
<tr>
<th>Classes of Community</th>
<th>Protein</th>
<th>Vitamin A</th>
<th>Thiamine</th>
<th>Riboflavin</th>
<th>Niacin</th>
<th>Vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal Plain Villages</td>
<td>(Mean of 4)</td>
<td>83</td>
<td>176</td>
<td>98</td>
<td>39</td>
<td>96</td>
</tr>
<tr>
<td>ACCRA</td>
<td>156</td>
<td>335</td>
<td>96</td>
<td>52</td>
<td>159</td>
<td>120</td>
</tr>
<tr>
<td>Forest Villages</td>
<td>(Mean of 8)</td>
<td>86</td>
<td>165</td>
<td>93</td>
<td>41</td>
<td>103</td>
</tr>
<tr>
<td>Forest Towns</td>
<td>(Mean of 5)</td>
<td>105</td>
<td>249</td>
<td>117</td>
<td>54</td>
<td>147</td>
</tr>
<tr>
<td>Northern Region Villages</td>
<td>(Mean of 4)</td>
<td>170</td>
<td>34</td>
<td>247</td>
<td>75</td>
<td>167</td>
</tr>
<tr>
<td>DAKONGO</td>
<td>98</td>
<td>18</td>
<td>147</td>
<td>44</td>
<td>130</td>
<td>48</td>
</tr>
</tbody>
</table>
The general impression that there is protein shortage in Ghana should be qualified because various data collected in nutritional surveys show that the average adult has sufficient protein and the theoretical requirements are satisfied.

However, the protein supply of children of preschool age and of the farming villages in the south (both forest and coastal zones) is insufficient. This is due to the low intake of protein-rich foods (meat, fish, eggs etc.) Most clinical protein deficiency cases are encountered in children under five years of age. This is mainly due to the fact that their share of protein-rich foods is withheld for various reasons (Whitby 1969). According to Dovlo (1969) fish and meat are not given to children under five because they are expensive and chiefly because of the belief that the children suffer various illnesses such as indigestion and diarrhoea from eating these.

A greater proportion of vitamin A in the Ghanaian diet is obtained from palmnuts and palm oil. It can be seen from Table 3 that apart from the Northern Savanna zone where oil palm does not grow, there is ample supply of vitamin A in Ghana. Cases of clinical deficiency of vitamin A in Northern Ghana have been reported (Davey 1961; Rodgers 1957; Platt and Mayer 1959).

Plantains, starchy roots and tubers contribute significantly to the intakes of vitamin C in the south of Ghana. Intakes of this vitamin in Northern Ghana seem to be rather low because cereals, which are low in ascorbic acid, form the bulk of the staple foods.
Cereals are the major source of thiamine and niacin both vitamins of the B group. Theoretical requirements of vitamins of this group are proportional to calorie requirement. Therefore figures for the fulfillment of these are in Table 3 are related to the calorific intake. Both beriberi and pellagra, the deficiency diseases of thiamine and niacin respectively, are rare in Ghana.

On the other hand, nutritional data available, indicate that there is insufficient supply of riboflavin another B group vitamin, in every part of Ghana (Davey 1961. Colbourne and Eddington 1950). This is probably because most of the major sources of this vitamin are foods of animal origin (liver, kidney, milk etc.,) which are expensive in Ghana.

Recorded intakes of calcium are lower than the theoretical requirements, but there is no evidence of clinical problems associated with shortage of dietary calcium.

Dietary intakes of iron are very close to the recommended allowances but there is in Ghana, as in other parts of West Africa, widespread incidence of anaemia. This is probably due to malaria, hookworm and other such infections which cause anaemia.

It can be said in summary that there is protein insufficiency for children under five years. This condition is responsible for two main protein deficiency diseases found in children, namely kwashiorkor and marasmus. It also appears that serious shortages of riboflavin and vitamin A exist in Ghana.
1.1:3 Role of Koko in Ghana with particular reference to Protein and Vitamin Content.

Although Koko is eaten by adults in Ghana as a breakfast cereal, it is the traditional weaning food:

Williams (1933) observed that at the weaning stage of infants in Ghana, then known as the Gold Coast, breast-feeding was supplemented with "Koko".

Davey (1961) showed that infants in Ghana are given cereals (Koko and Kenkey) as their first solid food and starchy roots and tubers later when they are completely weaned. The cereals were given at the start of weaning mainly in the form of "Koko" which is prepared from maize in the South and from millet or sorghum in the north.

This means that for the first year or so, maize "Koko" supplies most of the nutrients required by infants in the South of Ghana.

The protein content of maize is adequate in quantity but is of poor quality. This is because maize protein is deficient in tryptophan and lysine, two of the eleven essential amino acids. Block and Michell (1946) concluded after experiments with rats that the biological value of maize protein is considerably lower than those of proteins of whole wheat and whole rice. Williams (1933) first associated maize diets of infants with a protein deficiency disease called Kwashiorkor.

Maize is fairly rich in thiamine and constitutes a major source of
this vitamin in places where it is eaten as a staple food. Golberg and Thorp (1945) concluded that African maize provide an adequate level of thiamine. The effect of Koko preparation on Thiamine content of maize has been investigated in this thesis.

The niacin content of whole maize is much lower than that of whole wheat or whole rice. Pellagra, a niacin deficiency disease is often encountered in maize eating populations in the world (F.A.O. 1953). It is generally accepted that niacin deficiency would not cause pellagra if maize were rich in tryptophan which can be converted to niacin in the human body. The availability of niacin in maize is increased by treatment with strong acid or weak alkali (Chandhuri and Kodicke 1959; Krehl et al 1944 and Harper et al 1953). This explains why in Mexico where maize is treated with lime-water in the preparation of tortillas, little or no pellagra is experienced. The fact that pellagra is of little importance also in Tropical Africa could be due to the traditional preparations which usually involve sprouting and alcoholic or lactic acid fermentation of the maize (Miracle 1966). Various workers have found that these processes increase the B group vitamins in maize (Goldberg and Thorp 1946).

The riboflavin content of maize is so low that it is estimated to be often less than half of the theoretical requirement (Anderson et al 1948 and Calvo et al 1946). Riboflavin deficiency is often encountered in maize eating regions of the world both independently and in association with pellagra (F.A.O. 1953). Golberg and Thorp (1946) showed that
there is an increase of 92% riboflavin in the preparation of leting, a lactic acid fermented maize product eaten in South Africa. They went on to recommend widespread use of fermented cereal foods in achieving vitamin balance in areas with incidence of riboflavin and niacin deficiencies. The probability of such high increases in riboflavin in the preparation of Koko, a Ghanaian fermented maize product has always been suggested by various workers (Whitby 1969). In this thesis, investigations have been carried out to determine how riboflavin content is affected in the preparation of Koko.

Vitamin C content of maize is very low in the dried mature kernels although moderately higher levels are found in immature kernels and in varieties of sweet-maize (Dunken et al 1937; Van Duyne et al 1945).

The amount of vitamin A found in maize depends on whether it is of yellow or white variety. High levels of vitamin A are found in yellow maize whilst white maize contains no appreciable amount of this vitamin. (Fraps and Kummerer 1941; Fraps 1931). In regions where white maize is eaten with inadequate supplementary foods which provide vitamin A, signs attributed to its deficiency have been reported (Hassan 1944; Miranda 1944; Raymond 1941).

It can be said in conclusion that white maize is deficient in proteins, riboflavin, ascorbic acid and vitamin A. Infants' diets based on maize, such as Koko, have to be supplemented with other foods which provide these nutrients. With the exception of ascorbic acid, these nutrients could be supplied by the addition of milk, soya flour or peanut flour to the
maize diet.

In this thesis, the traditional method of Koko preparation has been followed and the fate of thiamine and riboflavin has been investigated.
1.2. TRADITIONAL PREPARATION OF KOKO.

Almost all the maize grown in Tropical Africa is used as human food. Virtually insignificant quantities are used for feed by African farmers. Flint maize is the commonest type grown in West Africa. There is also some floury or soft type. Figure 2 shows a small plot of maize plants in an African Village. The mature kernels are used in staple diets based on maize and in West Africa these are usually fermented and eaten as gruel or dumplings.

Various writers have described maize preparations in south western Nigeria (Akinrele 1965, Banigo 1969, Bascom 1951 and Oke 1965). The bulk of maize consumed in this area is in the form of "Ogi", a fermented paste very much like soured wet starch. "Ogi" is cooked into a gruel called "Eko" or a thick paste, like blancmange, called "agidi" which is sold wrapped in leaves.

Adande (1953) gave a very good account of maize preparations in his study of maize in Dahomey, a French-speaking country in West Africa. He described in detail the preparation of a fermented maize paste called "Ogi". As the Nigerian equivalent with the same name, this product is usually eaten as a gruel or a cooked thick paste.

Alldridge (1910) observed that "agidi", a fermented maize dish, was the principal maize preparation in Sierra Leone.

In the south of Ghana maize is a staple food and a variety of dishes are prepared from it. Maize is usually made into a fermented dough
Fig. 2. SMALL PLOT OF MAIZE AT ENTRANCE TO WEST AFRICAN VILLAGE.
(Courtesy H.C. Muller)
called "mbor" which forms the basis of the various dishes. The timing of operations varies depending on the final use of the dough. "Koko" is a Hausa name for a gruel prepared from "mbor", and it has different names in different tribes (Akasa - Ga and Fante; Akatsa - Ewe).

In the account given below the general method of "mbor" preparation is described with particular reference to Koko. Figure 3 shows a flow diagram of the traditional method of Koko preparation.

The maize grain is cleaned by winnowing to remove light particles of dirt. Other pieces of dirt and substandard grains are separated by hand. The cleaned maize is then poured into either a metal container or an earthenware pot and fresh water is used to cover the grain.

The container is loosely covered and kept in a corner of the kitchen at room temperature of about 30°C. Steeping is usually done overnight in Koko preparation but a period of up to 3 days has been reported by Whitby (1968) in the preparation of dough for other purposes. The steeping water is decanted and the steeped maize is either poured into a basket to allow the rest of the steeping water to drain off or it is scooped into a dry container. With exceptionally dirty grain a scum forms on steeping and the grain is washed after steeping to clean it.

The steeped maize is then milled into a wet wholemeal. A powered mill such as the one shown in Figure 4 is usually used for the grinding but in villages where such mills are not available the traditional method of milling is employed. This involves pounding of the grain with pestle and mortar Figure 5 and finally grinding on a stone.
Fig. 4: Powered grinding of maize in an urban community.
Fig. 5: PESTLE AND MORTAR MILLING OF MAIZE IN A WEST AFRICAN VILLAGE.

(Courtesy H.G. Muller)
The ground maize is mixed with water into a dough which is left at room temperature to ferment for 1-3 days. Fermentation is always spontaneous and is carried out for 2 days if Koko is to be the final product. It is not possible to control the flavour of "mbor" since the fermentation process is spontaneous. This could be done with moisture and temperature control if starters were used.

Pyler (1952) observed that bakers in Germany and other European countries used to rely on spontaneous bacterial fermentation in the preparation of rye bread. The rate of fermentation was slow then and the flavour characteristics of rye bread could not be controlled. Later, the "Berlin Short Sour Method" was adopted in Germany. In this method, a fresh batch of dough or sponge is inoculated with a portion of the previous day's sour dough. Thus a sufficient amount of the bacterial flora is introduced into the new sponge or dough to produce the desired effect in the course of normal fermentation. By judicious selection of temperature and absorption conditions of the sour i.e. the starter, a baker is able to control to a considerable degree, the flavour characteristics of his rye bread and also to shorten the fermentation time.

Hicka (1942, 1955) studied lactic acid fermentation of soda crackers and found that sterile mixing troughs, slowed down fermentation and affected the flavour of the finished product. He recommended that troughs should not be thoroughly cleaned in order to allow traces of the old sponge to act as starters.

In this thesis, some experiments were carried out to find the effect
of starters on the fermentation of "mbor" prepared in the laboratory.

After fermentation, the dough is used to prepare various dishes some of which will be described later. In the preparation of "Koko" the fermented maize-dough is mixed with water into a slurry which is sieved using a piece of muslin cloth or a fine sieve to separate the particles of bran. Figure 6 shows this stage of the process. The overtails from the sieve are finally washed with water and discarded.

The sieved slurry is boiled with constant stirring to prevent formation of lumps. Stirring is normally done with a wooden stirrer very much like a small paddle Figure 7. The cooked product is in the form of a thin gruel and is called "Koko" (Hausa) or Akasa (Ga and Fante). Sugar is always added before it is eaten. Milk may also be added where it is available.

Sieving is sometimes omitted, in which case fermented maize-dough is mixed with water and boiled, under stirring, into a thick pap. This is called "mpampa" (Fante) or "Kpokponsu" (Ga) and is of a thicker consistency.

Kenkey, the principal maize dish in Ghana is prepared from the fermented dough. There are different varieties of this, some of which are associated with particular tribes. The dough is usually fermented for 3 days and is divided into two parts. One half is boiled, under stirring, into a thick half-cooked paste called "aflata". This is thoroughly mixed with the uncooked half and made into balls, weighing a pound each. These are wrapped in either maize husk or plantain leaves.
Fig 6: WIT-SIEVING OF GROUND MAIZE.

(Courtesy Kano Information Office)
Fig 7: COOKING OF KOKO PORRIDGE.

(Courtesy H.C. Muller.)
and steamed until cooked (about 2 hours). Owing to the difficult labour of stirring, only a third of the dough may be cooked into 'aflata'. With this modification of the method, the texture of the Kenkey is not as sticky as desired. Kenkey is eaten with soup or stew.  Alyward 1961 reported that on the eastern coast of Ghana, Kenkey was characteristically more sour than in the other parts.

The Ga and Fante tribes on the coastal plains have another staple dish called "banku". This has the consistency of dumplings. Maize-dough is usually fermented for 3 days then dropped into boiling water and vigorously stirred to prevent lumping. Salt is always added. The cooked product is shaped into balls and eaten with soup or stew.

"Akpler" is a dish mainly eaten by the Ewes on the eastern coast. It is softer than banku in consistency and is prepared from a mixture of fermented maize-dough and fermented cassava-dough. The method of cooking is the same as for banku.

There are also various sweet dishes prepared from mixtures of maize-dough and pounded ripe plantain. The fermentation period of the dough in these preparations is always very short (about a day).

This account indicates the versatility of the fermented maize-dough among maize eating communities in Ghana. Unfortunately, it cannot be kept for more than 4 days. After the third day of fermentation moulds start to grow on it and off-flavour develops. Some preliminary investigations were carried out in this thesis on the preservation of "Koko" by dehydration and canning.
A FLOW DIAGRAM OF KOKO PREPARATION.

1. Cleaned Maize
2. Steeping (Overnight)
3. Grinding
4. Doughing (Mbor)
5. Fermentation (2 days)
6. Wet Sieving
7. Boiling

Overtails

Koko
1.3 FOOD PRESERVATION.

All foods for mankind are perishable and are likely to deteriorate after harvest or slaughter. Food deterioration is usually accompanied by losses in nutritive value and sometimes by the production of poisonous substances. Man has however, learnt to control some of the deteriorations by various methods of preservation.

In this thesis, some preliminary studies were carried out on the preservation of "Koko" by dehydration and by canning.

One of the oldest methods of preservation by man is sun-drying which is also the most widely used method of food preservation. Drying is a natural process of preservation for foods such as cereals, legumes, nuts and certain fruits. In the food industry drying is effected by supplying heat from a source other than sunlight and this is called dehydration. Different types of driers are employed in the food industry and no single drier is universal in its application.

1.3.1 Roller-drying.

In the roller-drier or drum-drier the food is usually spread in a thin film on the outer surface of steam or water heated drums revolving at atmospheric pressure or under vacuum. Heat is transferred through the drum wall to the product film. Heating is almost entirely by conduction. The dried product is in the form of flakes which are removed by suitably designed scraper blades. The flakes may then be
ground to a fine powder. Drying is controlled by varying the temperature of the heating medium, the speed of the rotating drums and the thickness of the layer applied to the surface.

1. **Spray-drying.**

A spray-drier is an example of the second group of driers. In this drier heat is carried into the drying chamber by hot gas, usually air which gives up heat to the water in the food and carried out the water vapour produced. Spray-driers are used to dry liquid foods and slurries.

The liquid food may be dispersed into the drying chamber by a pressure nozzle or a centrifugal disc atomizer. Here the liquid impacts upon a rotating disc, and the resultant "sheet" of fluid breaks up into small liquid particles at the periphery of the rotating disc.

The particle size of the food is very important since the smaller the droplets the more rapidly moisture may be removed. Most spray-dried powders are in the particle size range of 50-125 microns but powders from small test driers are in the range of 5-50 microns (Seltzer and Settelemeyer 1949). Slurries or suspensions that cannot be atomized so finely are likely to deposit on the chamber walls in a partly dried condition.

Laboratory spray-driers are less flexible as an experimenter may encounter cases where it is not possible to dry certain materials. Seltzer and Settelemeyer 1949 recommended that tests should be carried out with commercial size driers before concluding that the material cannot be
Freeze-drying is another method of dehydration. The principle of freeze-drying is very simple. The food is frozen and water vapour is removed by sublimation i.e. water changes from the solid phase to the vapour phase without going through the liquid phase. The temperature must be kept below the melting point to avoid melting the ice phase.

With some biological materials it is necessary to maintain the temperature well below the freezing point in order to preserve biological activity. For food products the temperature should be held as near the melting point as possible in order to obtain the maximum drying rate.

There are two main methods of freezing the sample: prefreezing and evaporative freezing. In prefreezing the material is frozen before being placed under vacuum. This is usually done in conventional freezing equipment. Special methods have been designed by Greaves 1954 for prefreezing of pharmaceutical and biological materials. In evaporative freezing the unfrozen material is placed in the drier in which a vacuum is then created and evaporative cooling causes freezing. This method of freezing is suitable for liquid foods (Harper and Tappel 1957).

The material being dried should be held under vacuum during the entire drying process. The pressure in the system should not be above 4.7 mm of mercury since at pressure above this the liquid phase of water can occur (Desrosier 1959).
There are two types of equipment available for obtaining the vacuum required for freeze-drying namely mechanical vacuum pumps and steam jet ejectors. The water vapour formed by sublimation can either be removed directly by the vacuum pump or ejector or be removed ahead of the pump by condensation. The latter method of removing the water vapour is usually used in small-size laboratory freeze-driers. In these, dry ice is usually used to provide the low temperature on the condenser surface. The condenser is placed so that all vapours flow past it in order to reach the vacuum pump.

Heat of sublimation must be provided if drying is to take place. However, no special provision is made for this in some laboratory driers, dependence being placed on heat gained by natural transfer from the surroundings.

Floisdorf 1949 and Harris 1954 have given summaries of the literature on laboratory freeze-driers in their books. The importance of laboratory freeze-driers in food research is illustrated by the extensive use of such equipment in freeze drying of foods (Gane 1951, 1954, particularly beef (Wang et al 1954) eggs, (Rolfe et al. 1955) citrus juices (Campbell et al 1943) and milk (Nickerson et al 1952).

As with any method of preservation, the quality of dehydrated food is lower than that of the original foodstuff. Discolouration, loss of texture, loss of volatile flavours and poor rehydration ability have been known to influence the acceptability of some dehydrated foods.

The nutritive value of dried protein is dependent on the method of
drying. Prolonged exposure to high temperatures can render the protein unavailable to the consumer. Freeze-drying and spray-drying of raw milk do not affect the availability of lysine, whilst roller drying may result in heavy losses depending on roller speed and temperature (Bujard et al 1967)55.

Vitamins are the most sensitive nutrients in dehydrated products. The extent of vitamin destruction will depend on the degree of caution exercised during preparation of foodstuff for dehydration, the dehydration process selected and conditions of storage. Denton et al (1944)56 found that there was no loss in Vitamin A, D and riboflavin content of eggs during spray drying. Vitamin content of dried meat is usually less than in fresh meat. Great losses of thiamine occur at high drying temperatures whilst small losses of riboflavin and niacin occur (Desrosier 1959 46).

1. 3:4 Canning.

In the thermal processing of canned foods, the main objective is to destroy living organisms capable of causing deterioration of the food or endangering the health of the consumer through toxic hazards. It must also be borne in mind that the organoleptic and nutritive properties of the food should not be adversely affected by the heat treatment in canning.

In order to achieve a compromise between these needs, some information on the heat-resistance of the contaminating micro-organisms, the chemical and physical nature of the food and the rate at which heat penetrates the contents of the can is essential.
At pH values below 4.5 the growth of Clostridium botulinum, the most heat-resistant of food poisoning-organisms, is generally regarded as inhibited. Because of this, for foods with pH values below 4.5, pressure cooking (above 100°C) is considered unnecessary. This means that processing of 'Koko' (pH 4.0) need not exceed 100°C.

The rate of heat penetration in the product is measured by means of a thermocouple. Solid foods transfer heat by conduction and liquid foods mainly by convection. Some foods exhibit what is known as a broken heating curve. In these types of food an initial period of relatively rapid heat transfer ceases abruptly and is followed by an period of much slower heat penetration. Jackson and Olson 1940 in their report on the mechanisms of heat transfer in canned foods mentioned thick soups, brine-packed whole-grain corn and certain tomato juices as foods which gave broken heating curves. They concluded that the "break" was due to the product undergoing a sol-gel transition. Heat transfer is by convection in the first stage of heating but when the product changes to a gel form, heating continues mainly by conduction. "Koko" thickens when heated so its heat penetration curve is likely to be a broken one. It will be shown later that "Koko" does in fact give a broken heat curve.

To formulate the heating time and temperature for low acid canned foods, the lethal effect of the temperatures during heat-penetration can be calculated using various standard methods. However, according to Townsend et al 1954 there are not sufficient data, at present, to enable the suggestion of standard processing times and temperatures for acid
products as has been done for low acid foods, because acid products vary greatly in acidity, texture, cooking procedure and so forth.

The usual method of calculating the process time for acid foods is by inoculated pack studies. In this method the food is inoculated with specific micro-organisms before the cans are sealed. The canned product is sterilized at a particular temperature for different periods and observed for blown cans. They are then examined by microbial count to determine which processing time gives the least count considered to be safe for consumption.

Beadle et al (1943) showed thiamine to be more stable in solution of low pH than in neutral or alkaline solutions. Clifcorn and Heberlein (1944) found that commercial sterilization processes destroyed significant amounts of thiamine in vegetables but a high degree of thiamine retention was observed in canned tomatoes and juices. This was explained by the fact that tomatoes and tomato juice have low pH values and so require comparatively low sterilizing temperature which does no affect the thiamine content. Hilleres and Fellers (1949) showed that significant amounts of thiamine are destroyed during canning of chicken meat. They found that losses were dependent on time and temperature required for the process. Such losses were explained by the instability of thiamine at high temperatures in media with high pH values. There was evidence for retention of riboflavin and niacin in canned chicken meat since these are stable at relatively high temperatures.

The effect of canning on heat sensitive nutrients seems to depend on
temperature and duration of sterilization and the pH of the food being canned.
EXPERIMENTAL.
This section is divided into three main groups dealing with the Laboratory Preparation of 'Koko', its Preservation and finally with the Fate of Thiamine and Riboflavin, two vitamins normally contained in it.

2.1. LABORATORY PREPARATION OF 'KOLO'.

2.1.1. Materials.

White maize of flint variety was obtained from Ghana through the courtesy of the Food Research Institute, Accra, Ghana. This sample of maize was the only type used in the following studies. Proximate analysis of this sample of maize is shown in Table 4.

All chemicals used were of Analar or Laboratory Reagent grade.

In the fermentation experiment (section 2.1.9), the Lactobacillus acidophilus was a commercial preparation sold by Boots, the Chemists.

Table 4. Proximate Analysis of White Maize from Ghana (Dry weight basis)

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content</td>
<td>13.2</td>
</tr>
<tr>
<td>Crude Protein (N x 6.25)</td>
<td>10.5</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>4.3</td>
</tr>
<tr>
<td>Crude Fibre</td>
<td>1.7</td>
</tr>
<tr>
<td>Carbohydrate (by difference)</td>
<td>82.0</td>
</tr>
<tr>
<td>Ash Content</td>
<td>1.45</td>
</tr>
</tbody>
</table>
2.1.2. Methods.

Grinding.

In the preparation of 'Koko' maize is steeped before it is ground (section 1.2). This is necessary to soften the grain so that the traditional milling methods can deal with it. An electric Coffee grinder (2 Horse Power) supplied by Hobart Manufacturing Co. Ltd., was found suitable for grinding the steeped maize. This grinder can be adjusted to different settings to give different particle sizes of ground material.

Sieving.

To determine the particle size distribution of ground maize (section 2.1.3), sieving was done with a nest of 5 wire sieves with diameter 22 cm. and mesh sizes 10, 20, 30, 40 and 60. The sieves were supplied by Townson and Mercer Ltd., Croydon.

All proximate analyses were carried out using the Cereal Laboratory Methods of the American Association of Cereal Chemists (1962), modified whenever necessary.

(1) Determination of Moisture Content.

For maize and samples with moisture content below 13% the determination was done by oven drying in stainless steel moisture dishes at $130 \pm 5^\circ C$ for one hour (A.A.C.C. 44-15).

The moisture content of samples with % moisture higher than 13%,
was determined in two stages i.e. by air drying on top of a heated oven, followed by oven drying for one hour at $130 \pm 3^\circ C$ (A.A.C.C. 44 - 17).

(ii) **Determination of Crude Protein Content.**

The samples were digested with 25 ml concentrated $H_2SO_4$ and two catalyst tablets consisting of 5.0 g potassium sulphate and 1 g Copper sulphate, supplied by Thompson and Copper Ltd., Liverpool, until a clear light green solution was obtained. This was cooled, made up to a standard volume and distilled by the macro-Kjeldahl method. (A.A.C.C. 46 - 11).

(iii) **Determination of Ash Content.**

The samples were first ignited in silica dishes on a bunsen-burner. They were then ashed at a temperature of 550°C to a constant weight in a Gallenkamp muffle furnace (A.A.C.C. 68 - 01).

(iv) **Determination of Crude Fat.**

The samples were dried to constant weight in a laboratory forced-air oven at 100°C. Crude fat was then extracted from the dried sample in a soxhlet extraction unit with petroleum spirit, B.Pt. 40-60°C (A.A.C.C. 30-25).

(v) **Determination of Crude Fibre.**

The defatted residue from the crude fat determination was dried at
100°C to constant weight. The dried residue remaining after standard digestion of this sample with dilute sulphuric acid and dilute sodium hydroxide was ashed at 600°C to constant weight. The loss on ashing was taken as the crude fibre (A.A.C.C. 32-15) 62.

(vi) Determination of pH Values.

The samples were suspended in previously boiled distilled water. The suspension was agitated for 30 minutes and left to stand for 10 minutes. The supernatant was decanted and pH values determined with a Cambridge pH meter (A.A.C.C. 02-52) 62.

(vii) Determination of Total Titratable Acidity.

Samples were suspended in distilled water and shaken for 15 minutes. The suspension was then filtered and aliquots of the filtrate titrated against 0.1 N NaOH. The results were expressed as mg. NaOH per 100 g sample. (A.O.A.C. 1965) 63.
2.1.3. **Effect of Grinding on Particle Size Distribution.**

Maize used in this experiment was steeped before grinding as would be done in 'Koko' preparation.

**Method.**

400 g of maize were steeped in 600 ml of distilled water overnight at 30°C in an incubator supplied by Hearson Laboratory Equipment Ltd., London. The steeping water was decanted and the steeped maize was divided into 5 portions. Each portion of the steeped maize was ground at a different setting on the grinder.

The particle size distribution for each ground portion was determined using a nest of 5 wire sieves (section 2.1.2). 50 g of ground maize was weighed into the top sieve and the nest of sieves was shaken for 15 minutes on a vibrator supplied by Townson and Mercer Ltd., Croydon. The amount of material on each sieve and in the receiver was weighed separately and calculated, on dry weight basis, as a percentage of the sum of all separations. Five determinations were carried out on each sample.

Because the sieves used were not of British Standard, the aperture size for each mesh was measured with a travelling microscope attached to a vernier scale. Ten measurements were taken from different parts of each sieve and the average taken as the aperture size.

The particle size distribution of ground maize is shown in Table 5.
**Results and Discussion.**

**Table 5:** Particle Size Distribution of Ground Maize (Percent weight on dry basis) at Different Grinder Settings.

<table>
<thead>
<tr>
<th>Mesh Size Apperture (µm)</th>
<th>10 detns. Mean of 10 detns.</th>
<th>Grinder Setting 1</th>
<th>Grinder Setting 2</th>
<th>Grinder Setting 3</th>
<th>Grinder Setting 4</th>
<th>Grinder Setting 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2050</td>
<td>0</td>
<td>0.6</td>
<td>2.2</td>
<td>2.5</td>
<td>5.3</td>
</tr>
<tr>
<td>20</td>
<td>820</td>
<td>2.9</td>
<td>14.6</td>
<td>30.8</td>
<td>47.8</td>
<td>55.4</td>
</tr>
<tr>
<td>30</td>
<td>580</td>
<td>22.3</td>
<td>25.5</td>
<td>25.5</td>
<td>18.8</td>
<td>17.6</td>
</tr>
<tr>
<td>40</td>
<td>430</td>
<td>45.6</td>
<td>32.5</td>
<td>25.9</td>
<td>18.6</td>
<td>13.1</td>
</tr>
<tr>
<td>60</td>
<td>240</td>
<td>4.2</td>
<td>9.8</td>
<td>4.1</td>
<td>2.3</td>
<td>1.6</td>
</tr>
<tr>
<td>1240</td>
<td>25.0</td>
<td>17.0</td>
<td>11.5</td>
<td>10.0</td>
<td>7.0</td>
<td></td>
</tr>
</tbody>
</table>

Total 100 100 100 100 100 100
From these results the percentage undersize for each mesh was calculated. This is plotted against particle size in microns for the different grinder settings in Figure 8.

The significance of these results will be discussed in the next section, 2.1.4.

2.1.4. **Effect of Grinding on Protein Content.**

Mesh 20 was used in this experiment because it was found to sieve out the coarsest bran and let in the maximum throughs.

**Method.**

Some maize was steeped as described in section 2.1.3. It was divided into 5 portions and each was ground at a different setting on the grinder, 40 g of each ground portion was wet sieved with 200 ml distilled water through mesh 20. The throughs were collected and dried (section 2.1.2). Each sample was analysed for moisture content and crude protein using A.A.C.C. methods 44-15 and 46-11 respectively. Each determination was carried out in triplicate and the percent crude protein was calculated on dry weight basis (\( \times 6.25 \)). The results are shown in Table 6.
Figure 8.4 PARTICLE SIZE DISTRIBUTION CURVES

(1, 2, 3, 4, & 5 refer to grinder settings)
Results and Discussion.

Table 6. Percent Crude Protein in the Throughs of mesh 20. (on dry weight basis)

<table>
<thead>
<tr>
<th>Grinder Setting</th>
<th>Percent Crude Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Setting 1</td>
<td>10.6</td>
</tr>
<tr>
<td>Setting 2</td>
<td>10.2</td>
</tr>
<tr>
<td>Setting 3</td>
<td>9.3</td>
</tr>
<tr>
<td>Setting 4</td>
<td>8.3</td>
</tr>
<tr>
<td>Setting 5</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Figure 9 shows % weight undersize of mesh 20 plotted against % crude protein.

The results show that the finer the grinding the higher the % crude protein in the throughs. This seems to agree with the observation made by Kent-Jones (1950) that the protein level in wheat flour increases as the extraction os increased.

This finding is of considerable nutritional importance and will be discussed in section 3.

It was concluded that in order to retain as much of the nutrients as
Figure 2. % Crude Protein in Undersize of Mesh 20. (8804)

as given by 5 settings on the Grinder

(1, 2, 3, 4, 5 refer to grinder settings).
possible setting 1 on the grinder should be used and mesh 20 for wet sieving.

2. 1.5. General Preparation of 'Koko' in the Laboratory.

In the laboratory preparation of 'Koko', the traditional method described in section 1.2. and Figure 2 was followed as closely as possible.

Steeping.

400 g of cleaned maize were steeped in a 5 litre flat-bottomed flask with 600 ml distilled water. The mouth of the flask was plugged with a piece of cotton wool. The flask was kept in an incubator at 30°C overnight. After steeping the contents of the flask were transferred to a coarse sieve to drain the steeping water. This was collected and stored at -20°C for analysis while the steeped maize was ground at grinder setting 1.

Doughing and Fermentation.

The ground maize was mixed into a dough with distilled water (40 ml for every 100 g wet, ground maize). The dough was then placed in a 1 litre beaker, covered with a watch-glass. The dough was then left in an incubator at 30°C for 2 days to ferment.

Wet Sieving.

The fermented dough was mixed with water (200 ml to 40 g dough) into
a slurry which was sieved using 20 mesh. The throughs were collected in a container and the overtails stored at -20°C for analysis.

**Cooking.**

200 ml of the sieved slurry (throughs of 20 mesh) were cooked for 15 minutes in a 250 ml beaker on a boiling water bath.

2.1.6 **Proximate Analysis.**

**Method.**

Samples collected at the various stages of koko preparation were analysed to find how the proximate composition was affected by processing.

The A.A.C.C. methods described in section 2.1.2 were used for the analysis. All determinations were carried out in triplicate.

Table 7 shows the proximate composition of the samples analysed.
Results and Discussion.

Table 7: Proximate Composition of Fermented Maize-dough, Sieved Slurry and Overtails.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Crude Protein (N x 6.25) % dry wt.</th>
<th>Ash % dry wt.</th>
<th>Crude Fat % dry wt.</th>
<th>Crude Fibre % dry wt.</th>
<th>Carbohydrate (by difference) % dry wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermented</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize-dough</td>
<td>10.5</td>
<td>1.50</td>
<td>4.2</td>
<td>1.9</td>
<td>81.9</td>
</tr>
<tr>
<td>Sieved Slurry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(uncooked Koko)</td>
<td>10.1</td>
<td>1.62</td>
<td>4.0</td>
<td>1.0</td>
<td>83.3</td>
</tr>
<tr>
<td>Overtails</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(20 mesh)</td>
<td>13.2</td>
<td>0.56</td>
<td>4.1</td>
<td>4.4</td>
<td>77.7</td>
</tr>
</tbody>
</table>

Comparing the results in Table 4 with those shown in Table 7, it seems the proximate composition of maize is virtually unchanged by steeping and fermentation.

Although the protein content of maize does not seem to be affected by fermentation, wet-sieving reduces it slightly.
Kerr, (1950) and Rooney and Clark (1968) found that the germ contained about 80% of the total minerals in maize. In the milling of steeped maize, the germ is squashed and so passes through the sieve into the throughs. This probably explains the high ash content in the sieved slurry.

2.1.7. Yield of Material in the Laboratory.

This experiment was carried out in order to determine losses of total solids which occur during the laboratory preparation of Koko.

Method.

The whole procedure of the laboratory preparation of Koko was followed and the following samples were obtained: maize, steeped maize, steeping water, fermented dough, sieved slurry (uncooked Koko) and overtails.

The moisture content of the samples was determined using the A.A.C.C methods 44-15 and 44-17 which are described in section 2.1.2.

The dry weight of each sample was expressed as the percentage of the dry weight of starting material i.e. maize (Pearson, 1962).

The results are shown in Table 3.
Results and Discussion.

Table 6: Losses of Total Solids in the Laboratory Preparation of Koko.

<table>
<thead>
<tr>
<th>Material</th>
<th>Percent total solids content (on dry wt. basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>100</td>
</tr>
<tr>
<td>Steeping water</td>
<td>0.4</td>
</tr>
<tr>
<td>Steeped maize</td>
<td>99.0</td>
</tr>
<tr>
<td>Fermented maize dough</td>
<td>96.2</td>
</tr>
<tr>
<td>Overtails (20 mesh)</td>
<td>16.8</td>
</tr>
<tr>
<td>Sieved slurry (uncooked Koko)</td>
<td>79.5</td>
</tr>
</tbody>
</table>

Not all of the ground maize could be recovered from the grinder. This would account for the apparent loss of about 3.5% total solids from the steeped maize to the fermented dough.

The commercial extraction of wheat when milled into flour is 68-77% (Hlyaka, 1964).
The extraction of maize in the laboratory preparation of Koko would therefore be slightly higher at 79.5%.

2.1.8. **Total Protein Yield in the Laboratory.**

**Method.**

Samples obtained at each stage of Koko preparation were analysed to find losses in total nitrogen content due to processing methods.

The crude protein content in each sample was determined on dry weight basis ($N \times 6.25$) in triplicate. Each value of % crude protein was then multiplied by its equivalent value of % yield of total solids (Table 8) to obtain the yield of total protein.

Table 9 shows the losses of Total Protein during the preparation of Koko.
Results and Discussion.

Table 9: Losses of Total Protein during Laboratory Preparation of Koko.

<table>
<thead>
<tr>
<th>Materials</th>
<th>% of Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>10.5</td>
</tr>
<tr>
<td>Steeping water</td>
<td>0.1</td>
</tr>
<tr>
<td>Steeped maize</td>
<td>10.4</td>
</tr>
<tr>
<td>Fermented maize–dough</td>
<td>10.2</td>
</tr>
<tr>
<td>Overtails (20 mesh)</td>
<td>2.4</td>
</tr>
<tr>
<td>Sieved slurry (uncooked Koko)</td>
<td>8.0</td>
</tr>
</tbody>
</table>

There seems to be an overall loss of 2.5% total protein during processing. Wet-sieving seems to account for almost all the losses in total protein since the overtails contain as much as 2.4% of the total protein.

Nutrient losses could be greatly reduced if wet-sieving could be
eliminated in 'Koko' preparation by grinding the maize finer than it is normally done.

2.1.9. The Use of Starters.

In the traditional method of 'Koko' preparation, the fermentation of maize-dough is spontaneous (section 1.2, page 7). The following experiment was therefore carried out to find the effect of various starters on the fermentation of freshly prepared maize-dough.

Method.

Some fresh (unfermented) maize-dough was prepared as described in section 2.1.5. This was then divided into 4 portions. The first maize-dough had no starter added and was used as control. The second consisted of fresh dough containing 7% (w/w) of dough previously fermented for two days. To the next dough 0.7% (w/w) of Lactobacillus acidophilus was added. 1.2% (w/w) freeze-dried fermented maize-dough was added to the last dough.

Each of the 4 doughs was then divided into 5 portions. One portion was set aside for analysis and the other four portions were fermented for varying periods of 1-4 days at 30°C.

The pH values for each sample were determined with a Cambridge pH meter (A.A.C.C. 1962). The total titratable acidity was also determined using A.O.A.C. method. Aliquots of extracts from these samples were titrated against 0.1N NaOH and the results expressed as mg NaOH per 100 g sample.
The results for pH values are shown in Table 10. Values for titratable acidity are given in Table 11 and Figure 10.

Organoleptic tests were carried out on each sample, after cooking.

Results and Discussion.

Table 10: pH Values of Maize-dough Fermented with Starters.

<table>
<thead>
<tr>
<th>Time</th>
<th>Spontaneous Fermentation</th>
<th>With 7% Lactobacillus fermented dough</th>
<th>With 0.7% Lactobacillus preparation</th>
<th>With 1.8% freeze-dried dough</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Mixture</td>
<td>6.2</td>
<td>5.7</td>
<td>5.9</td>
<td>6.2</td>
</tr>
<tr>
<td>1 day</td>
<td>4.0</td>
<td>3.9</td>
<td>4.0</td>
<td>3.8</td>
</tr>
<tr>
<td>2 days</td>
<td>3.9</td>
<td>3.8</td>
<td>3.7</td>
<td>3.8</td>
</tr>
<tr>
<td>3 days</td>
<td>4.0</td>
<td>3.8</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>4 days</td>
<td>4.0</td>
<td>3.8</td>
<td>3.8</td>
<td>3.7</td>
</tr>
</tbody>
</table>
Figure 10: Total Titratable Acidity of Maize-dough Fermented with Starters.

1. Natural Fermentation
2. 7% Fermented dough as starter
3. 0.7% Lactobacillus preparation as starter
4. 1.6% Freeze-dried dough as starter
### Table 11: Total Titratable Acidity (mg. NaOH per 100 g) of Maize-dough Fermented with Starters.

<table>
<thead>
<tr>
<th>Fermentation Time</th>
<th>Spontaneous Fermentation</th>
<th>With 0.7% Lactobacillus preparation dough</th>
<th>With 0.7% freeze-dried dough</th>
<th>With 1.8% freeze-dried dough</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Mixture</td>
<td>72.18</td>
<td>83.22</td>
<td>96.24</td>
<td>88.22</td>
</tr>
<tr>
<td>1 day</td>
<td>360.90</td>
<td>384.96</td>
<td>348.87</td>
<td>433.08</td>
</tr>
<tr>
<td>2 days</td>
<td>384.96</td>
<td>497.24</td>
<td>449.12</td>
<td>469.17</td>
</tr>
<tr>
<td>3 days</td>
<td>384.96</td>
<td>537.34</td>
<td>517.29</td>
<td>517.29</td>
</tr>
<tr>
<td>4 days</td>
<td>429.07</td>
<td>617.52</td>
<td>533.33</td>
<td>545.36</td>
</tr>
</tbody>
</table>

The results show that the pH values of the fresh dough are lowered by the production of acid during fermentation. However, pH determination does not seem sensitive enough to give a clear indication of the rate of acid production in the various mixtures. This may be due to the buffering action of proteins and other soluble constituents in the dough (Zeleny, 1949 and Banigo, 1969).
The values for total titratable acidity show a higher rate of acid production in samples containing starters. Maize-dough inoculated with freeze-dried, fermented dough reached an acidity of 433.08 mg. NaOH per 100 g in 1 day but the naturally fermented dough required 4 days.

In the early stages of spontaneous fermentation, the bacteria that predominate, produce small amounts of acid but their activity is suppressed when an inoculum is added (Nabors and Salunkhe, 1969). This probably accounts for the slow initial production of acid found in mixtures inoculated with the Lactobacillus preparation.

The organoleptic tests showed that the flavour of the mixture inoculated with Lactobacillus acidophilus was sour but the typical 'Koko' flavour was absent.

Hence it appears that the fermentation period can be reduced by the use of appropriate starters.
2.2. PRESERVATION OF 'KOKO'.

2.2.1. Equipment

A laboratory size roller-drier, supplied by the Kestner Evaporator and Engineering Co. Ltd., was used for the roller-drying experiments. The roller is steam heated. The steam pressure is indicated on a pressure gauge graduated in 0.1406 kg/cm$^2$ (2 p.s.i.)

Figure 11 shows a diagram of the Spray Drier which was used in these trials. Liquid foods are dried by being sprayed into a chamber where electrically heated air is circulated to remove water rapidly from the suspended droplets. The inlet and outlet temperatures of the air circulating in the drying chamber are registered by thermo couples.

The liquid feed is dispersed into the drying chamber by a centrifugal disc-atomiser which is driven by an air turbine. The air pressure to the atomiser is indicated on a pressure gauge graduated in 0.3515 kg/cm$^2$ (5 p.s.i.). The dried product is carried by the hot air into the cyclone separator where it is allowed to settle.

In the freeze-drying experiments a "Quickfit" drier was used. An oil vacuum pump supplied by Edwards High Vacuum Ltd., was used to create the vacuum. As mentioned in section 1.3.3. The vapour formed by sublimation of moisture from the sample can be removed by condensation ahead of the pump. A mixture of acetone and dry ice was used in the condenser.
FIGURE 11: DIAGRAM OF THE KESTNER LABORATORY SPRAY DRIER.
Retorting of canned 'Koko' was carried out in a laboratory size rotary sterilizer (68.6 cm. diameter 91.4 cm. long). This retort is supplied by John Fraser and Son Ltd., London.

In the canning experiments a Cambridge Minican Temperature Recorder (made by Cambridge Instrument Company Ltd.,) was used to measure heat penetration into canned 'Koko' during retorting. In this instrument, changes in temperature detected by a heat-sensitive bulb, are converted into proportional movement of the recorder stylus. The fine stylus traces its movement on a miniature drum chart.

2.2.2. **Roller-drying.**

**Method.**

100 g of fermented maize-dough were wet-sieved with 250 ml distilled water. The sieved slurry was cooked (in 250 ml Beakers) in a boiling water-bath for 10 minutes. The cooked product, in the form of a thick paste, was applied in a film to the steam heated roller with a hand roller. The roller of the drier which was revolving at 1.4 rev./mins. at atmospheric pressure was heated at a steam pressure of 1.4060 Kg/cm² (equivalent to a temperature of 126°C).

The dried product was collected and stored in a bottle at 5°C.

The experiment was repeated with the same roller speed but a reduced steam pressure of 1.0545 Kg/cm² (equivalent to 121°C).
The dried products of the two trials were reconstituted and tested for flavour and colour by a panel of tasters.

Four African students familiar with the product were used as tasters. Particular precautions were not taken in choosing the panel because the results were obvious.

Results and Discussion.

Table 12: Organoleptic test on Reconstituted Roller-dried 'Koko'.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colour</th>
<th>Flavour</th>
<th>Rehydration ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roller-dried</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 126°C</td>
<td>Very dark colour</td>
<td>All 'typical' Koko flavour</td>
<td>Incomplete rehydration resulted in &quot;lumpy&quot; product</td>
</tr>
<tr>
<td></td>
<td>(not acceptable)</td>
<td>lost</td>
<td></td>
</tr>
<tr>
<td>Roller-dried</td>
<td>Dark colour</td>
<td>'Typical' Koko flavour</td>
<td>&quot;lumpy&quot; product</td>
</tr>
<tr>
<td>at 121°C</td>
<td>(not acceptable)</td>
<td>flavour lost</td>
<td></td>
</tr>
</tbody>
</table>

The results will be discussed in section 2.2.3 together with those of the spray-drying experiments.
2. 2.3. 

Spray-drying.

Method.

Some maize-dough was wet-sieved using mesh 20 (40 g dough to 400 ml water). The particles of maize in the sieved slurry settled rapidly on standing. In order to obtain particles in suspension, the mixture was heated for 10 minutes at 70°C to gelatinised the starch. The suspension was then spray-dried with an inlet temperature of 175°C and an outlet temperature of 90°C.

The experiment was repeated with an inlet temperature of 110°C and an outlet temperature of 70°C.

In the third trial, maize-dough was first ground with a colloid mill to make it smoother before its suspension was dried. An inlet temperature of 180°C and outlet temperature of 80°C were used. In all the trials, the feed was made to flow into a graduated funnel from which it entered the drier. The rate of feed flow was maintained at 30 ml/min. The atomiser speed was 4.2260 Kg/cm² (60 p.s.i.) which is equivalent to 22500 r.p.m. 71.

Samples which were successfully dried were tested for flavour and colour by a taste panel.

Results and Discussion.

With an inlet temperature of 110°C and an outlet temperature of 70°C
the material adhered to the walls of the drying chamber and did not dry adequately.

On each occasion of the other trials most of the dried particles collected on the wall of the drying chamber and had to be removed by hand.

Table 13: Organoleptic Test of Reconstituted Spray-dried 'Koko'.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colour</th>
<th>Flavour</th>
<th>Rehydration ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet temp. 175°C</td>
<td>Dark colour</td>
<td>All 'typical'</td>
<td>&quot;lumpy&quot; product</td>
</tr>
<tr>
<td>Outlet temp. 90°C</td>
<td>(unacceptable)</td>
<td>Koko flavours</td>
<td>(unacceptable)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lost</td>
</tr>
<tr>
<td>Inlet temp. 180°C</td>
<td>No colour</td>
<td>Tasted sour</td>
<td>Complete</td>
</tr>
<tr>
<td>Outlet temp. 80°C</td>
<td>change</td>
<td>but all flavour</td>
<td>rehydration</td>
</tr>
<tr>
<td></td>
<td>(acceptable)</td>
<td></td>
<td>was lost</td>
</tr>
</tbody>
</table>

The range of particle size in 'Koko' is about 240-380 microns (Table 5 and Figure 8). But the particle size of powders dried in small test driers fall in the range of 5-50 microns and slurries that cannot be
atomised so finely tend to be deposited on the wall of the drying chamber (Seltzer and Settlomeyer, 1949).

Goldblith (1963) reported that the presence of water in food contributes to quality: structure, nutritive value and flavour, and during dehydration the loss of water may cause undesirable, irreversible changes. He further stated that foods high in acid are among those more sensitive to change during conventional hot air dehydration.

Banigo (1969) and Alcinrele (1967) identified lactic and acetic acids as the major acids produced in the fermentation of 'Ogi', a maize preparation very similar to 'Koko'. In lactic acid fermentation, the sour taste is largely due to lactic acid whilst the more volatile acetic acid is mostly responsible for the flavour (Saint-Mont, 1949). In 'Koko', acetic acid is probably a major flavour constituent. There is loss of volatile acetic acid when sauerkraut is dehydrated, resulting in loss of flavour (Nabors and Salunkhe, 1969). The loss of flavour in dehydrated 'Koko' could be explained in the same way.

2. 2.4. Freeze-drying.

Method.

80 g of maize-dough was mixed with 10 ml water into a very thick slurry. The slurry was poured into 6 round-bottomed "Quickfit" flasks (250 ml). Each flask was rotated to have the slurry in a thin layer on
the walls of the flask and was then dipped in a mixture of acetone and dry ice to freeze the sample. The flasks were quickly fitted to a column of the drier which was then placed under vacuum. Drying of the samples was carried out for 5 hours.

The dried samples were combined and reconstituted with boiling water. It was then tested for colour, flavour and texture using a taste panel. The moisture content of the dried sample was determined.

Results and Discussion.

The moisture content of the freeze-dried sample was 7.8%.

Table 14: Organoleptic Test of Reconstituted Freeze-dried 'Koko'.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colour</th>
<th>Flavour</th>
<th>Rehydration ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried for 5 hours</td>
<td>No colour</td>
<td>Typical 'Koko'</td>
<td>Complete rehydration.</td>
</tr>
</tbody>
</table>

Of the three methods of dehydration used in the experiments freeze-drying seems to be the only one that does not adversely affect the organoleptic properties of 'Koko'.

Bird (1964) concluded that freeze-dried foods have better
organoleptic properties, high nutritional value and better storage characteristics. Unfortunately freeze-drying is a very expensive process and while preservation of 'Koko' by this means appears to be excellent for laboratory purposes it does not presumably lend itself to commercial exploitation.

2. 2.5 Canning.

Heat Penetration.

Some maize-dough was wet-sieved (40 g to 250 ml) using mesh 20. 5 g of sugar were added to every 40 g of sieved dough. The pH value of this mixture was 4.0. One can (Size A 1) was filled with some of the mixture leaving a head space of 2.3 cm (½ in.). The can was sealed, punctured at the centre of the lid and fitted with a Kinican Temperature Recorder. The can was then fixed in the centre of a rotary sterilizer and sterilized for 20 minutes at 100°C.

The heat penetration data is shown in Table 15 and Figure 12.

Some of the canned 'Koko' was tested for flavour, colour and texture by a taste panel.

Some cans of 'Koko' were sterilized for varying times of 0, 5, 10, 15, 20 and 25 minutes, at 100°C. The cans were then stored at 30°C for 3 months to observe which cans would "blow".
FIG. 12: HEAT PENETRATION CURVE OF CANNED 'KOKO'.
Results and Discussion.

Table 15: Rate of Heat Penetration During Retorting of Canned 'Koko'.

<table>
<thead>
<tr>
<th>Time (mins.)</th>
<th>Temp. °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25.0</td>
</tr>
<tr>
<td>1</td>
<td>54.4</td>
</tr>
<tr>
<td>2</td>
<td>67.8</td>
</tr>
<tr>
<td>3</td>
<td>75.0</td>
</tr>
<tr>
<td>4</td>
<td>79.0</td>
</tr>
<tr>
<td>5</td>
<td>81.7</td>
</tr>
<tr>
<td>6</td>
<td>84.2</td>
</tr>
<tr>
<td>10</td>
<td>90.6</td>
</tr>
<tr>
<td>15</td>
<td>95.6</td>
</tr>
<tr>
<td>18</td>
<td>97.8</td>
</tr>
<tr>
<td>20</td>
<td>98.3</td>
</tr>
<tr>
<td>25</td>
<td>100.0</td>
</tr>
</tbody>
</table>
When the time-temperature curve is plotted on a logarithmic scale (Figure 12) there is a "break" in the heating curve at a temperature of 75°C i.e. after 3 minutes retorting. The heating rate seems to slow down after this point. Kite et al (1957) found that the gelatinization temperature of maize meal falls within the range of 62-72°C. The point of the "break" in the curve seems to correspond to the stage when gelatinization of the 'Koko' mixture would occur. A "break" in the heat transfer curve is typical for materials which exhibit a sol-gel change on heating (section 1.3.4).

The fact that the mixture is not very viscous and that it was agitated in the rotary sterilizer during retorting would explain the rapid transfer of heat.

The texture, colour and flavour of 'Koko' was not adversely affected by canning. All the volatile flavour was retained. On the whole, the canned 'Koko' was very much like the laboratory cooked 'Koko'. Canning of 'Koko' on an industrial scale appears to be possible if Ghana develops economically and technologically.

In the storage experiments only the unsterilized cans "blew" after one week. The other cans did not "blow" after 3 months storage. These results are not conclusive since a detailed microbiological analysis would be required to determine which sterilization time gives a product safe for consumption.
2.3 FATE OF THIAMINE AND RIBOFLAVIN DURING MANUFACTURE AND PROCESSING OF KOKO.

2.3.1. The Chemistry of Thiamine and Riboflavin and Methods Available for their Analysis.

Chemistry of Thiamine

Thiamine occurs in natural foods and other biological materials in the free or a combined form – as a protein complex, or as a phosphorous-protein complex or as the pyro-phosphoric acid ester, cocarboxylase.

The chemical structure of thiamine has a pyrimidine and a thiazole ring (section 2.3.2). In neutral or alkaline solutions thiamine is rapidly destroyed, presumably because of decomposition of the thiazole ring. However, in acid solutions (pH 3.5) it can withstand temperatures up to 120°C. (Beadle et al 1943; Pike and Brown).

In solution it is quite sensitive to oxidation and reduction. In vitro, oxidation of the vitamin yields thiochrome which is biologically inactive. The ease of this oxidation has been the basis of the thiochrome method for determining thiamine (Williams, 1939).

The daily requirements of thiamine is 0.6 mg./1,000 non-fat calories (Bender, 1967). It is so defined because it is not needed for fat metabolism.

Chemistry of Riboflavin

Riboflavin is a fluorescent, yellow-green, water soluble pigment.
found in plant and animal cells. In living cells it usually occurs combined with either phosphoric acid or phosphoric acid and adenylic acid, both of which may be combined with specific proteins to form oxidative enzymes, namely flavin mononucleotide (FMN) and flavine adenine dinucleotide (FAD).

It is very sensitive to both visible and ultra-violet light. Even subdued light may cause destruction of this vitamin (Williams and Cheldelin 1942; DeKere and Brown 1944).

It is reversibly reduced to leucoriboflavin, a colourless compound, either by hydrogen ions in the presence of a catalyst, or sodium hydro-sulphite and other reducing agents. This reaction forms the basis of the fluorometric method of riboflavin analysis (2.3.2).

It is stable in neutral or acid solutions. It is highly soluble in alkaline solutions but not stable to heat under this condition. Recommended intakes are 0.6 mg./1,000 Calories (Bender, 1967).

Methods Available for Analysis of Thiamine and Riboflavin.

Methods available for thiamine and riboflavin can be divided into animal, microbiological and chemical. Each class of methods has certain disadvantages which limit its usefulness.

Animal methods have the disadvantage of being expensive and time consuming for the assay of both vitamins.

The microbiological methods consume much less time, are less expensive
and yield reproducible results. However the microbiological methods for thiamine assay tend to give values that are too high because breakdown products of thiamine respond in the same way as the vitamin.

Chemical analyses can be carried out rapidly and economically and are more applicable to routine determinations than most of the other methods. In conclusion, both the microbiological and chemical methods give comparable results for the assay of riboflavin and are not difficult to carry out. But only the chemical method is recommended by the Association of Vitamin Chemists (1966) for thiamine analysis.

2. 3.2. Materials and Methods.

Materials.

For the determination of thiamine (Section 2. 3.3) four samples of maize were steeped at 30°C over a period of 1-4 days. Each sample of steeped maize was ground, thus yielding 4 samples of ground steeped-maize with different steeping periods.

For the determination of thiamine and riboflavin (Sections 2. 3, 4, and 2. 3. 5.) canned 'Koko' (15 minutes retorting) and samples collected during the laboratory preparation of 'Koko' (2. 1.5) were analysed.

All chemicals used in the analysis were of Laboratory Reagent Grade.

The Hilger and Watts Fluorimeter H 960 was used to measure fluorescence of both thiamine and riboflavin extracts.
Methods.

The Thiochrome Method for Thiamine Assay.

The thiochrome method depends on the oxidation of thiamine to thiochrome which fluoresces under ultra-violet light.

![Thiamine to Thiochrome](image)

Under standard conditions and in the absence of other fluorescing substances, the fluorescence of thiochrome in ultra-violet light is proportional to thiamine in solution.

The bound form of thiamine in the samples was released by treatment with an enzyme solution. Filtration was then carried out to remove solid particles. To separate thiamine from interfering substances in the filtrate, it was adsorbed into a cation exchange resin (Decalso) and then
eluted. This was followed by oxidation in alkaline potassium ferri-cyanide to thiochrome which was extracted by isobutyl alcohol and its fluorescence measured.

The thiochrome method which is applicable to cereals and cereal products (A.A.C.C. 86-80) was used in these determinations.

The Fluorometric Method for Riboflavin Assay.

The fluorometric method for the determination of riboflavin (A.A.C.C. 86-70) was used in all the determinations carried out in section 2.3.5. In this method bound riboflavin in the samples was released by heating in acid with boiling water-bath. The pH of the mixture was adjusted to 4.5 with 2.5 M sodium acetate solution. The mixture was filtered to remove solid particles. Impurities in the filtrate were separated by oxidising with potassium permanganate solution. Hydrogen peroxide was then used to decolorise the purified riboflavin solution the fluorescence of which was measured before and after reduction with sodium hydrosulphite. The fluorescence of the reduced riboflavin corresponds to the blank reading.

\[
\text{Riboflavin} \quad \overset{+2H}{\longrightarrow} \quad \text{Leucoriboflavin}
\]
2.3.3. **Thiamine Content in maize steeped over a period of 1-4 days.**

**Method.**

4 samples of maize steeped over a period of 1-4 days were analysed for thiamine content using the thiocchrome method of the A.A.C.C.

Decalso Y was the cation exchange resin used to remove interfering substances from the thiamine extracts. The efficiency of the Decalso columns was determined using a standard solution of thiamine and found to be 95%. This level of recovery from the column is recommended as satisfactory by the A.V.C. 20 ml of each extract was passed through a column and was eluted with 25 ml of 25% potassium chloride solution.

Determinations were carried out in triplicate and calculations were done on dry weight basis.

**Results and Discussion.**

**Table 16: Thiamine in Steeped Maize.**

<table>
<thead>
<tr>
<th>Steeping Time</th>
<th>Thiamine ug/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>327.3 (327.9, 328.5, 325.7)</td>
</tr>
<tr>
<td>2 days</td>
<td>329.4 (330.5, 329.6, 328.1)</td>
</tr>
<tr>
<td>3 days</td>
<td>333.5 (329.0, 334.2, 337.3)</td>
</tr>
<tr>
<td>4 days</td>
<td>339.7 (335.9, 343.5, 339.7)</td>
</tr>
</tbody>
</table>
The results seem to suggest that there is leaching of thiamine from the maize during the first day of steeping. After this, the thiamine content in the maize starts to increase as the period of steeping is increased.

Adam et al (1942) 83, Lee (1958) 74 and Aykroyd et al (1940) 85 found that wet operations such as washing and blanching cause the leaching out of water soluble vitamins. This would probably explain the loss of thiamine from maize on steeping for one day.

Fermentation was observed in the steeping water during the wet processing of maize for the manufacture of starch (Matz. 1959) 86.

Akinrele and Bassir (1967) 87 identified some lactic acid bacteria in the steeping water of maize. Akinrele (1967) 73 and Banigo (1969) 34 reported the presence of carboxylic acids - lactic, acetic and butyric - in the steeping water of maize after 3 days steeping.

The apparent increase in thiamine after one day steeping could probably be due to the fact that there is synthesis of thiamine during fermentation at the steeping stage.

2. 3.4. Thiamine Content at Different Stages of 'Koko' Preparation.

Method.

The following samples, collected at different stages of laboratory preparation and processing of koko were analysed for their thiamine content;
maize, steeped maize, steeping water, fermented maize-dough, sieved slurry, overtails, cooked 'Koko' and canned 'Koko'.

The same procedure used in experiment 2. 3. 3. was employed. Because extracts from the steeping water and overtails contained lower concentrations of thiamine, 50 ml of each were passed through a Decalso column instead of 20 ml in the case of the other samples.

Each determination was carried out in triplicate and calculations done on a dry weight basis.

Results and Discussion.

Table 17: Thiamine Content at Different Stages of Koko Preparation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ug Thiamine / 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>339.1 (340.0, 340.7, 349.8)</td>
</tr>
<tr>
<td>Steeped Maize (18 hours steeping)</td>
<td>318.0 (316.5, 316.5, 316.1)</td>
</tr>
<tr>
<td>Steeping water</td>
<td>685.0 (685.0, 685.0, 685.0)</td>
</tr>
<tr>
<td>Fermented maize-dough</td>
<td>383.5 (383.4, 383.4, 383.4)</td>
</tr>
<tr>
<td>Sieved slurry (uncooked Koko)</td>
<td>385.5 (385.4, 385.4, 385.4)</td>
</tr>
<tr>
<td>Cooked Koko</td>
<td>383.5 (383.4, 383.4, 383.4)</td>
</tr>
<tr>
<td>Canned Koko</td>
<td>383.5 (383.4, 383.4, 383.4)</td>
</tr>
</tbody>
</table>
The value of thiamine in the overtails was too low and therefore could not be measured accurately.

Although 18 hours of steeping reduces the thiamine content in the maize, the loss is not serious. Fermentation seems to result in considerable increase of the thiamine content. This result seems to agree with the observation made by Golberg and Thorp (1946) that there is about 30% increase in thiamine content during the fermentation of 'leting', a fermented maize product eaten in South Africa.

The changes in the value of thiamine due to wet-sieving and cooking of the fermented dough are not considerable. Because thiamine is water soluble it is probably effectively washed into the throughs during wet-sieving. Even the mild alkalinity of natural waters results in destruction of thiamine in boiled rice (Roy and Rao, 1963). But short cooking of breakfast cereals with pH 5.5 cause no destruction of thiamine (And Lund and Goddard, 1945. Lincoln et al, 1944; White et al, 1946) 89-91.

The cooking of 'Koko' does not seem to destroy thiamine because it has a low pH of 4.0.

2.3.5. Riboflavin Content at Different Stages of 'Koko' Preparation.

Method.

The samples analysed in the previous experiment (2.3.4) were analysed also for riboflavin content.
analysis on each sample was carried out in triplicate and calculations were done on dry weight basis.

Results and Discussion.

Table 18: Riboflavin Content at Different Stages of 'Koko' Preparation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ug Riboflavin/100 g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>149.6 (146.8, 150.9)</td>
</tr>
<tr>
<td>Steeped Maize</td>
<td>104.0 (106.0, 105.5)</td>
</tr>
<tr>
<td>Steeping water</td>
<td>321.1 (320.9, 318.6)</td>
</tr>
<tr>
<td>Fermented maize–dough</td>
<td>108.6 (106.3, 109.4)</td>
</tr>
<tr>
<td>Sieved slurry (uncooked Koko)</td>
<td>92.6 (90.1, 93.7, 94.1)</td>
</tr>
<tr>
<td>Cooked Koko</td>
<td>89.5 (90.3, 88.3, 89.9)</td>
</tr>
<tr>
<td>Canned Koko</td>
<td>90.7 (92.8, 89.2, 90.1)</td>
</tr>
</tbody>
</table>

Steeping seems to reduce the riboflavin content in maize because this vitamin is water soluble.

Fermentation increases the riboflavin content very slightly. Either the fermentative micro-organisms do not produce riboflavin in any great
amounts, or possibly, exposure of the dough to light could conceivably destroy the vitamin. Gleim et al (1944) observed losses of 5-22% riboflavin when crates of some vegetables were exposed to light.

Sieving and cooking do not seem to affect the riboflavin content considerably. Just as in the case of thiamine, wet-sieving probably tends to wash the riboflavin into the throughs.
Laboratory Preparation of 'Koko'. (pp. 28-45)

In the preparation of 'Koko', the throughs of wet-sieved, fermented maize-dough are cooked into 'Koko' (pp. 17 and 19). The results of the particle size distribution of ground maize (pp. 32a) show that the finest grinding gives the highest amounts of throughs using different mesh sizes for sieving. Hence it appears that in order to have as much throughs as possible, grinding of maize should be done as fine as possible.

When maize was ground at different settings on the grinder and sieved with mesh 20 (which was found to sieve out the coarsest fraction thus giving the maximum amount of throughs) it was found that the finer the grinding, the higher the protein content in the throughs (page 34). This finding is nutritionally very important. Most protein deficiency cases in Ghana are encountered in children of preschool age who are fed on mainly maize preparations (Davey 13). For these children as well as most adults in Ghana, staple foods provide much of the required protein. Hence, methods of food preparation should be carefully controlled to preserve as much protein as possible.

The results of the proximate analyses (Tables 4 and 7) show that fermentation does not change the proximate composition of maize. Wet-sieving, however, seems to reduce the protein and fibre contents slightly. Fermentation increases the protein content in some fermented foods (Platt
This does not seem to be the case with maize-dough, probably because the micro-organisms responsible for the fermentation do not synthesize protein in sufficient quantity to make it obvious.

Tables 8 and 9 show the losses that occur in Total Solids and Total Protein during the laboratory preparation of 'Koko'. There seems to be about 20% loss of total solids. This extraction of about 80% is not satisfactory because in Ghana, food production has not developed well enough for the country to afford this wastage. Table 9 shows that a protein content of 10.5% in maize is reduced to 6% in the sieved slurry which is cooked into 'Koko'. Losses of total solids and protein could be reduced if the maize could be ground finer than it is normally done so that sieving would be eliminated.

The results of the starter experiments 2. 1.9 (page 44) show that the rate of fermentation is higher in inoculated samples of maize-dough. A total acidity of over 400 mg. NaOH per 100 g. was recorded in inoculated samples of maize-dough after 2 days fermentation, whilst the naturally fermented dough required 4 days to attain this level of acidity.

Organoleptic tests carried out on cooked inoculated samples showed that those inoculated with fermented dough and freeze-dried, fermented dough tasted the same as the naturally fermented product. But, although the sample which was inoculated with Lactobacillus acidophilus tasted sour, the typical 'Koko' flavour was absent.

It appears that fermentation period could be shortened, but the
appropriate starters should be used to give the right flavour.

**Preservation of 'Koko'. (pp. 46-55)**

The organoleptic tests on both the roller-dried and spray-dried samples of 'Koko' indicate that the typical flavour of 'Koko' is lost (pp. 48-50). This is probably because the flavour constituents of 'Koko' are volatile and are driven off by the heat. Lactic acid and acetic acid have been identified as the major acids in fermented 'Ogi', a Nigerian cereal product, 34 and 75. Since 'Ogi' and 'Koko' have similar flavour, these two acids probably contribute largely to the flavour characteristics of 'Koko'. It is also interesting to note, that when fermented products like sauerkraut are dehydrated, the volatile acetic acid is lost resulting in a loss of flavour 70. Adeyinka 97, however, reported successful dehydration of 'Ogi' by spray-drying without loss of flavour. Maize-meal is eaten as a staple without fermentation in Tanzania. This product lacks the volatile acids which fermentation produces in 'Koko' and there is no problem of loss of flavour during dehydration by roller-drying (Nosha, 1970) 93. Freeze-drying seems to retain the flavour characteristics of 'Koko' (page 52). Both the colour and texture of 'Koko' are also not affected by the process. The low temperature which the process is carried out helps to reduce the destruction of heat sensitive nutrients such as proteins and vitamins. In fact, freeze-dried foodstuffs have better nutritional value compared to other dehydrated products 74. This process is however a very expensive one and has so far been used in developed countries mainly for research purposes. Because freeze-drying produces
very little deterioration, it has found wide acceptance in preparing samples for research purposes. Unfortunately, the severe economic disadvantages of the process make it impossible for the preservation of 'Koko' on commercial scale.

As regards the canning experiments, the heat penetration curve of canned 'Koko' (Figure 12) is a "broken" one. The "break" which occurs at a temperature of about 75°C seems to coincide with the gelatinization temperature of maize-meal. During the heat treatment of canned foods, solid foods transfer heat by conduction whilst liquid foods do so mainly by convection. Olson and Jackson (1940) found that some foods which undergo a sol-gel change when heated exhibit what is known as a "broken" heat curve. Heat is transferred by convection at the initial stage of heating but when the product changes to a gel form the rate of heat transfer is slowed down and heat transfer is mainly by conduction.

The main objective of thermal processing of canned foods is to destroy micro-organisms capable of causing deterioration. In order to achieve this, some information on the heat-resistance of the contaminating micro-organisms and the rate at which heat penetrates the contents of the can is essential. Some work on the heat resistance of micro-organisms in 'Koko' is necessary to give a more conclusive meaning to the above results on heat-penetration.

There was no change of texture, colour and flavour characteristics in the canned 'Koko' which made it very acceptable. It appears that the canning of 'Koko' on a commercial scale is possible. However, more
research work on the microbiological and processing aspects is necessary to evaluate processing conditions.

**Fate of Thiamine and Riboflavin during manufacture and Processing of 'Koko'** (pp. 56-66)

The steeping of maize is carried out for about 18 hours (overnight) in the traditional preparation of 'Koko'. The time of steeping is sometimes extended up to 3 days if the final product is to be a more sour product like 'Kenkey'. The results on thiamine in steeped maize (page 51) seem to suggest that thiamine level in maize is lowered after steeping overnight. This is not unexpected since thiamine is water soluble and would therefore leach out into the steeping water unless bound.

Fermentation has been observed in the steeping water during the wet processing of maize for the manufacture of starch. Some workers on the fermentation of 'Ogi' have reported the presence of lactic acid bacteria and some carboxylic acids in the steeping water of maize. One would expect the loss of thiamine on steeping to increase as the steeping time is increased. But the results of page 61 show that after one day steeping, there is an increase in thiamine content of maize as the steeping time is prolonged. This is probably due to the fact that there is synthesis of the vitamin during fermentation at the steeping stage by micro-organisms.

Table 17 shows the results of the experiment to determine the effect
of 'Koko' preparation on thiamine.

Fermentation of maize-dough for 2 days seems to increase the thiamine considerably. Fermented maize-dough forms the basis of various staple dishes in the south of Ghana. In fact, maize and cassava are the two main staple foods in southern Ghana. Since cassava, a starchy root tuber, is generally considered a very poor source of thiamine, one would conclude that maize forms the major source of thiamine in this region.

The results in Table 17 show that wet-sieving reduces the thiamine only slightly. Thiamine is stable in acid media up to temperatures of 120°C. Temperatures taken in the laboratory during cooking of 'Koko' show that the highest temperature the product attained was 85°C. This comparatively low temperature and the acidity of the product probably explain why there is hardly any difference in the thiamine content of the cooked and uncooked products. The high retention of the vitamin in the canned product can also be explained by the same reason.

As in the case of thiamine, steeping overnight seems to reduce the riboflavin in maize (Tables 17 and 18). This is probably because the riboflavin is also water soluble. Although fermentation seems to increase the riboflavin, this is not high enough to make up for the amount lost on steeping. This is rather unfortunate because riboflavin is the one vitamin which seems to be in short supply throughout Ghana.

The original amount in maize is supposed to be less than half the theoretical requirement. The vitamin is, however, supposed to be increased by some fermentation process. Various workers in Ghana have
always suggested the probability of high increases in riboflavin during
the fermentation of some traditional foods such as maize-dough. It is
suggested that these traditional foods should be analysed to find which
ones are rich in riboflavin so that they could be exploited. The point
to stress now is that analysis of foodstuffs is not sufficient. It is
recommended that traditional methods of preparation should be studied to
find where losses or increase of nutrients occur so that these methods
could be modified where necessary. For instance, maize-dough could be
inoculated with micro-organisms capable of increasing the riboflavin
level during fermentation.
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