Irradiation of ready meals for microbiological safety and shelf-life extension. 1. Microbiological quality of waakye and other ready-to-eat meals

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ABSTRACT
Waakye bought from the open market and 14 meals prepared under the hazard analysis and critical control point (HACCP) plan were microbiologically investigated. The aerobic mesophilic count (AMC) (10^7 - 10^8 CFU g^-1) and coliform count (10^6 - 10^7 CFU g^-1) for complete waakye meals, including macaroni, fried fish, sauce and vegetable salad, exceeded the microbiological standards for such ready-to-eat meals. The AMC (10^1 - 10^4 CFU g^-1) and coliform count (10^1 - 10^2 CFU g^-1) for all the ready meals prepared under HACCP and stored (-5 to 0 °C) for up to 5 days were within the standards. Potential pathogens isolated from waakye and the meals prepared under HACCP plan included Escherichia coli, Klebsiella spp., Serratia spp., and Enterobacter spp. Subsequent studies will exploit the potential of irradiation to eliminate pathogens and ensure the microbiological safety of ready-to-eat meals.

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Introduction
Current trends in particular developed countries suggest increasing demand for convenient foods such as ready meals and those that require minimum preparation before consumption. In Ghana, the trend is the same, with “street foods” (ready-to-eat meals sold in the informal sector) playing a major role in meeting the nutritional needs of the people (GLSS, 1988/89, 1998/99). In most developing countries including Ghana, these ready meals are prepared daily. With increasing urbanization and increased use of refrigerators for food preservation in developing countries, “street foods” could soon be sold in supermarkets.

The hygienic limitations of “street foods” in Ghana and elsewhere have been recognized and reported (Mosupuye & von Holy, 1999; Bryan et al., 1997; FAO/GHANA, 1997; Nkansah, 1996; Swanepoel et al., 1995). Ready-to-eat smoked mackerel served in “chop bars” and chicken pieces from food service outlets in Accra have been reported to harbour varying levels of indicator or-
ganisms, sometimes as high as 10^9 CFU g^{-1} (Adu-Gyamfi, 2000; Hayford, 1979). Organisms of public health concern isolated from these foods included faecal *Streptococci*, coagulase-positive *Staphylococcus aureus* and faecal coliforms, notably *Escherichia coli* and *Shigella flexneri*. Studies on waakye (co-boiled rice and cowpea), a ready-to-eat meal, indicated that the cooking step applied is adequate for inactivating vegetative bacteria, but not spores (FAO/GHANA, 1997). Consequently, *Staphylococcus aureus* and *Clostridium perfringens* have been isolated from waakye and gravy during 2 to 7 h holding under ambient conditions, although counts did not exceed 10^3 CFU g^{-1} (FAO/GHANA, 1997). The implication of the heat stability, survival of spores of the indicator bacteria, and their subsequent germination and proliferation during holding of waakye is a serious public health concern. Because waakye may not be consumed immediately after purchase, but may be held under ambient conditions for many more hours. Other researchers have also reported proliferation of microbes on ready-to-eat meals during holding (Bryan et al., 1997).

Before consumption, waakye may be reheated. The fact that reheating reduces microbial counts on waakye to < 10 CFU g^{-1} (FAO/GHANA, 1997) might not necessarily render the food safe, especially if heat-stable toxins have already been produced by pathogens. It is imperative that meals are prepared by hygienic and preventative procedures, coupled with effective treatments for eliminating vegetative cells and spores for safety assurance. The preparation of meals by the HACCP approach improves their microbiological quality. The HACCP, a systematic approach to hazard identification, assessment and control, also involves identifying critical control points of hazards and establishing procedures to monitor these points (ICMSF, 1989). However, HACCP might not ensure the safety of meals if the consumer does not strictly follow the recommended post-processing handling instructions. Irradiation is widely recognized as an effective control measure for inactivating pathogenic bacteria and parasites from food, especially those eaten raw or minimally processed (IAEA, 2003).

This study investigated the microbiological quality of waakye and other ready-to-eat meals prepared under HACCP from selected sources, aiming at considering irradiation as a potential treatment for ensuring their microbiological safety.

**Materials and methods**

Samples of waakye (co-boiled rice and beans) complete with their accompaniments (sauce/gravy/pepper, fried fish, macaroni, gari and uncooked salads (lettuce, tomato, onion, cabbage) were bought from street vendors from three suburbs (Dome, Madina and Haatso) of Accra in Ghana.

Meals, prepared under HACCP plan, were also bought from two caterers. Altogether, 14 meals were randomly selected by the chefs after preparation and held under the normal storage conditions for hot meals (-5 to 0 °C) until they were collected and transported on ice to the laboratory for analysis.

Twenty-gram portions of the samples were homogenised in a stomacher (Mix 2, AES Laboratoire, France) for 1 min. Ten grams of each sample was added to 90-ml peptone water (1 % peptone water + 0.5 % NaCl) and placed on a mechanical shaker (Junior Orbit Shaker, Lab-Line Instruments, USA) for 30 min. Standard methods (APHA, 1976) were used in determining the microbiological indices of AMC (Plate Count Agar; Oxoid, UK; incubated at 36 °C/48 h); general coliform count (Eosin Methylene Blue Agar; Difco, USA; incubated at 36 °C/48 h); *Salmonella/Shigella* count (*S/S* Agar with Selenite Broth enrichment; Difco, USA; incubated at 36 °C/24-48 h); mould and yeast count (Oxytetracycline (0.01%) Glucose Yeast Agar; Merck, Germany; incubated at 28 °C/72 h).

Representative colonies from the plate counts were purified by sub-culturing and identified using morphological characteristics and standard biochemical tests. The tests used were Gram stain, catalase, oxidase, motility, nitrate, carbohydrate fer-
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Results

Waakye and its accompaniments

The AMC for complete waakye meals ranged between 8.1 × 10^7 and 1.3 × 10^8 CFU g⁻¹ (Table 1), whilst coliform count ranged between 5.8 and 9.7 × 10⁶ CFU g⁻¹. These values are comparable to the counts recorded for macaroni (9.6 × 10⁷ - 9.9 × 10⁸ CFU g⁻¹) and salad (5.7 × 10⁷ - 5.4 × 10⁸ CFU g⁻¹). Lower AMC (3.9 × 10⁴ - 1.8 × 10⁵ CFU g⁻¹) and coliform count (7.0 × 10⁴ - 3.3 × 10² CFU g⁻¹) were recorded for waakye only. The fried fish also had high coliform count (3.5 × 10⁴ - 6.3 × 10⁶ CFU g⁻¹).

Mould and yeast counts were highest on the raw vegetable salad, but could not be detected on waakye alone. Although Salmonella and Shigella could not be detected on any of the samples, the results have serious implications because the counts exceeded the microbiological standards for such short shelf-life foods (< 10⁶ CFU g⁻¹ at 36°C/48 h AMC; < 10⁴ CFU g⁻¹ at 36°C/48 h coliforms).

Identification of isolates from positive coliform plates showed that Klebsiella spp., Enterobacter spp., and Citrobacter freundii were present on the complete waakye meals.

| Sample                  | Aerobic mesophilic | Coliform       | Mould and yeast |
|-------------------------|--------------------|----------------|-----------------|
| Complete                | 8.1 × 10⁷          | 5.8 × 10⁶      | 3.5 × 10³       |
| Waakye                  | 1.3 × 10⁸          | 9.7 × 10⁴      | 4.2 × 10³       |
| Waakye only             | 3.9 × 10⁷          | 7.0 × 10⁴      | ND              |
|                         | 1.8 × 10⁷          | 3.3 × 10⁵      | ND              |
| Fried fish              | 8.3 × 10⁴          | 3.5 × 10⁴      | 1.5 × 10⁴       |
|                         | 1.2 × 10⁴          | 6.3 × 10⁵      | 8.3 × 10⁴       |
| Hydrated gari           | 2.7 × 10⁵          | 1.0 × 10⁶      | ND              |
|                         | 1.8 × 10⁶          | 3.4 × 10⁷      | 9.0 × 10⁵       |
| Cooked macaroni        | 9.6 × 10⁴          | 9.5 × 10⁵      | 4.5 × 10³       |
|                         | 9.9 × 10⁴          | 9.5 × 10⁵      | 3.8 × 10²       |
| Vegetable salad         | 5.7 × 10⁷          | 8.5 × 10⁴      | 5.5 × 10³       |
|                         | 5.4 × 10⁸          | 6.5 × 10⁷      | 6.0 × 10⁴       |

Discussion

The AMC (10⁴ - 10⁸ CFU g⁻¹) on waakye (Table 1) agreed with those reported (FAO/GHANA, 1997). However, because waakye is eaten with all its accompaniments, the high TVC (10⁷ - 10⁹ CFU g⁻¹) and coliform count (10⁶ - 10⁷ CFU g⁻¹) for the complete waakye (Table 1) show the microbiological quality of waakye better. High coliform counts (10⁶ - 10⁸ org g⁻¹) have also been reported for ready-to-eat chicken pieces after 8 h holding during marketing under ambient conditions (Hayford, 1979).

The observed high count on the vegetable salad and macaroni (Table 1) and the
| Meal code | Days holding | AMC<sup>a</sup> (CFU g<sup>-1</sup>) | Coliform<sup>b</sup> (CFU g<sup>-1</sup>) | Isolate |
|-----------|--------------|---------------------------------|-----------------------------------|---------|
| HAP-1<sup>c</sup> | 2 | 8.5 × 10<sup>4</sup> | 7.8 × 10<sup>2</sup> | Pseudomonas spp. |
|          |               | 9.4 × 10<sup>5</sup> | 8.8 × 10<sup>3</sup> | Alkaligenes spp. |
| HAP-2    | 3 | 6.4 × 10<sup>5</sup> | 2.0 × 10<sup>3</sup> | E. coli, Enterobacter spp. |
|          |               | 7.3 × 10<sup>3</sup> | 3.0 × 10<sup>4</sup> |           |
| HAP-3    | 4 | 2.0 × 10<sup>4</sup> | ND | No coliform isolates |
|          |               | 2.3 × 10<sup>2</sup> | ND |           |
| HAP-4    | 2 | 4.9 × 10<sup>2</sup> | 1.0 × 10<sup>2</sup> | Acinetobacter spp. |
|          |               | 5.2 × 10<sup>4</sup> | 2.0 × 10<sup>3</sup> |           |
| HAP-5    | 3 | 1.1 × 10<sup>4</sup> | 3.0 × 10<sup>2</sup> | Acinetobacter spp. |
|          |               | 1.2 × 10<sup>2</sup> | 8.5 × 10<sup>3</sup> | Serratia spp. |
| HAP-6    | 4 | 3.2 × 10<sup>4</sup> | 1.8 × 10<sup>3</sup> | Acinetobacter spp. |
|          |               | 3.7 × 10<sup>3</sup> | 2.6 × 10<sup>3</sup> |           |
| HAP-7    | 4 | 7.6 × 10<sup>4</sup> | 4.7 × 10<sup>2</sup> | Enterobacter spp. |
|          |               | 8.5 × 10<sup>4</sup> | 7.8 × 10<sup>2</sup> |           |
| HAP-8    | 4 | 4.9 × 10<sup>4</sup> | 4.0 × 10<sup>2</sup> | Serratia spp. |
|          |               | 5.4 × 10<sup>4</sup> | 4.5 × 10<sup>2</sup> | Enterobacter spp. |
| HAP-9    | 5 | 5.6 × 10<sup>4</sup> | 1.2 × 10<sup>3</sup> | E. coli, Klebsiella spp. |
|          |               | 5.9 × 10<sup>4</sup> | 1.4 × 10<sup>3</sup> | Acinetobacter spp. |
| HAP-10   | 0 | 5.3 × 10<sup>4</sup> | 4.0 × 10<sup>3</sup> | Acinetobacter spp. |
|          |               | 5.4 × 10<sup>4</sup> | 6.0 × 10<sup>3</sup> | Serratia spp. |
| HAP-11   | 2 | 9.8 × 10<sup>2</sup> | 2.5 × 10<sup>2</sup> | Acinetobacter spp. |
|          |               | 1.0 × 10<sup>3</sup> | 2.7 × 10<sup>2</sup> | Serratia spp. |
| HAP-12   | 2 | 1.5 × 10<sup>4</sup> | 2.0 × 10<sup>3</sup> | Acinetobacter spp. |
|          |               | 2.1 × 10<sup>2</sup> | 3.0 × 10<sup>3</sup> |           |
| HAP-13   | 4 | 1.4 × 10<sup>4</sup> | 4.0 × 10<sup>3</sup> | Enterobacter spp. |
|          |               | 5.7 × 10<sup>4</sup> | 6.0 × 10<sup>3</sup> | Klebsiella spp. |
| HAP-14   | 2 | 1.1 × 10<sup>3</sup> | ND | Enterobacter spp. |
|          |               | 1.2 × 10<sup>2</sup> | 4.0 × 10<sup>3</sup> | Acinetobacter spp. |

<sup>a</sup> AMC - Aerobic mesophilic count  
<sup>b</sup> Actual counts for replicate tests  
<sup>c</sup> Meal ingredients:
- HAP-1: Fish fillet, cheese, sauce, oil, potato, spices, vegetables
- HAP-2: Deboned chicken, spices, oil, plain rice, potato
- HAP-3: Chicken, spices, sauce, oil, steamed rice, potato
- HAP-4: Beef steak, spices, oil, potato, tomato, peppers
- HAP-5: Beef steak, spices, sauce, oil, steamed rice
- HAP-6: Chicken breast, spices, oil, boiled rice, sauce
- HAP-7: Fish fillet, spices, oil, sauce, potato
- HAP-8: Chicken, peppers, curry, oil, steamed rice
- HAP-9: Fish, spices, oil, potato, sauce
- HAP-10: Eggs, salt, oil
- HAP-11: Eggs, salt, oil
- HAP-12: Lamb, spices, oil
- HAP-13: Chicken, spices, oil, vegetables, rice
- HAP-14: Biscuit, fruits, cheese, cream

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fact that they reflected the high count for complete *waakye* deserve further investigation. Uncooked vegetable salads have high microbial load unless strict procedures, including thorough washing and sanitization, are followed to reduce microbial load while subsequent refrigeration or chilling is required to control microbial growth (Farkas *et al.*, 1997; Beuchat, 1996). Discussions with and observation of *waakye* processors showed that the vegetable salads were washed with potable water, comminuted and displayed together with other accompaniments of *waakye*, during marketing. No effective sanitizing treatment is applied to the salads. Earlier studies (FAO/GHANA, 1997) also indicated that accompaniments of *waakye* such as salads and pepper sauce, which are minimally processed, had higher counts ($\text{AMC} > 4 \times 10^3 \text{ CFU g}^{-1}$; *Staphylococcus aureus* $> 3 \times 10^4 \text{ CFU g}^{-1}$, and *Bacillus cereus* $> 2 \times 10^5 \text{ CFU g}^{-1}$).

Direct observation of *waakye* sellers also suggested cross-contamination resulting from the use of fingers or the same ladle to serve the salads, cooked macaroni and *waakye*. Although the use of different ladles would largely prevent cross-contamination of the bulk of the meal composites, this would not prevent the complete *waakye* meal from being contaminated as the results of this study indicate (Table 1). The only measure for the safety of any complete meal is to ensure that each accompaniment, especially the minimally processed such as uncooked salads, are microbiologically safe. Isolates from the positive coliform plates of complete *waakye* included *Citrobacter freundii*, *Klebsiella* spp., and *Enterobacter* spp.; these have public health implications (Anon, 1996; Klipstein, Engert & Short 1977).

The poor microbiological quality of many street foods might result from the combined effect of using ineffective sanitary procedures to prepare uncooked or minimally processed foods, post-processing contamination, and long holding under conditions which enhance proliferation of microorganisms. Improving sanitary conditions under which ‘street foods’ are prepared and marketed and assisting stakeholders to adopt effective preventive procedures, based on the HACCP approach, to convert the raw food ingredients into ready-to-eat meals are important in ensuring food safety and good public health.

The microbiological quality of 14 ready-to-eat meals prepared under HACCP plan indicated that for all the meals, the counts recorded (Table 2) did not exceed the microbiological standards of short shelf-life foods ($< 10^7 \text{ CFU g}^{-1}$ at 36 °C/48 h AMC and $< 10^6 \text{ CFU g}^{-1}$ at 36 °C/48 h coliforms). These results show the important contribution that the HACCP plan can make toward ensuring food safety when it is implemented effectively.

Table 2 further shows that holding the meals at 0 to -5 °C controlled the proliferation of microbes. This is supported by the observation that HAP-10, which was analysed soon after production (i.e., 0 holding time), had higher AMC and coliforms than HAP-11, which is the same meal held (-5 to 0 °C) for 2 days. Although all the meals are supplied to clients within 24 h of preparation, it is important to investigate the microbiological quality of some meals if they are held for over 24 h. The counts for meals which were held for up to 5 days were also within the microbiological standards. Potential pathogens isolated from the meals prepared under the HACCP plan (Table 2) included enterotoxigenic bacteria such as *E. coli*, *Serratia* spp., and *Klebsiella* spp. (Anon, 1996; Klipstein *et al.*, 1977). The last two species were also isolated from the complete *waakye* meals. The results suggested that implementing HACCP reduced microbial counts substantially; however, some potential pathogens survived.

Although the HACCP plan requires that the ready meals are held at -5 to 0 °C to suppress growth of survivors, they could proliferate if the meals are subjected to temperature abuse such as during power outages. It is necessary to adopt processes and technologies which can eliminate potential pathogens from prepared meals and improve their microbiological safety. Food irradiation is a proven technology for eliminating pathogens and ensuring food safety.
Conclusion
The microbiological count on all samples of waakye bought from the open market exceeded the microbiological standards for such short shelf-life foods. Although microbiological counts on meals prepared under HACCP agreed with the requisite microbiological standards, the potential pathogens identified on some meals is noteworthy. Potential pathogens need to be eliminated from waakye as well as ready meals prepared under HACCP to ensure their microbiological safety. Radiation processing is a proven technology for eliminating potential pathogens, but has no adverse effects on nutritional and sensory quality of meals. Subsequent studies will exploit the potential of irradiation to eliminate pathogens and ensure the microbiological safety of ready-to-eat meals.

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