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Effects of Processing on Antibiotic Residues (Streptomycin, Penicillin-G and Tetracycline) in Soft Cheese and Yoghurt Processing Lines

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Abstract: Fifty-eight samples (40 cheese and 18 yoghurt samples) were collected from five soft cheese and two conventional yoghurt processors. The samples were collected along the processing lines of these dairy products and analyzed for antibiotic residues (streptomycin, penicillin-G and tetracycline). The mean residual levels for cheese was; streptomycin (0.0040±0.0018), penicillin-G (0.0062±0.0026) and tetracycline (0.0023±0.0008), while levels in yoghurt was; streptomycin (0.0014±0.0010), penicillin-G (0.0017±0.0017) and tetracycline (0.0011±0.0071). Penicillin-G exceeded Maximum Residue Limit (MRL) in raw milk and cheese for 4 out of the 5 processors. In yoghurt processing only powdered milk for processor F exceeded MRLs for penicillin-G. Significant differences ($p<0.05$) was obtained along the processing lines for all processors. Generally antibiotic residue levels reduced along the processing line of soft cheese and yoghurt. The presence of penicillin-G above MRLS in some of the samples poses a threat to the consumers.

Key words: Antibiotics residues, cheese, yoghurt, processing lines

INTRODUCTION

The economic benefits of veterinary drugs make their use a widespread practice in food-producing animals, this practice may constitute a consumer health risk, as an incorrect use of these pharmacologically active drugs causes residues to remain in edible parts.

Antibiotics are widely used in dairy cattle management for the treatment of disease and as dietary supplements. They may be administered orally, as feed additives or directly by injection. The use of antibiotics may result in drug residues being present in the milk and subsequently; interference with starter cultures for cheese, yoghurt and other dairy products, or indicate that the milk may have been obtained from an animal with a serious infection (Schenck and Callery, 1998; Choma *et al*., 1999; Phillips *et al*., 2004). There are also concerns that the usage of antibiotics may be responsible for the promotion of resistant strains of bacteria (Neu, 1992; Brady and Katz, 1992; Brady *et al*., 1993; Caswell *et al*., 2000; Torrence, 2001) resulting in untreatable infections in both animals and humans.

The presence of drug residues in milk supplies and products is of public health implications and are perceived by consumers as undesirable (Mcewen *et al*., 1991; Bencini and Pulina, 1997). Today, it is the general consensus that even slight traces of antibiotics in milk and food for human consumption should not be tolerated (Jepsen, 1990). Allergic reactions in highly sensitive consumers and potential carcinogenicity, mutagenicity, teratogencity and long-term toxic effects of the residues of all classes of antibiotics (Enomoto and Saito, 1972; Epstein *et al*., 2000) are of public health concern. Adetunji (2008) reported various levels of antibiotics in milk products in Southwestern Nigeria, similar results were obtained by, Dipeolu and Alonge (2001, 2002) who recorded residues of antibiotics; tetracycline and streptomycin in beef samples from cattle marketed in Ogun and Lagos states of Nigeria, respectively.

Although reports on antibiotics residues in milk and milk products did not eliminate the antibiotics especially with products that had undergone heat processing like cheese, evaporated milk and yoghurt. These findings agree with an earlier study by O'Keefe and Kennedy (2008), who reported very little or no effect on antibiotics residues after processing. The presence of residues of these antibiotics in some milk products at a level exceeding the MRLs of FAO/WHO (Joint Food and Agricultural Organization) Expert Committee on Food Additives Standards (1998) is probably due to injudicious use of antibiotics in the treatment of infections in cattle and lack of adherence to withdrawal period before milking. Dina and Arowolo (1991) reported the widespread misuse of veterinary drugs in Nigeria due to inadequate monitoring and prescription by untrained personnel. The objective of this study is to observe the effects of processing on the antibiotics residues in cheese and yoghurt by analyzing samples taken along the processing line.

MATERIALS AND METHODS

Sampling protocols: Five cheese processors and two yoghurt processors were selected for this study, a total of fifty-eight samples were collected (40 cheese and 18 yoghurt samples), along the processing lines of cheese and yoghurt into sterile sample bottles and kept in a cooler with ice pack on transport to the laboratory for analysis.

Cheese processing samples (10 gm or 10 ml) were collected at the following stages; Raw Milk (Rm), addition of coagulant (using extract from *Calotropis procera* or *Carica papaya* or others) (Ac), Curdling point (Cp) and the Cheese in mould (Cm). While, the yoghurt processing samples (10 gm or 10 ml) were collected at the following stages; Powdered milk (Pm), Pasteurization (Pt), Inoculation of starter culture (In), Fermentation (Ft) and Finished product (Fp). Each sample was collected in replicates.

Antibiotic residue analysis of samples: High performance liquid chromatography standard methods were used for antibiotic residue analysis. Penicillin-G, streptomycin and tetracycline were analyzed according to Shaikh and Moats (1993); Clark (1977) and Posyniak *et al*. (1998) respectively.

RESULTS

The mean levels of antibiotics and standard deviation were: streptomycin (0.0040±0.0018 ppm), penicillin (0.0062±0.0026 ppm) and tetracycline (0.0023±0.0008 ppm) in cheese processing stages (Table 1), while, in yoghurt processing stages the mean levels and standard deviation were; streptomycin (0.0014±0.0010 ppm), penicillin (0.0017±0.0017 ppm) and tetracycline (0.0011±0.0071 ppm) (Table 2).

Cheese: There were no significant differences for streptomycin levels in Raw milk (Rm) and Addition of coagulant (Ac) in processors A, C and E and for processors B and D at Cp and Cm stages. But at the Curdling point (Cp), all processors had significant differences ($p<0.05$) as values were observed to reduce. For penicillin-G, there were no significant differences for all the cheese processors between Cp and Cm; between Rm and Ac for processors A, D and E. Processors B and D are not different at Ac stage ($p<0.05$).

Values obtained for tetracycline residues in Rm and Ac were not significantly ($p<0.05$) different for processors, A, D and E, while Cp and Cm were not significantly different for processors A, B and E. At Curdling point (Cp) reduction in tetracycline levels from all the processors was observed.

Yoghurt: During yoghurt processing it was observed generally for all antibiotics evaluated that the Pasteurization (Pt) stage had a significant effect ($p<0.05$) on the values obtained as compared to values observed in Powdered milk (Pm), Fermentation (Ft) stage was not different significantly from the Final product (Fp) stage for all antibiotics for processor G, while, no significant differences ($p<0.05$) were observed between stages I, II and III in both processor for all the antibiotics.

For processor F, streptomycin and penicillin levels in samples taken at inoculation (In) stage was not significantly different ($p<0.05$) from samples taken at Ft (fermentation) stage.

Significant finding in this study is that penicillin-G exceeded Maximum Residue Limit (MRL) in raw milk and cheese for 4 out of the 5 processors. In yoghurt processing only powdered milk for processor F exceeded MRLs for penicillin-G but tetracycline and streptomycin were within MRLs.

DISCUSSION

Mean levels of antibiotic residues obtained from the processing stages of cheese and yoghurt are lower than values obtained by Adetunji (2008), who recorded residual levels of antibiotics of; streptomycin (0.008-0.1 ppm), penicillin (0.01-0.075 ppm) and tetracycline (0.08-0.1 ppm). These differences may be due to differences methods of analysis and sample types.

Similar levels of streptomycin observed between Rm and the Ac by processors A, C and E shows that the similar coagulants used (*Calotropis procera*) had little or no effects on antibiotics residual levels. The Cp had a significant reduction effect on antibiotics (streptomycin and tetracycline) residue levels. Similar reports were made by Javadi *et al*. (2011) where cooking processes reduced the levels of antibiotic residues. However, Posyniak *et al*. (1998) suggested that the difficulty in isolating tetracycline from tissue is associated with the propensity of the compound to bind with sample protein and to form chelate complexes with metal ions. This also explains our observation because at curdling point there is coagulation of protein fraction of the milk resulting into formation of curds, hence reduction in antibiotic residual levels. No significant difference ($p<0.05$) was observed between stages Cp and Cm for A and C with penicillin levels and processors A, B and E for tetracycline levels, most likely because of drop in temperature from the Cp stage to the (Cm) stage.

The reduction in antibiotic levels observed at Pt stage for yoghurt processing is similar to the effect of heat at Cp for cheese. It can be suggested that proper pasteurization will be of help in reducing antibiotic levels to the bearest minimum. But for processor G the fermentation and finished product stages were not affected by processing significantly for all the antibiotics and all the antibiotics were not affected by processing significantly at Pm, Pt and In stages for both processors this explains the fact that processing does not fully breakdown these drugs. Also the insignificant difference observed between In stage and Ft stage reveals that, the starter culture used was resistant (i.e. contains resistant bacteria culture Torrence, 2001) to the antibiotic residues levels present in the samples such that levels remain unchanged during fermentation. This agrees with contributions from (Neu, 1992; Brady and Katz, 1992; Brady *et al*., 1993; Caswell *et al*., 2000) that

Table 1: Levels of antibiotic residues in samples taken along the processing stages of the five cheese processors

Processor stages	A	B	C	D	E
Streptomycin					
Rm	0.0071±0.0001^{aA}	0.0033±0.0002^{aB}	0.0063±0.0002^{aC}	0.0045±0.0001^{aD}	0.0020±0.0001^{aE}
Ac	0.0070±0.0001^{aA}	0.0030±0.0002^{abB}	0.0059±0.0001^{aC}	0.0040±0.0002^{bD}	0.0022±0.0001^{aE}
Cp	0.0047±0.0001^{bA}	0.0027±0.0001^{bcB}	0.0053±0.0002^{bC}	0.0030±0.0001^{cB}	0.0017±0.0001^{abD}
Cm	0.0046±0.0001^{bA}	0.0028±0.0001^{cB}	0.0063±0.0002^{aC}	0.0028±0.0001^{cB}	0.0015±0.0001^{bD}
Penicillin-G					
Rm	0.0130±0.0002^{aA}	0.0053±0.0001^{aB}	0.0077±0.0003^{aC}	0.0050±0.0001^{aD}	0.0039±0.0001^{aE}
Ac	0.0128±0.0001^{aA}	0.0050±0.0001^{bB}	0.0073±0.0001^{abC}	0.0047±0.0001^{aB}	0.0037±0.0004^{aD}
Cp	0.0070±0.0002^{bA}	0.0042±0.0001^{cB}	0.0070±0.0002^{bA}	0.0054±0.0001^{aC}	0.0044±0.0001^{abB}
Cm	0.0066±0.0001^{bA}	0.0044±0.0001^{cB}	0.0068±0.0001^{bA}	0.0050±0.0001^{aC}	0.0042±0.0001^{abB}
Tetracycline					
Rm	0.0040±0.0001^{aA}	0.0016±0.0001^{aB}	0.0026±0.0001^{aC}	0.0017±0.0002^{aB}	0.0034±0.0025^{aB}
Ac	0.0042±0.0001^{aA}	0.0020±0.0001^{bB}	0.0031±0.0002^{bC}	0.0017±0.0001^{aB}	0.0018±0.0001^{aB}
Cp	0.0026±0.0001^{bA}	0.0012±0.0001^{cB}	0.0027±0.0001^{abA}	0.0022±0.0001^{bC}	0.0021±0.0001^{aC}
Cm	0.0028±0.0001^{bA}	0.0011±0.0001^{cB}	0.0025±0.0000^{aC}	0.0019±0.0001^{abD}	0.0023±0.0001^{aC}

Valures are means ± standard deviation in µg/ml. Processing stages: Raw milk (Rm), Addition of coagulant (Ac), Curdling point (Cp), Cheese in mould (Cm). Values with similar uppercase letter in the same row are not significantly different with respect to the processing stage across the processor for the antibiotics analyzed. Values with similar lowercase letter in the same column are not significantly different with respect to the processor along the processing stages for the antibiotics analyzed

Table 2: Levels of antibiotic residues in samples taken along the processing stages of the tow yoghurt processors

Processor stages	F	G
Streptomycin		
Pm	0.0029±0.0001^{a}	0.0015±0.0004^{a}
Pt	0.0000±0.0000^{b}	0.0012±0.0000^{ab}
In	0.0015±0.0001^{c}	0.0007±0.0001^{c}
Ft	0.0020±0.0001^{d}	0.0010±0.0001^{bc}
Fp	0.0013±0.0001^{c}	0.0008±0.0000^{bc}
Penicillin-G		
Pm	0.0057±0.0001^{a}	0.0031±0.0001^{a}
Pt	0.0000±0.0000^{b}	0.0011±0.0001^{b}
In	0.0018±0.0006^{c}	0.0003±0.0001^{c}
Ft	0.0023±0.0001^{c}	0.0008±0.0001^{d}
Fp	0.0011±0.0001^{d}	0.0006±0.0001^{d}
Tetracycline		
Pm	0.0019±0.0008^{a}	0.0025±0.0001^{a}
Pt	0.0000±0.0000^{b}	0.0012±0.0001^{b}
In	0.0011±0.0001^{c}	0.0004±0.0001^{c}
Ft	0.0013±0.0002^{ad}	0.0008±0.0000^{d}
Fp	0.0008±0.0001^{bcd}	0.0009±0.0001^{d}

Values are means ± standard deviation in µg/ml. Processing stages: Powdered milk (Pm), Pasteurization (Pt), Inoculation of starter culture (In), Fermentation (Ft), Finished product (Fp). Values with the same lowercase letter in the same column are not significantly different with respect to the processor along the processing line

antibiotics residues promotes development of resistant strains of bacteria and (Schenck and Callery, 1998; Choma *et al*., 1999; Phillips *et al*., 2004) that; antibiotic residues interferes with starter cultures for processing of cheese, yoghurt and other dairy products.

In general, the results obtained from this study reveals that antibiotics are present at different levels at the various stages of processing of cheese and yoghurt. Also, it is important to note that the processing stages of these products did not eliminate the antibiotics considering the fact that these products have undergone heat treatment. These findings agree with earlier report by O'Keefe and Kennedy (2008) that recorded very little or no effect on antibiotics residues after processing.

Moreover, several methods like fluorimetry (Wilson *et al*., 1972) and chromatography (Wagman and Westein, 1983) have been used to detect antibiotic residue levels in substances but each method has its own limitations, this may be responsible for the variations in levels observed among the various processors.

The finding of penicillin-G residues above MRLs in some samples is of public health concern since this will result in development of drug resistant strains of microorganism and allergic reactions in hypersensitive individuals. Therefore the judicious use of antibiotics and proper screening of food items before sales to the public is paramount.

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