INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 2, p. 116-125, 2022

RESEARCH PAPER OPEN ACCESS

# Antibiotic residues in food of animal origin: effect of heat treatment on some antibiotic molecules used in veterinary medicine

**Serge Samandoulougou[1*], Hamidou Compaore[1], Fidèle Wend-Bénédo Tapsoba[1], André Jules Ilboudo[2], Hagretou Sawadogo/Lingani[1]**

[1]*Institut de Recherche en Sciences Appliquées et Technologies/Département Technologie Alimentaire, (IRSAT/DTA) 03 BP 7047 Ouagadougou 03, Burkina Faso*

[2]*Laboratoire de Biochimie, Biotechnologie, Technologie Alimentaire et Nutrition (LABIOTAN), Département de Biochimie-Microbiologie (DBM), Université Joseph KI ZERBO, 03 BP. 7021 Ouagadougou, Burkina-Faso*

**Key words:** Veterinary drugs, Antibiotic residues, Heat treatment.

http://dx.doi.org/10.12692/ijb/20.2.116-125 Article published on February 7, 2022

## Abstract

Foods of animal origin containing residues of antibiotics are mostly not consumed raw. They undergo heat treatment through unit operations such as cooking, pasteurization, frying etc. These heat treatments induce effects on antibiotic residues in food of animal origin. The objective of this study is to assess the effect of heat treatment on antibiotic molecules that may be found as residues in meat, milk or eggs consumed. The effects of heat treatments on oxytetracycline, chlortetracycline, penicillin G, streptomycin and chloramphenicol were evaluated. The evaluation was made by visual observation to appreciate the color changes and the formation of precipitates. Also, microbiological methods have use to measure the inhibitory capacity of antibiotics subjected to thermal treatment. Spectrophotometry and high performance liquid chromatography (HPLC) have permit to quantify and identify antibiotics. It emerges from this evaluation that heat treatment can cause, on the one hand, a degradation of certain antibiotics with an increase or decrease in their inhibitory capacity. On the other hand, antibiotic molecules can change their conformation or remain stable under different heat treatments. Residues of antibiotics which may be found in foods of animal origin are not removed by heat treatments; but they remain stable or undergo modifications which can make them more or less toxic.

* **Corresponding Author:** Serge Samandoulougou ✉ serge1rech@gmail.com

## Introduction

Foods of animal origin contain high concentrations of nutrients such as proteins, essential fatty acids, vitamins and certain minerals (Nys and Sauveur, 2004; Kouakou *et al.*, 2015). The intensification of the production of meat, milk and eggs over the last decades has been favored by the use of veterinary drugs, in particular, anti-infective drugs in modern farming (Moretain, 2005; Tatsadjieu *et al.*, 2009). These drugs are used either in curative treatments applied individually or collectively to animals suffering from microbial diseases, or in preventive treatments to avoid the appearance of certain pathologies or, in some extreme cases, to overcome deficiencies in hygiene in livestock (Sanders, 2005). In addition, regulations concerning the possession, distribution and use of veterinary drugs are lagging far behind in developing countries. In these countries, many herders treat their animals themselves (Biagui, 2002). Even if the type of molecules used is the same as that used by veterinarians, notions of the conditions and methods of administration as well as the quantities to be administered or the withdrawal periods are ignored. In addition, certain practices consist in administering ruminant drugs intended for another species. In addition, farmers' knowledge of food safety remains quite limited (Mensah *et al.*, 2014). The uncontrolled use of antibiotics can lead to the formation of residues in the foodstuffs produced by these animals, especially when the withdrawal periods are not respected by the breeders (Alambedji, 2008). The potential risks associated with the presence of antibiotic residues in foodstuffs of animal origin are of several types: carcinogenic risks (nitrofurans), allergic risks (Penicillins, Streptomycin), toxic risks (Chloramphenicol), modification of the intestinal flora (Tetracyclines), selection of bacteria resistant to antibiotics (Kabir *et al.*, 2004; Alambedji *et al.*, 2008; Persoons, 2011) and inhibitions of fermentation phenomena in the dairy industry (Biagui, 2002). In the West African sub-region, the presence of antibiotic residues in foods of animal origin is common (Hao, 2000; Nhiem *et al.*, 2006). The prevalence rate of residues of veterinary drugs in foods of animal origin is less than 1% in Europe, while it reaches 94% in some African countries (Mensah *et al.*, 2014). Foods containing residues of antibiotics are mostly not eaten raw. They undergo heat treatment through unit operations such as cooking, pasteurization, frying, etc. What effect can these heat treatments have on antibiotic residues in foods of animal origin? The objective of this study is to assess the effect of heat treatment on antibiotic molecules that may be found as residues in meat, milk, or eggs consumed. This will help to know if the antibiotic residues found in meats, milk and eggs can be destroyed during cooking.

## Material and methods

*Preparation of antibiotic solutions*

A mixture of 5 mg of antibiotics with 50 ml of Mili-Q water (demineralized and deionized water) was made to obtain antibiotic solutions of 100 µg / ml. These are the following antibiotics: oxytetracycline hydrochloride, chlortetracycline hydrochloride, penicillin G potassium salt, chloramphenicol and streptomycin in 1 mg / mL solution in 1 mM EDTA.

*Methodology of heat treatments*

Each prepared antibiotic solution was divided into 5 lots numbered 1 to 5. Lots 1, 2, 3, 4 and 5 of each antibiotic were placed in a water bath and in an autoclave, for 30 minutes respectively at 35 ° C, 45 ° C, 65 ° C, 100 ° C and 120 ° C.

*Visual observations*

Observations of color change and precipitation formation after heat treatments of each batch of antibiotics were made with the naked eye.

*Methodology for evaluating areas of inhibition of treated antibiotics*

The microbiological test on a Petri dish was carried out with Geobacillus stearothermophilus ATCC 10149 as the test microorganism. The preparation and inoculation of the culture media were carried out as described above. Twenty (20) µl of the heat-treated antibiotic solutions (35 ° C, 45 ° C, 65 ° C, 100 ° C, 120 ° C) are used to impregnate the sterile 6.13 Whatman paper discs mm in diameter. Discs soaked in

antibiotics are placed on the boxes already inoculated. The boxes were then incubated at 55 ° C for 24 hours. Clear zones of inhibition around the discs were measured using an electronic caliper (LR44, ISO metric thread, model 0-150mm, 1.55v) after 24 h incubation.

*Methodology for evaluating the concentration of treated antibiotics*

In order to assess the concentration of antibiotics subjected to the different heat treatments, we performed a calibration curve (concentration, absorbance) for each antibiotic. Curves were plotted using absorbance versus known concentrations of each antibiotic. Concentrations of 1.56 µg / ml; 3.12µg / ml; 6.25 µg / ml; 12.5 µg / ml; 25 µg / ml; 50 µg / ml; 100 µg / ml; and 150 µg / ml of each antibiotic were used to read absorbances at 200 nm. A 96-well Epoch BioTeK brand spectrophotometer coupled to a computer was used.

The different evolutions and variations of the chromatograms of the antibiotics subjected to the heat treatments (35 ° C and 120 ° C) were evaluated using high-performance liquid chromatography (HPLC). A PerkinElmer 2000 series HPLC chain was used. The program selected for the analysis is shown in Table 1 below.

*Data analysis*

The data collected was analyzed by ANOVA using XLSTAT software version 7.5.2. Descriptive statistics (mean ± standard deviation) were given for each variable. The means were compared by a Z test. The differences were declared significant at the 5% level.

**Results and discussion**

*Visual observations of color changes and/or precipitate formation*

It emerges from Table 2 above that no change in color and/or formation of precipitate was visible after the various heat treatments on penicillin G, streptomycin and chloramphenicol. On the other hand, a color change was observed on oxytetracycline and chlortetracycline subjected to 65 ° C and 100 ° C, respectively (Fig. 2). The change in color of oxytetracycline after heating to 65 ° C corroborates the finding made by Randame (2015) in Algeria.

Chlortetracycline and oxytetracycline are from the tetracycline family. Antibiotics in this family are heat sensitive resulting in color change Randame (2015).

This change in color is probably due to a change in the structure of the molecule under the action of heat (Lederer, 1986). Fig. 1 below shows a color change in an oxytetracycline solution at 65 ° C.

**Table 1.** Antibiotic analysis program at 35 ° C and 120 ° C.

| Parameters | |
|---|---|
| Concentration SM (mg / ml of eluent) | 2 mg / ml and 0.2 mg / ml (For streptomycin) |
| Injection volume (µl) | 10 |
| UV-Vis detection wavelength (nm) | 325 |
| Debit (ml/min) | 1,5 |
| Analysis time (min) | 5 |
| Stationary phase | Reverse |
| Eluent A | MQ water + 0,1% AP |
| Eluent B | Acétonitrile |
| Elution gradient | Isocratique à 95%A + 5%B |
| Pump equilibrium time (minute) | 10 |

SM: Mother Solution; PA: Phosphoric Acid; MQ : water: mili-Q water.

*Evolution of the concentration of antibiotics subjected to heating*

The concentrations of the heated antibiotics were determined and reported in Table 3 below. Fig. 2 below gives the evolution of the concentration of oxytetracycline and chloramphenicol subjected to heat treatments and Fig. 3 gives the evolution of the concentration of penicillin, streptomycin and chlortetracycline subjected to heat treatments. It can be seen from Fig. 2 and 3 that the concentrations of

oxytetracycline and penicillin G increase under the effect of heating temperature. Statistical analyzes have shown that this increase is significant (Table 3). This same observation was observed by Ibrahim and Moats (1994). On the other hand, those of streptomycin, chloramphenicol and chlortetracycline decrease slightly with the increase in temperature; this could be explained by a degradation of the molecules, either precipitation of the molecules or a change in the spatial conformation of the antibiotic molecules. For chloramphenicol, the concentration increases around 45 ° C and decreases at temperatures above 45 ° C. Despite the fact that chlortetracycline and oxytetracycline belong to the same family, their molecules did not react in the same way to the effect of temperature.

**Table 2.** Visual observations of color changes or precipitate formation.

| | Different processing temperatures | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 35 °C | | 45 °C | | 65 °C | | 100 °C | | 120 °C | |
| Parameters | C.C | F.P | C.C | F.P | C.C | F.P | C.C | F.P | C.C | F.P |
| Oxytetracycline | - | - | - | - | + | - | + | - | + | - |
| Chlortetracycline | - | - | - | - | - | - | + | - | + | - |
| Penicillin G | - | - | - | - | - | - | - | - | - | - |
| Streptomycin | - | - | - | - | - | - | - | - | - | - |
| Chloramphenicol | - | - | - | - | - | - | - | - | - | - |

CC = color change; F.P = formation of precipitate; + = presence of color changes;
- = absence of color changes and / or formation of precipitate.

**Table 3.** Concentration of heated antibiotic solutions.

| | Average concentrations in µg / mL (average of 3 concentrations) | | | | |
|---|---|---|---|---|---|
| | 35°C | 45°C | 65°C | 100°C | 120°C |
| Oxytetracycline | $143,33^{a}$ ±0,001 | $139,52^{b}$ ±0,005 | $143,57^{c}$ ±0,00 | $143,65^{d}$ ±0,00 | $145,23^{e}$ ±0,001 |
| Chlortetracycline | $11,45^{a}$ ±0,000 5 | $13,07^{b}$ ±0,00 | $12,99^{c}$ ±0,0005 | $11,57^{a}$ ±0,002 | $10,32^{d}$ ±0,0009 |
| Penicillin G | $24,42^{a}$ ±0,00 | $23,94^{a}$ ±0,000 5 | $22,69^{b}$ ±0,0005 | $25,29^{c}$ ±0,003 | $24,93^{d}$ ±0,0015 |
| Streptomycin | $6,82^{a}$ ±0,005 | $7,96^{b}$ ±0,003 | $5,91^{c}$ ±0,00 | $7\,964^{b}$ ±0,00 | $6,64^{a}$ ±0,00 |
| Chloramphenicol | $82,20^{a}$ ±0,01 | $107,11^{b}$ ±0,003 | $82,20^{a}$ ±0,001 | $57\,861^{c}$ ±0,0005 | $57\,149^{d}$ ±0,001 |

Values with the same letters on the same row are statistically identical at the 5% threshold (P> 0.05).

The increase in the concentration of oxytetracycline following heat treatment suggests cyclization and/or a change in molecular conformation absorbing more than the initial molecule. Likewise, the concentrations of penicillin solutions increased slightly at elevated heating temperatures. The decrease in the concentration of antibiotic solutions could be explained by the degradation of molecules under the effect of heat. We then asked ourselves whether the effects of heat treatment on antibiotic molecules influence their inhibitory capacity or not?

*Evolution of the bacterial inhibitory capacity of antibiotics*

Table 4 shows a variation of the zones of inhibition depending on the heat treatments. Fig. 4 below shows the evolution of the zone of bacterial inhibition of antibiotics as a function of the heating temperature.

**Table 4.** Zone of inhibition obtained for the antibiotic solutions subjected to different temperatures for 30 minutes.

| Temperatures | 35°C | 45°C | 65°C | 100°C | 120°C |
|---|---|---|---|---|---|
| Average diameters of the zones of inhibitions in mm (oxytetracycline) | $15^{cd}$ ±0,5 | $16^{bc}$ ±1 | $14^{d}$ ±0,5 | $17^{ab}$ ±0,0 | $18^{a}$ ±1 |
| Average diameters of the zones of inhibitions in mm (penicillin G) | $23^{bc}$ ± 0,5 | $26^{a}$ ± 0,5 | $25^{ab}$ ± 2 | $22^{c}$ ± 1 | $21^{c}$ ±0,0 |
| Average diameters of the zones of inhibitions in mm (streptomycin) | $9^{a}$ ± 1 | $7^{b}$ ± 0,0 | $4^{c}$ ±1 | $4^{c}$ ±0,0 | $6^{b}$ ±0,5 |
| Average diameters of the zones of inhibitions in mm (chlortetracycline) | $14^{a}$ ±1 | $12^{b}$ ± 0,5 | $13^{ab}$ ± 0,0 | $14^{a}$ ± 1 | $13^{ab}$ ± 0,0 |
| Average diameters of the zones of inhibitions in mm (chloramphenicol) | $10^{a}$ ±1 | $10^{a}$ ±0,5 | $11^{a}$ ±0,0 | $10^{a}$ ±0,5 | $11^{a}$ ±0,0 |

Values with the same letters on the same row are statistically identical at the 5% threshold (P> 0.05).

The inhibitory capacity of penicillin G increases and peaks at 45 ° C and decreases with higher temperatures (Fig. 4). As for oxytetracycline, its inhibitory capacity increases at 45 ° C, decreases at 65 ° C and then increases considerably at 100 ° C and 120 ° C. The inhibitory capacity of streptomycin decreases significantly from 35 ° C to 100 ° C, but from 100 ° C to 120 ° C, it increases slightly.

**Table 5.** Oxytetracycline Chromatogram Parameters at 35 ° C and 120 ° C.

| | Parameters | 35°C | 120°C |
|---|---|---|---|
| Oxytétracycline | Retention Time (min) | 3,57 | 3,68 |
| | Area | 302 501,21 | 500 552,67 |
| | Height | 26 494,35 | 46 885,02 |

**Table 6.** Parameter of the chromatogram of penicillin at 35 ° C and at 120 °C.

| | Parameters | 35°C | 120°C |
|---|---|---|---|
| Penicillin | Retention Time (min) | 4 185 | 3 951 |
| | Area | 184 336,31 | 190 123,26 |
| | Height | 14 183,22 | 15 010,43 |

The inhibitory potency of chlortetracycline and that of chloramphenicol were generally stable with heat treatments despite slight fluctuations noted from one temperature to another. Statistical analysis does not show a significant difference in the evolution of the zones of inhibition induced by heated chloramphenicol. With regard to Figs 2, 3 and 4, it is plausible to assert that chlortetracycline, streptomycin, chloramphenicol partially degrade under the effect of heat treatment (100 ° C and 120 ° C), but their inhibitory capacity on the microorganisms remains. An increase in the concentration of oxytetracycline and penicillin residues in prepared meat and eggs (thermal cooking) constitutes a risk for the consumer, in particular the modification of the intestinal flora and allergies due to the new, more active molecules (Fabre *et al.*, 2000; Gysi, 2006).

**Table 7.** Parameters of the chromatograms of streptomycin, chloramphenicol, chlortetracycline at 35 ° C and at 120 ° C.

| | Parameters | 35°C | 120°C |
|---|---|---|---|
| Streptomycine | Retention Time (min) | 1,845 | 1,845 |
| | Area | 78 928,46 | 40 246,75 |
| | Height | 11 242,70 | 9360,60 |
| Chloramphénicol | Retention Time (min) | 2,81 | 2,81 |
| | Area | 4 853 722,66 | 2 788 079,33 |
| | Height | 619 789,40 | 377 225,65 |
| Chlortétracycline | Retention Time (min) | 2,44 | 2,44 |
| | Area | 232 890,51 | 148 825,81 |
| | Height | 45 098,91 | 25 103,05 |

The weak change in the concentration of chlortetracycline and streptomycin solutions (Fig. 4) and the overall stability of their inhibitory capacity under the effect of heat treatment (Fig. 5) suggests a low degradation of these molecules with heat treatments. This result corroborates that of Van Egmond *et al.* (2000), who reported low degradation and even stability of some antibiotic molecules after treatment at 130 ° C for 20 minutes. The effects of heat treatment on oxytetracycline found in this study corroborate those reported by Van Egmond *et al.* (2000) and Ramdane (2015).

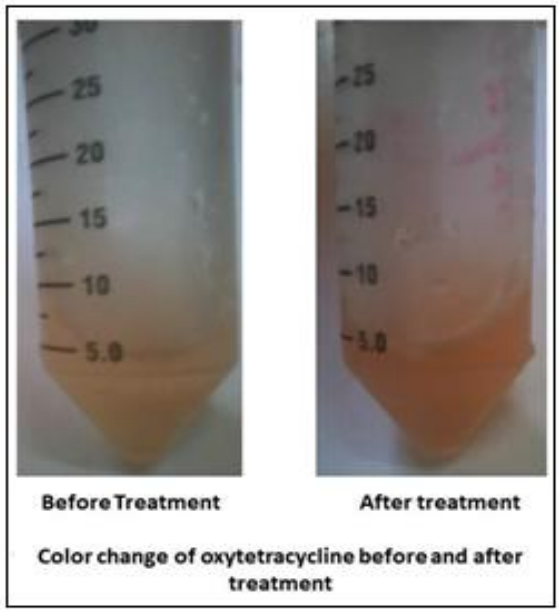


**Fig. 1.** Color change of oxytetracycline before and after heat treatment at 65°C.

Their studies showed that oxytetracycline subjected to 130 ° C for 20 minutes still had inhibitory properties. So pasteurization or sterilization, light frying and cooking of foods (milk, egg, meat) do not completely destroy the antibiotic residues that they may contain.

Comparative observations of the chromatograms of antibiotic molecules heated to 120 ° C and those kept at 35 ° C will allow a better appreciation of the impact of heat treatment on the antibiotic molecules.

*Chromatograms of antibiotics at 35 ° C and 120 ° C*

Fig. 5 below shows the chromatograms of the same oxytetracycline solution subjected to 35 °C and 120 °C for 30 minutes. Also, Table 5 below gives the parameters of the chromatogram at 35 ° C and 120 ° C.

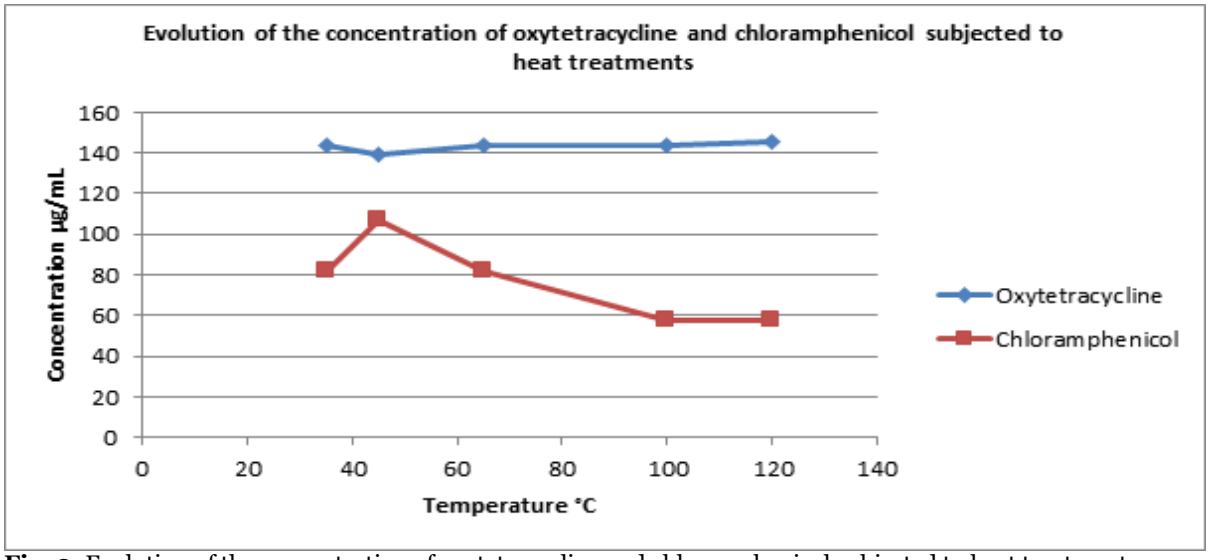


**Fig. 2.** Evolution of the concentration of oxytetracycline and chloramphenicol subjected to heat treatments.

The variation in retention times (RT) of the oxytetracycline molecule at 35 ° C and 120 ° C suggests a change in the conformation and/or cyclization of the molecule (Rouessac, 2004).

The peak area increases with temperature. The peak area is directly proportional to the absorbance and the concentration (Rouessac, 2004).

This increase in peak area with increasing temperature justifies the increase in the concentration of oxytetracycline found above. At 120 ° C for 30 minutes, the oxytetracycline molecule likely undergoes a conformational change and/or cyclization with an increase in its inhibitory capacity. Fig. 6 below shows the chromatograms of the penicillin solution subjected to 35 ° C and 120 ° C.

The parameters of the penicillin chromatograms at 35 ° C. and at 120 ° C. are given in Table 6 below. The variation in the retention times (RT) of the penicillin molecule at 35 ° C and at 120 ° C suggests a change in the conformation and/or cyclization of the molecule. The peak area increases with temperature.

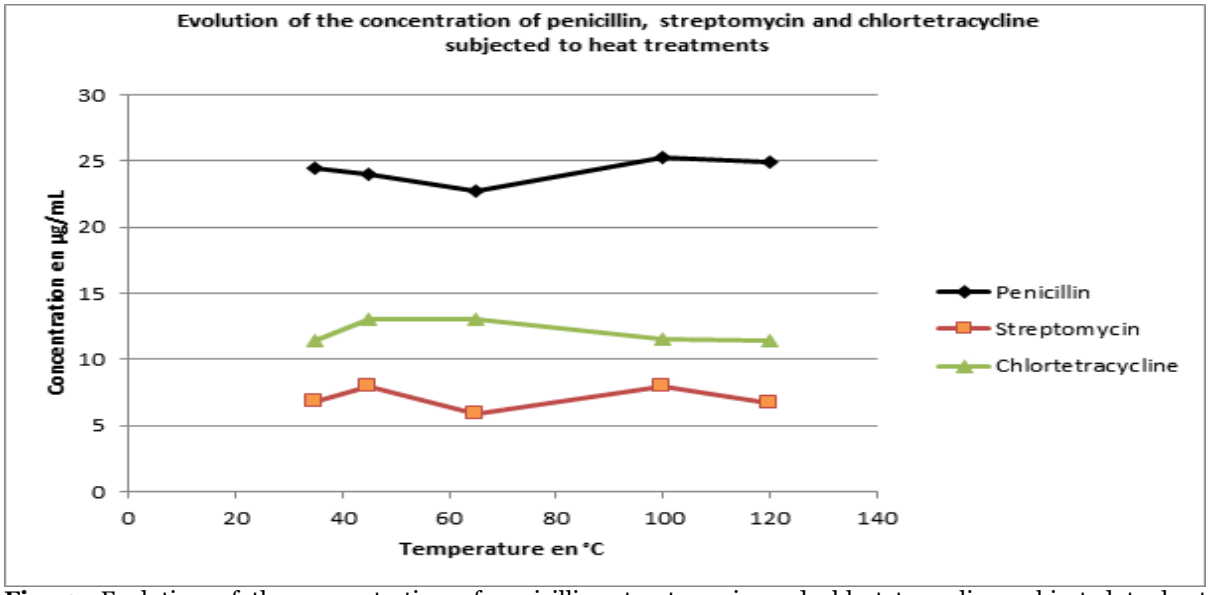


**Fig. 3.** Evolution of the concentration of penicillin, streptomycin and chlortetracycline subjected to heat treatments.

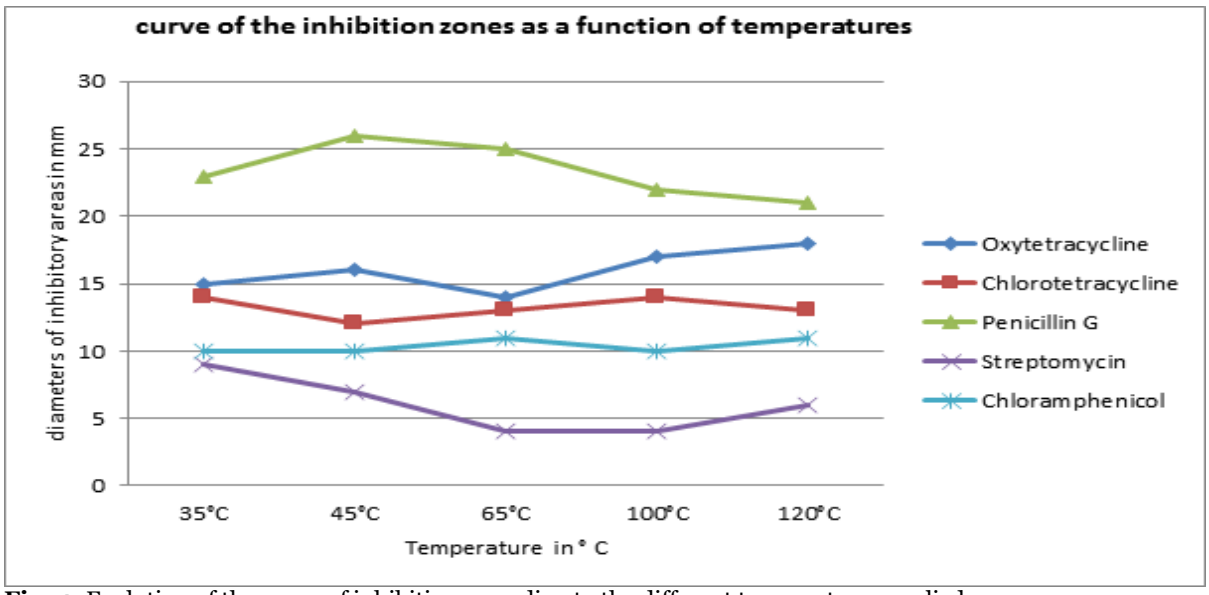


**Fig. 4.** Evolution of the zones of inhibition according to the different temperatures applied.

The peak area is directly proportional to the absorbance and the concentration (Rouessac, 2004). This finding is similar to that obtained by spectrophotometry, which concluded that the concentration increased with increasing temperature.

After heating at 120 ° C for 30 minutes, the penicillin molecule probably undergoes a conformational change with a decrease in its inhibitory capacity.

The effects of heat treatment on the streptomycin, chloramphenicol and chlortetracycline molecules seen on the chromatograms are very similar. Thus, the retention times (RT) of streptomycin, chloramphenicol, chlortetracycline at 35 ° C and 120 ° C did not change.

Also, the peak area of these molecules decreases at 120 ° C. Figs 7, 8, 9 below are the overlay views of streptomycin, chloramphenicol and chlortetracycline at 35 ° C and 120 ° C, respectively. Table 7 below gives the parameters of the chromatograms of streptomycin, chloramphenicol and chlortetracycline at 35 ° C and at 120 ° C.

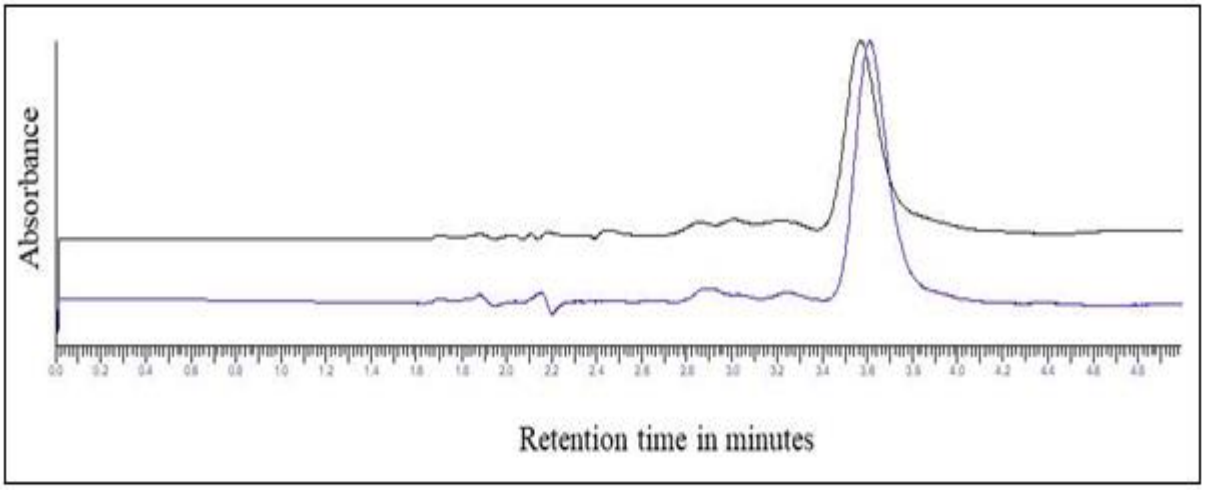


**Fig. 5.** Overlay view of oxytetracycline at 35 ° C (in black) and 120 ° C (in blue).

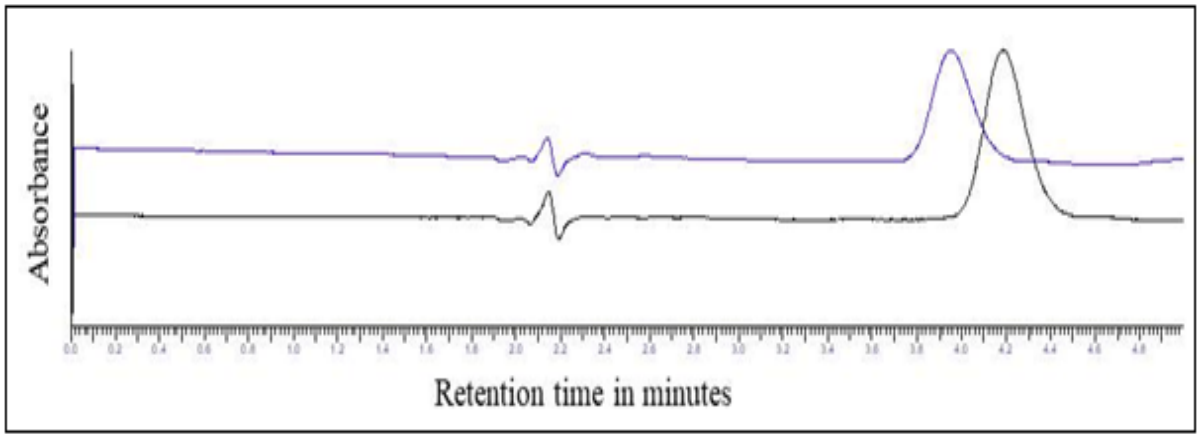


**Fig. 6.** Overlay view of penicillin at 35 ° C (black) and 120 ° C (blue).

The molecules of streptomycin, chloramphenicol, chlortetracycline did not undergo a change in conformation and/or cyclization after heating because their retention time remained the same at 35°C as at 120°C (Rouessac, 2004). However, the decrease in the peak area of the chromatograms of these antibiotics with increasing temperature is explained by partial degradation of these antibiotics due to heating.

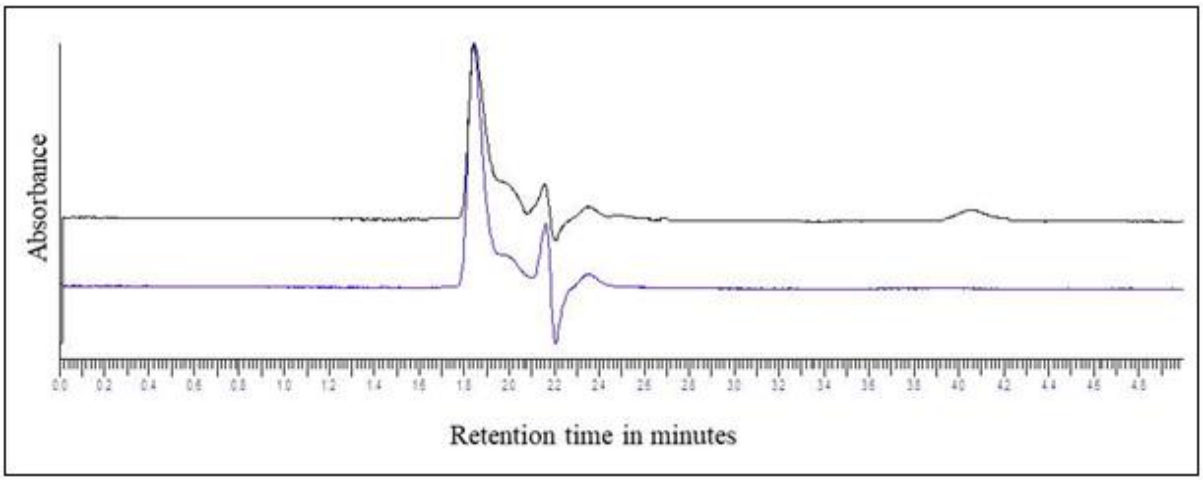


**Fig. 7.** Overlay view of streptomycin at 35 ° C (black) and 120 ° C (blue).

The degradation of streptomycin and chlortetracycline at 120 ° C is followed by a decrease in their inhibitory capacity. On the other hand, the inhibitory capacity of chloramphenicol remains stable despite its partial degradation at 120 ° C. This latter finding could be justified by a higher sensitivity of the test microorganism used (Geobacillus stearothermophilus) to chloramphenicol than to the two other antibiotics.

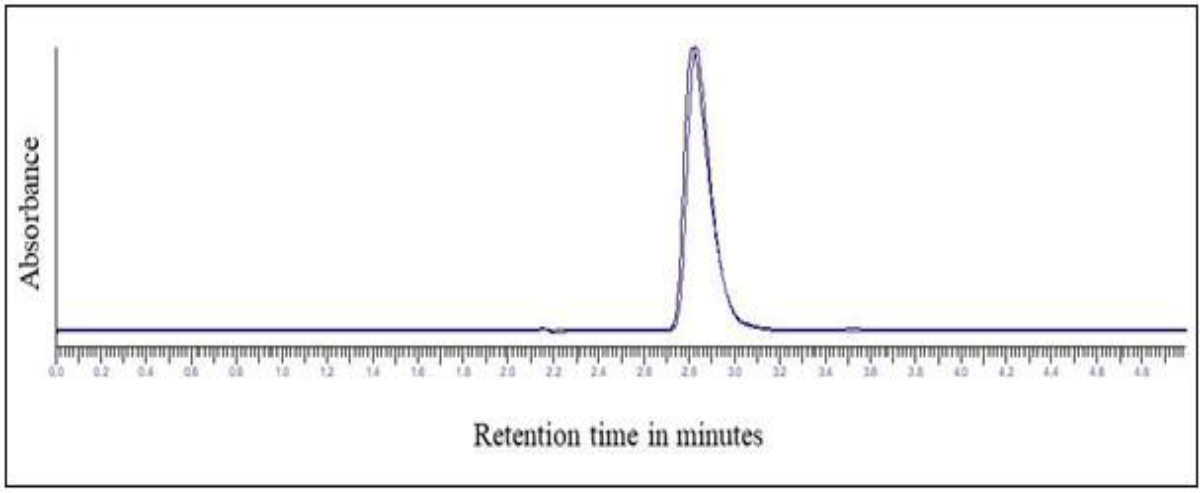


**Fig. 8.** Overlay view of chloramphenicol at 35 ° C (black) and 120 ° C (blue).

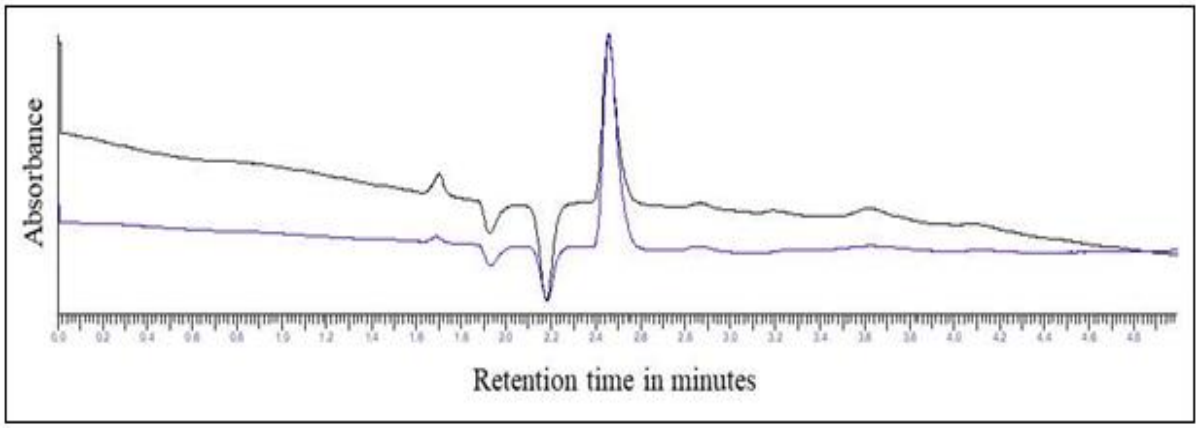


**Fig. 9.** Overlay view of chlortetracycline at 35 °C (black) and 120 °C (blue).

## Conclusion

Also, the effects of heat treatments on 5 antibiotics (oxytetracycline, chlortetracycline, penicillin G, streptomycin, chloramphenicol) were evaluated. It emerges from this evaluation that these antibiotic molecules are either partially degraded with an increase or decrease in their inhibitory capacity, or change of spatial conformation, or stable to different heat treatments. From one antibiotic molecule to another, the effect of the same heat treatment differs. The temperatures of cooking, pasteurizing and frying foods of animal origin would not remove antibiotic residues in these foods if they are present.

## Acknowledgment

We would like to thank the Laboratory of Biochemistry & Applied Chemistry (LABIOCA), of Burkina Faso, which allowed us to characterize certain parameters

## References

**Alambedji RB, Akakpo AJ, Teko-Agbo A, Chataigner B, Stevens B, Gadin B.** 2008. Contrôle des résidus : exemple des antibiotiques dans les aliments au Sénégal. *Conférence de l'Organisation mondiale de la santé animale sur les médicaments vétérinaires en Afrique, Dakar,* p 11.

**Biagui C.** 2002. Utilisation des médicaments vétérinaires en élevage avicole dans la région de Dakar ; qualité de la viande à travers la recherche de résidus de substances à activité antimicrobienne (Antibiotiques). *Thèse, École Inter-États des Sciences et Médecine Vétérinaires, Université Cheick Anta Diop de Dakar, Sénégal*, p 153.

**Fabre JM, Gardey L, Lherbette L, De Boisseson M, Berthelot X.** 2000. Détection des résidus de céfalexine dans le lait en cas d'allongement de la durée du traitement par voie intramammaire. Revue de Médecine Vétérinaire **151(10),** p 965-968.

**Gysi M.** 2006. Antibiotiques utilisés en production laitière en 2003 et 2004. Suisse. Agriculture **38,** p 215-220.

**Hao DN.** 2000. Report on real situation evaluation results and draff safe food production programme in Hanoi. *In Vietnamese,* p 49-60.

**Kabir J, Umoh VJ, Audu-Okoh E, Umoh JU, Kwaga JKP.** 2004. Veterinary drug use in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State, Nigeria. Food Control **15,** p 99-105.

**Kouakou NDV, Traore GC, Angbo-Kouakou CEM, Kouame KB, Adima AA, Assidjo NE, Grongnet JF, Kouba M.** 2015. Essai préliminaire de production d'œufs des poules pondeuses (ISA Warren) enrichis en acides gras polyinsaturés oméga 3 avec les graines de *Euphorbia heterophylla* L. International Journal of Biological and Chemical Sciences **9(4),** p 1902-1909. http://dx.doi.org/10.4314/ijbcs. v9i4.15

**Lederer J.** 1986. Encyclopédie modern de l'hygiène alimentaire. Paris. Nauwelaerts, p 289.

**Mensah SEP, Koudandé OD, Sanders P, Laurentie M, Mensah GA Abiola FA.** 2014. Résidus d'antibiotiques et denrées d'origine animale en Afrique : risques de santé publique . *Revue scientifique et technique - Office international des épizooties* **33(3).**

**Moretain JP.** 2005. Les résidus d'antibiotiques dans les aliments. Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants. *AFSSA, Fougères*, France, p 18.

**Nhiem DV, Peter P, Witaya S, Frans JMS, Moses NK, Maximilian POB, Karl HZ and Ngan PH.** 2006. Preliminary Analysis of Tetracycline Residues in Marketed Pork in Hanoi, Vietnam. Annals of the New York Academy of Sciences **1081,** p 534–542.

**Nys Y, Sauveur B.** 2004. Valeur nutritionnelle des œufs. Institut National de la Recherche Agronomique **17,** 385-393. https://hal.inrae.fr/hal-02681912/document

**Persoons D**. 2011. Antimicrobial use and resistance in Belgian broiler production. PhD thesis, Ghent University, Belgium.

**Ramdane MS.** 2015. Etudes qualitatives et quantitatives des résidus d'antibiotiques dans la viande de volaille et les œufs dans la région de la Mitidja. Utilisation des probiotiques comme alternative. Thèse de doctorat, Université de Mouloud Mammerie d' Algérie, p 159.

**Sanders P.** 2005. L'antibiorésistance en médecine vétérinaire : enjeux de santé publique et de santé animale. *Bulletin de l'Académie Vétérinaire de France* **158 (2),** 137–142.

**Tatsadjieu NL, Tanedjieu KS, Mbofung CMF.** 2009. Impact de l'utilisation des antibiotiques sur la sensibilité des bactéries pathogènes de poules dans la ville de Ngaoundéré. Cameroon Journal of Experimental Biology **5(2),** 52–61.

**Van Egmond HJ, Nouws JFM, Schilt R, van Lankveld-Driessen WDM, Streutjens-van Neer EPM, Simons FGH.** 2000: Stability of antibiotics in meat during a simulated high temperature destruction process. Euro Residue IV **29,** p 430–437.