

Effect of Cooking Temperature on Residual Oxytetracycline Hydrochloride in Cooked Chicken

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Abstract

The concentration in samples cooked at 115 ° C is of 73.71 µg/mg either 49.40% reduction and at 145 ° C is 66.03 µg/mg representing a 54.68% reduction. Bake at 145 ° C during 20 minutes is enough to destroy almost 55% of the initial quantity of residues in chicken hence HCL OTC residues are degradable thermo.

Keywords: *antibiotics, residues, livestock, chicken meat*

Introduction

The aim of this study is the detection and the quantification of «Oxytetracycline Hydrochloride» antibiotic residues in cooked chicken meat and the heat effect on its molecule.

Few works described the thermal analysis of these antibiotics. These include the thermostability of oxytetracycline, tetracycline and doxytetracycline at high and ultrahigh temperatures (110–140°C), showing a first order reaction kinetic (Hassani *et al.*, 2008).

Oxytetracycline Hydrochloride (OTC HCl, C₂₂H₂₅ClN₂O₉) is commonly used in poultry farming for preventive and curative treatment but mainly as a growth factor in Algeria.

Tetracycline compounds (TCs) are common antibiotics used for human therapy, for veterinary purposes and as agricultural feed additives (Coustès, 2016, Li *et al.*, 2011).

However, the misuse of this compound may result in its presence in chicken meat, especially if waiting times are not respected, and may therefore pose a threat to the consumers health.

Moreover, the abundant and improper use of TCs may result in the presence of their residues in edible animal tissues, which can be toxic and potentially cause allergic reactions (Cetinkayaa

et al., 2012). It is recalled that the maximum residue limits (MRL) for oxytetracycline (OTC) are 100 µg.kg⁻¹ for chicken muscle (EU Regulation 37/2010) (Shahid *et al.*, 2007).

Material and methods

Analysis of turbidity

The detection of OTC HCl residues in fresh and turbidimetric chicken is based on the following principle: the microorganisms growth in a clear liquid medium (meat juice) containing a constant concentration of antibiotic residues will result in by a «change in the environment turbidity». This change in turbidity or absorbance is measured using a spectrophotometer. The recorded absorbance is determined by the number of microorganisms present in the solution. Thus, the decrease in absorbance reflects the effect of the antimicrobial substance on the bacterial population present in the sample. The time required to achieve a certain absorbance under well-defined conditions may be proportional to the initial load of micro-organisms inoculated into the medium. Absorbance is measured at 530 nm (Jorgensen and Schulz, 1985). The turbidimetry technique requires a basic step which is the

extraction of the antibiotic contained in the meat, before reading on the spectrophotometer.

Analysis by HPLC

The quantification of the content of OTC HCl residues of fresh and cooked chicken by reverse phase HPLC is based on the following principle: the sample is associated with a solvent which passes under pressure a column containing what is called the stationary phase, a gel that has the property of retaining the molecules that pass through it. Depending on the affinity between the compounds of the sample and the stationary phase, these compounds will be retained more or less long in the column before coming out and being measured. A detector continuously monitors the liquid at the outlet of the column to detect the presence of the compounds. The most common is a UV-visible spectrophotometer, which measures the absorption of light by the product. The detector is linked to a computer that can display a quantization graph as a function of time. Each type of molecule transported by the mobile phase will appear as a peak in the graph whose area allows to quantify the quantity of these molecules (Meyer, 2010).

The column used is composed mainly of silicas grafted by linear chains of 18 carbon atoms (C18). This phase is apolar (hydrophobic) and therefore requires a polar solvent to convey the sample (acetonitrile, methanol, water). The polar compounds will be eluted first and the apolar (hydrophobic) compounds will be retained (Meyer, 2010). OTC HCl is a good example of these compounds (Aggarwal, 2015). The residues extraction from the meat is also required in the HPLC analysis. A volume of 20 μ L of standard OTC HCl solution and from each sample is injected.

Kinetics of the molecule

OTC HCl heating: a volume of 50 mL of OTC HCl (0.6 mg.mL⁻¹) is poured into 5 test tubes. Each tube is placed in a « bain-marie » preheated to a temperature and heating time according to Table 1.

Study of the OTC HCl molecule stability after heat treatment using the Agar diffusion method

A filter paper disk containing amounts of antibiotic is placed on a solid medium which has been widely seeded with test organisms. After incubation, the diameter of the clear inhibition

Table 1: Temperatures and heating times of OTC HCl solutions

Tube	Volume (mL)	Heating temperature (° C)	Heating time (min)
Control			
1	10	45	60
2		60	55
3		85	50
4		100	40
5		120	30

zone surrounding the disc is used to measure the antibiotic inhibitory potency against the test organism (Jawetz *et al.*, 1999). In this experiment, the aim is to study the heat effect on the inhibitory activity of the OTC HCl molecule by heating at different temperatures for specific times, which results in variations in the inhibition zone diameters.

Biological material

Strains: suspension of *Staphylococcus aureus* (ATCC 6538 P) is supplied by the laboratory.

Meat: 20 broilers, 35 days old, are taken from a farm in Medea (Algeria). Fifteen of them are isolated and put in a cage. OTC HCl is intentionally introduced into their diet at high doses (500mg) for 5 days (Protocol: 20 mg of oxytetracycline by kg of body weight per day for 3 to 5 days in drinking water, or about 400 mg of powder for oral solution per litre of drinking water). The waiting time was not respected to ensure the presence of residues in the muscle. It is to be on to find this residue of antibiotics in meat and see the effect of cooking on this molecule of oxytetracycline. It's from 35 days the chicken can be sold so the meat eating can hold at this age there.

The hygienic conditions of slaughter, evisceration and cutting have been respected in order to avoid any risk of contamination. The chicken part of interest is the wishbone. Ten wishbones of the treated animals are cooked: 5 in a pressure cooker at 115°C for 35 min (S(+)₁₁₅), the others in an oven at 145°C for 20 min (S(+)₁₄₅). A negative sample S(-) and a positive sample S(+) without heating were also used. Fifty grams portions of each fishbone are minced under sterile conditions. The samples, distributed as indicated in Table 2, are placed in sterile boxes at 4°C.

Table 2: Sampling

Sample	Quantity (g)	Cooking temperature (°C)	Duration (min)
S(-)	250		
S(+)	250		
S(+) ₁₁₅	250	115	35
S(+) ₁₄₅	250	145	20

Results of turbidimetric detection

The visual appearance of the solutions after incubation is presented in Figure 1.

This figure shows a difference in the solutions turbidity which intensifies when the antibiotic concentration in the mixture is low. The nearly unnoticed disorder in the S(+) and S(+)₁₁₅ solutions shows the presence of residues in the meat of large quantities treated animals.

The average absorbance values recorded in two tests are presented in Table 3.

The lowest absorbance value of the S(+) solution have confirmed the presence of a high antibiotic concentration in the positive sample. Conversely, the highest absorbance value of the S(-) solution have confirmed the absence of antibiotic in the sample. The comparison of the absorbance of the S(+)₁₁₅, S(+)₁₄₅ and S(+) solutions have shown that the heat has an effect on the concentration of antibiotic present in the muscle. Indeed, the concentration of this ATB versus absorbance decreases as the cooking temperature increases. The concentration in these

last two samples is less than 100 µg.mL⁻¹ since their absorbance is relatively high compared to that of the standard OTC HCl solution (100 µg.mL⁻¹).

The absorbances of the samples S(+), S(+)₁₁₅, S(+)₁₄₅ and standard OTC HCl solutions decreased because of the continuous inhibitory action of the antibiotics against the germs in these solutions. On the other hand, for the negative sample, the absorbance value has increased, so the germs can always multiply in the absence of antibiotic.

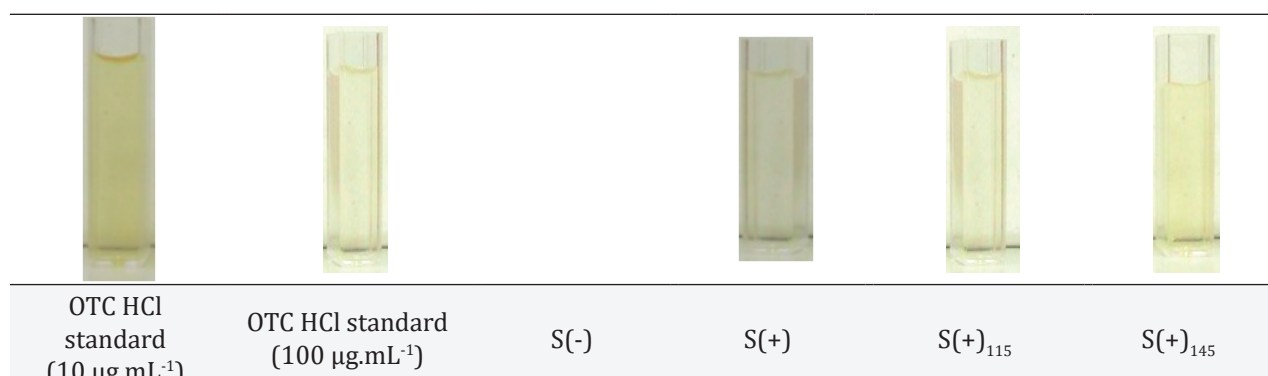
Quantification results by reverse phase HPLC

The following figures represent the peaks obtained after injection of the standard OTC HCl solution and from solutions containing the positive samples and the negative sample.

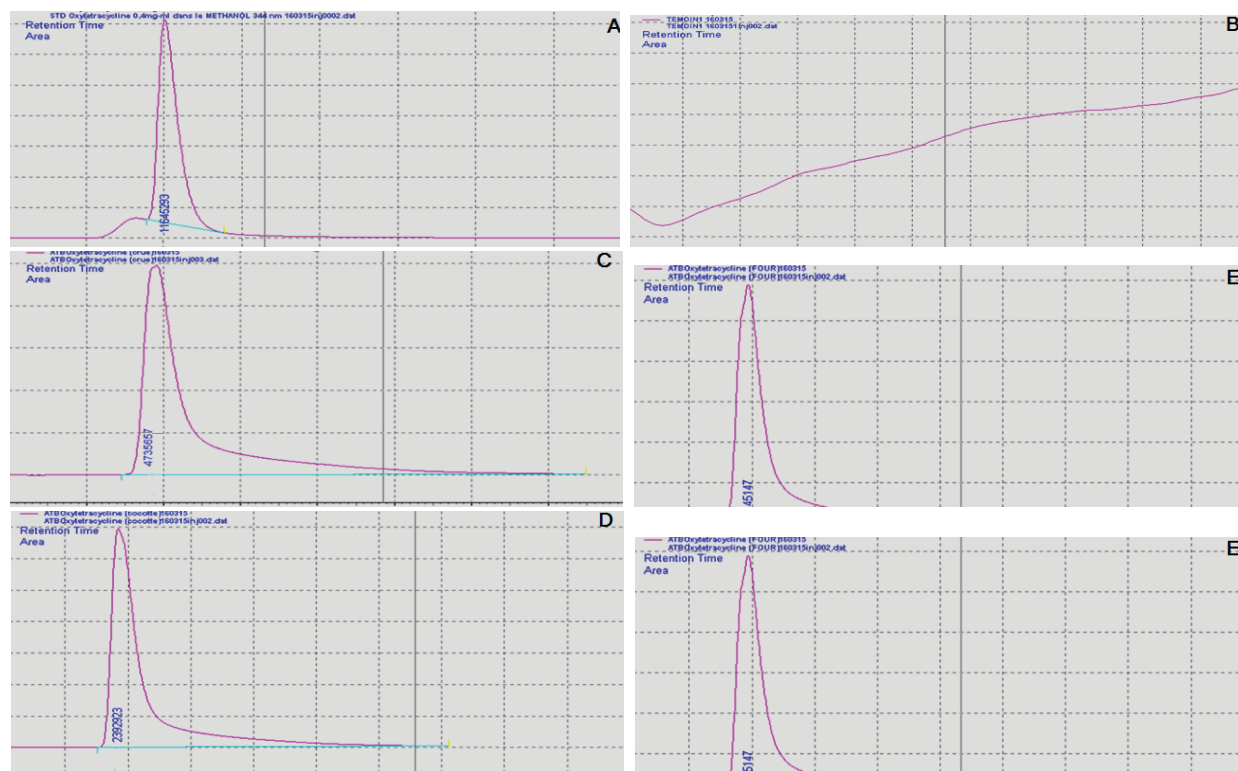
The injection of the standard solution OTC HCl (0.4 mg.mL⁻¹) has given a peak in the chromatogram after 2 min of analysis (Figure 2A), which is consistent with the OTC HCl analysis parameters mentioned in « Material and methods » paragraph. The area of this peak is 11645 293. No peak was recorded (Figure 2B) by injecting the S(-) solution which confirms the absence of the antibiotic in the negative sample. The S(+) solution (Figure 2C) has given a peak, area 4735 657, after 1.97 min of analysis. This is consistent with the OTC HCl assay

Quantification of the OTC HCl residues

The samples residues levels are calculated according to Pananookooln (2007)

**Figure 1: Samples to be analyzed after incubation****Table 3: Samples absorbances**

	OTC HCl standard (10 µg.mL ⁻¹)	OTC HCl standard (100 µg.mL ⁻¹)	S(-)	S(+)	S(+) ₁₁₅	S(+) ₁₄₅
Absorbance	0.098	0.031	0.374	0.020	0.049	0.067



Figures 2: A :OTC HCl standard chromatogram, B : Negative sample S(-) chromatogram, C : Positive sample S(+) chromatogram, D : Positive sample heated at 115°C S(+)₁₁₅ chromatogram, E : Positive sample heated at 145°C S(+)₁₄₅ chromatogram parameters and confirms the presence of the antibiotic in the analyzed sample. The S(+)₁₄₅ solution contains an OTC HCl amount which has given a peak (Figure 2D), area 2392 923, after 1.93 min of analysis. The S(+)₁₁₅ solution contains an OTC HCl amount which has given a peak (Figure 2E), area 2145 147, after 1.98 min of analysis.

Table 4: Data for the calculation of the residues concentrations (areas, weights and volumes)

	S(-)	S(+)	S(+) ₁₁₅	S(+) ₁₄₅
A _{Ech}		4 735 657	2 392 923	2 145 147
V _{Ech} (mL)	6	6	6	6
M _{Ech} (mg)*	6 934	6 926	6 917	6 922

* Mass of the solution = mass of the minced meat + total mass of solid reagents + mass corresponding to the total volume of the solvents (density*volume)

Table 5: Quantification of OTC HCl residues

	S(-)	S(+)	S(+) ₁₁₅	S(+) ₁₄₅
Cooking temperature (°C)			115	145
Duration (min)			35	20
Quantity (µg.g ⁻¹)		145.7	73.7	66.0
Residues destroyed by cooking (%)			49.4	54.7

$$C_r (\mu\text{g} \cdot \text{mg}^{-1}) = \frac{A_{\text{Ech}} \times V_{\text{Ech}} \times C_{\text{Std}} \times P_{\text{Std}}}{A_{\text{Std}} \times M_{\text{Ech}}}$$

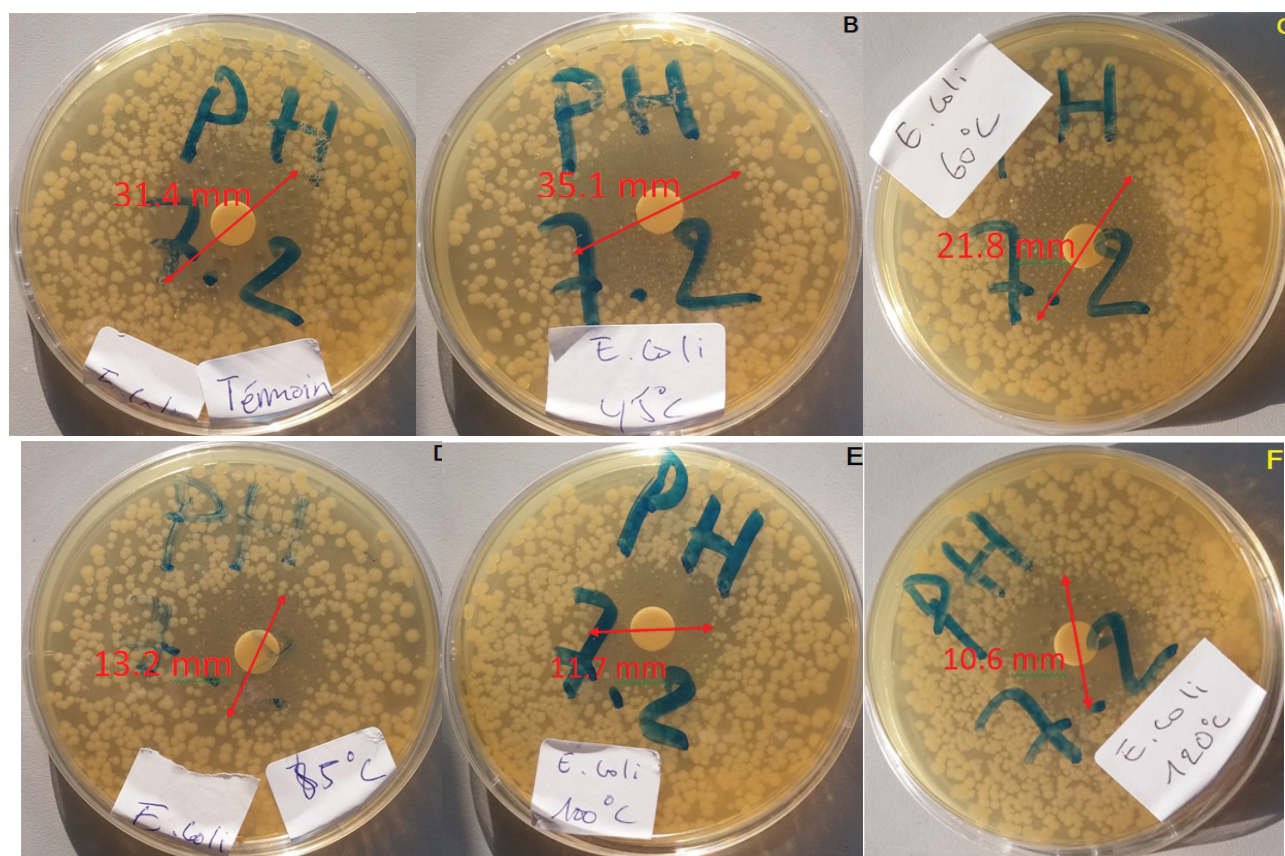
C_r: amount of residues in the sample (µg.mg⁻¹)

A_{Ech}: peak area of the injected sample solution

V_{Ech}: volume of the injected sample solution (mL)

C_{Std}: concentration of the OTC HCl injected standard solution (mg.mL⁻¹)

P_{Std}: standard power OTC HCl (µg.mg⁻¹)



Figures 3: Inhibition zones diameters after incubation. A: Control (31.4 mm), B: Inhibition zone (45°C, 35.1 mm), C: Inhibition zone (60°C, 21.8 mm), D: Inhibition zone (85°C, 13.2 mm), E: Inhibition zone (100°C, 11.7 mm), F: Inhibition zone (120°C, 10.6 mm)

A_{Std} : peak area of the OTC HCl injected standard solution

M_{Ech} : mass of the sample solution

$C_{Std} = 0.409 \text{ mg.mL}^{-1}$

$A_{Std} = 11645293$

$P_{Std} = 1011.58 \text{ } \mu\text{g.mg}^{-1}$

The collected data for the residue levels calculation (areas, weighings and volumes) are given in Table 4 and the results in Table 5.

The peak area of the S(+) solution has shown the existence of a very high concentration of OTC HCl residues in raw chicken ($>140 \text{ } \mu\text{g.g}^{-1}$), that is explained by the strong doses of medicines given to animals before slaughter. The residues levels in the S(+)₁₁₅ and S(+)₁₄₅ have confirmed the result of the detection, the decrease of the residues amount was noticeable: 49.4% and 54.7% for the S(+)₁₁₅ and the S(+)₁₄₅ samples respectively.

Results of the OTC HCl molecule kinetics after heat treatment by the Agar diffusion

The inhibition zones diameters after incubation are visualized in Figures 4A to 4F.

The diameters variation relative to control (in %) is given in Table 6.

Inhibition zone with a diameter $\geq 2 \text{ mm}$ is considered as positive (AFNOR, 2011). The control inhibition zone has given 31.4 mm. Heating the OTC HCl at 45°C for 1 hour has given a diameter larger than that of the control solution, indicating that increasing the ambient temperature can maximize the antibiotic inhibitory effect. On the other hand, heating at $T > 60^\circ\text{C}$ during more than 30 min has allowed the diameter (inhibitory effect) to be gradually reduced up to 66.2%. hence, it is easy to conclude that the OTC HCl is thermo-degradable and a treatment at 85°C for 50 min is sufficient to destroy more than half of its initial amount.

Discussion

In view of the results, the turbidity of the negative sample solution has shown that it did not contain antibiotic residues. However, the uncooked positive sample has shown a slight disturbance, evidence of a large quantity of

Table 6: Inhibition zone diameters according to temperature and heat exposure duration

	Control	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
T (°C)		45	60	85	100	120
Duration (min)		60	55	50	40	30
Diameter (mm)	31.4	35.1	21.8	13.2	11.7	10.6
Variation (%)		+ 11.8	-30.6	-58.0	-62.7	-66.2

antibiotic. For $S(+)$ ₁₁₅ and $S(+)$ ₁₄₅ samples, the cloudiness is much less obscure than that of the negative sample, evidence of antibiotic levels $<100 \mu\text{g.mL}^{-1}$. One explanation is that cooking (heat) has affected the initial antibiotic content of these samples, so the inhibitory effect decreased by destruction or transformation of the OTC molecule. To date, there are no similar studies that have used the turbidimetry technique in that sense. In reverse phase HPLC, the plots of the positive meat samples gave peaks consistent with the detection parameters of the OTC HCl antibiotic. No peak was recorded for the negative sample which has confirmed that it was free of residues. There have been a decrease of residues concentrations between the uncooked and the cooked samples, - 49.4% and -54.7% at (115°C, 35 min) and (145°C, 20 min) respectively (Table 5). This highlights the noticeable effect of cooking on the OTC HCl molecule. According to Ibrahim and Moats (1994), cooking for more than 30 min at 100°C completely degrades OTC residues. Our results have shown that cooking at 115°C for 35 min destroys almost 50% of the residues, which is consistent with the results of Van Egmond *et al.* (2000) who have found an incomplete residues destruction (53%), and their relative stability even if they undergo a cooking procedure at 130°C for 20 min. Nguyen *et al.* (2014) have found that cooking at 100°C for 15 min reduces the meat OTC content by 43% and generates a degradation product, α -apo-OTC, stated inactive and with no danger to the body. Both boiling and microwave treatments resulted in significant changes in OTCs and apo-OTCs concentrations. The formations of apo-OTCs, as well as reductions of OTCs, were dependent on the time of thermal treatments (Nguyen *et al.*, 2015). According to Abu-Raya *et al.* (2013), OTC residues are thermally unstable and a proper meat cooking over 140°C for 20 min can

avert the hazard what is similar to our results, baking at 145°C for 20 min having decreased the initial amount of residues by almost 55% and according to Ezenduka *et al.* in 2018 more than 70% of initial concentration destroyed after heating at 100°C for 40 min. In the heat treatment (Agar diffusion method), the gradual change in color probably indicates a change of the molecule in its three-dimensional structure or a fractionation into other compounds which is actually advanced by Kuhne *et al.* (2001).

The higher inhibition zone produced by the heated solution at 45°C relative to control (+11.8%, Table 6) shows that the inhibitory effect is more important at this temperature. The zones shrink and the inhibitory effect decreases above 60°C. The inhibition zones diameter decreases gradually as the temperature increases and the cooking time decreases, reaching - 66.2% relative to control inhibition zone. These variations have highlighted the negative correlation between the HTC OTC inhibitory activity and the temperature.

Hassani *et al.* (2008) stated that heating above 100°C for quite long periods (30 to 40 min) decreases the initial concentration but with negligible percentages ($\geq 2\%$), they suggest that high temperature treatments (UHT) are more effective than household cooking procedures in eliminating the risk of antibiotic residues in meat. In contrast, Hsieh *et al.* (2011) founded that a contact heating temperature for 35 min reduced by 60.5% the OTC inhibitory effect, percentage comparable to 62.7% of initial concentration destroyed after heating at 100°C for 40 min in our study. Kuhne *et al.* (2001) have found that the tetracycline (TTC) effect can increase after heat treatment at 100°C, which is due to the appearance of a degradation product, anhydro-TTC, more toxic than the parent compound, but which also degrades into inactive compounds, epianhydro-TTC over 120°C. This

was the case in our study, the heating of OTC HCl at 45°C increasing by more than 11% the inhibitory effect, but which gradually dropped as the temperature continued to rise (Ambrozini *et al.* 2016).

Our results are in agreement with those of Van Egmond *et al.* (2000), Abu-Raya *et al.* (2013), Nguyen *et al.* (2014) and Hsieh *et al.* (2011), and have confirmed that OTC HCl underwent significant but not complete degradation when heated. These results, however, are in contradiction with those of Ibrahim and Moats (1994).

In view of the kinetics results and those of the Agar diffusion method, it should be noted that the heat began to act positively on the molecule then negatively as the temperature increases. Indeed, the temperature first has acted positively on the inhibitory effect, its level being suitable for the germs development. Then, the inhibitory effect decreased when this one increased and beyond 100°C, the effect was greatly reduced, cause of the OTC molecule destruction in apparently more toxic elements. The fact remains that the molecule and its transformation effects must be the subject of further study to provide answers to many questions which remain in suspense.

Conclusion

Within sight of the results of the kinetics and those of the method in diffusion on agar, it should be noted that heat acts on the molecule on levels of temperature positively at the beginning then negatively as the temperature augment. Indeed temperature A acts positively on the inhibiting effect corresponding to a suitable temperature with the development of the germs. Then the inhibiting effect decreases when the temperature augment and beyond 100°C the effect is largely tiny room because of the destruction of the molecule of the OTC in elements apparently more toxic; But to become to it molecule and the effects of its transformation must be the subject of a thorough study in order to bring brief replies to much of questions.

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