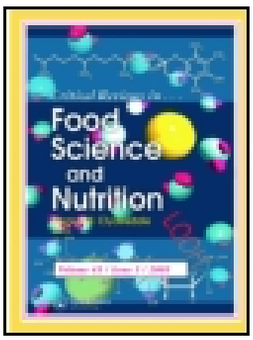

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Effect of thermal treatments on the degradation of antibiotic residues in food

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Abstract

The thermal stability of antibiotics commonly detected in food is reviewed. To quantify degradation, two major techniques have been reported: liquid chromatography-based methods and microbiological tests. As the degradation products may also display some antimicrobial activity, microbiological tests may not be considered accurate analytical methods for quantifying antibiotic residues' degradation. Degradation percentages are summarized for

different antibiotics and for various media (water, oil, milk and animal tissues). Studies presented in the literature confirm that the thermal degradation of β-lactams, quinolones, sulfonamides and tetracyclines can be described using a first-order kinetic model. Degradation rates, k, derived for this model for liquid matrix (water) at 100°C, followed the general trend amongst antibiotic classes: β-lactams = tetracyclines (most heat-labile) > lincomycin > amphenicols > sulfonamides > oxfendazole > levamisole (most heat-stable). Although thermal processing results in a decrease in the concentration of parent antibiotic residues, degradation by-products have not been properly characterized to date. As some of these products were shown to be hazardous, further investigation is needed to determine their impact on food safety and human health. It is therefore currently difficult to definitively conclude whether or not antibiotic degradation during food processing is necessarily beneficial in terms of food safety.

Key words

veterinary drugs, cooking, dietary exposure, food safety

1. Introduction

Antibiotics are used for medical treatment for both humans and animals, as well as for growth promotion in animal production. Major classes of antibiotics used in animal farming include tetracyclines, macrolides, sulfonamides and penicillins (Wang and Ma, 2008; Liu et al., 2014; FDA, 2014). Veterinary drug administration to animals, typically through animal feed, leads to residues of these antibiotics remaining in the animal tissue that is to be consumed. Furthermore, these residues may also be left in the environment such as water and land, after which they could be absorbed by other animals and human beings (Sarmah et al., 2006). As a result, antibiotic residues are regularly detected in many food categories including meat, eggs and seafood products (Donkor et al., 2011; Done and Halden, 2014). Some antibiotic residues can trigger adverse effects on human health including allergic reactions in hypersensitive individuals or the change of R+ enteric organisms' amount in human body (Levine, 1960; Franco et al., 1990). Besides potential direct effect on human health, the widespread use of antibiotic residues in food may also cause antibiotic resistance, an emerging public health issue of global concern

(McDermott et al., 2002; Grundmann et al., 2006). For this reason, regulations are in place to set the Maximum Residue Limits (MRLs) of antibiotic residues permitted in food products. However，MRLs apply to the quantity of residues in the raw food commodity, without systematically considering the changes that occur during processing. As most foods of animal origin are typically consumed after cooking or processing, knowing the effect of different thermal treatments on the residues is essential when assessing human exposure, determining MRLs, and evaluating toxicity. Shahani et al. (1956) first reported the effect of heating on penicillin G in water and milk. Since then, various studies have investigated the thermal stability of antibiotics under different heating conditions such as domestic cooking, commercial pasteurization and canning. The aim of the present paper is to review this literature to get a better understanding of the degradation of antibiotic residues in food and the implications for food safety. In particular, information on the degradation kinetics of antibiotics was compiled to assess the relationships between the degradation of antibiotics and processing time and temperature. Studies on the influence of other parameters (e.g. initial concentration of residue in

the raw food, the pH and the presence of additives) are also discussed. Finally, this paper reviews the current knowledge on the mechanisms of structural degradation and the identity of all the degradation products.

2. Material and methods

2.1 Selected literature

The scientific literature (1956-2015) was screened for information about the degradation of antibiotic residues in food using combination of keywords such as "*antibiotics"* (or the name of the chemical), "*cooking"*, "*thermal degradation"* and "*food."* Available literature published in Chinese was also screened. A total of 105 papers were evaluated out of which 84 papers are presented in this review.

2.2 Degradation percentage (DP) and degradation rate constant (k)

Quantitative information about the disappearance (degradation) of the antibiotic residue is mostly available in the literature through parameters such as degradation percentages (DP) or degradation rate constants (k).

For the biological test, the degradation was measured by the change of antimicrobial activity such as the minimal inhibitory concentration (MIC) and the inhibition zone diameter (detailed in section 3.1).

For the analysis based on chromatography, degradation percentages are calculated according to Equation (1), where C_0 and C_{final} are the concentrations of the chemicals before and after heating respectively. These concentrations are measured using various tools as discussed in section 3.1, and should account for the change in food weight during cooking (e.g. water loss). While the degradation percentage is relatively simple, it does not integrate any kinetic or mechanistic considerations. As a result, direct comparison of degradation percentages across studies is often inappropriate as experimental conditions (time, temperature, etc) are generally different.

$$Degradation\ percentage(\%) = \left(1 - \frac{C_{final}\left(corrected\ by\ weight\ change\right)}{C_0}\right) \times 100 \quad \text{(Equation 1)}$$

Alternatively, comparison of the various degradation experiments can be derived using the degradation rate constant k. To date, degradation rates in food have been calculated only in a few studies (Fuliaş et al., 2010; Roca et al., 2010; 2011; 2013).

Following the hypothesis that the degradation of antibiotic compounds follows a first-order model, k was first applied in studying antibiotic residues by using the following equation (Fuliaş et al., 2010) based on the equation developed by Martin (1993):

$$\frac{\partial[C]}{\partial t} = -k * [C] \quad \text{(Equation 2)}$$

where the t is the heating time and [C], the concentration of each compound in the sample at a specific point in time t. The integration of Eq. (2) leads to:

$$ln[C] = ln[C_0] - k \times t \quad \text{(Equation 3)}$$

where C_0 is the initial concentration of the antibiotic. Eq. (2) can be rewritten as:

$$k = \frac{ln[C_0] - ln[C]}{t}$$ (Equation 4)

The k value can then be computed for each antibiotic from experimental data. The examination of k values allows for a clearer comparison of the stability of antibiotics in food across studies for a specific set of conditions (e.g. for a particular temperature or a specific food matrix), independently of time. The suitability of the first-order model is discussed in section 3.3.1. To date, there is no sufficient kinetic data available for macrolides, aminoglycosides, amphenicols and lincomycin, and in this paper we hypothesized that they also obey the first-order kinetic model for the purpose of comparison across studies.

Some studies expressed the degradation kinetics using the D value, which is the amount of time required for one log reduction in the concentration of residues. D values are related to k values according to:

$$k = \frac{\ln(10)}{D}$$ (Equation 5)

2.3 Statistical analysis

k values were calculated using Microsoft Excel. For statistical analysis, t-test is employed and conducted by JMP statistical software (SAS Institute, North Carolina). $P<0.05$ is treated as significant.

3. Results and Discussion

3.1 Experimental assessment of antibiotic degradation

3.1.1. Experimental design of degradation studies

Generally, liquid samples (except those in water or salt water) were pretreated through deproteinization or extraction by solvent before analysis, while solid samples were always extracted by solvents and then analyzed by chromatography or MIC test to detect the concentration change of antibiotic residue. In solid samples that were treated by baking, microwave heating or frying, the water loss was taken into account by most of the authors for the correction of final results. For boiling samples, the boiling water was also analyzed. Sometimes, the experiment cannot be controlled well due to the specificity of grilling (e.g. the loss of juice during grilling was uncollectible) (Cooper et al., 2011).

In addition to the limitations of detection and weight loss, the procedures of experiments also present limitations in terms of achieving accurate results. Reported cooking temperatures for solid matrices do not, in most cases, reflect the actual temperature antibiotics are exposed to. This is because in most cases, the temperature in cooked solid matrices is not homogenous in comparison to liquid matrices. Furthermore, antibiotic residues may be unevenly distributed in treated animals (Moats, 1999). Even in the same body part or organ, the residue concentrations vary at different points (O'Brien et al., 1981). To achieve the homogeneous residue distribution, some studies used ground tissues before heat treatment (Moats, 1999), but this does not accurately represent real domestic cooking or commercial food processing. Thus, data for solid matrices are rejected from the comparison of k values.

3.1.2. Quantification of antibiotic degradation

The degradation of antibiotics is generally assessed by measuring the change in either the antimicrobial activity or the concentration of parent antibiotic residue in the food or the food extracts using analytical techniques based on chromatography.

Earlier studies used microbiological assays to determine the reduction in veterinary drug residues. These assays determine the difference in microbial activity before and after treatment, from which a degradation percentage in the biologically active compound was calculated. Tests such as the minimal inhibitory concentration test (MIC) (Traub and Leonhard, 1995) and the inhibition zone diameter (Javadi, 2011) have been applied to detect the changes of antimicrobial activity. Shit et al. (2008) reported the use of Delvotest ®(DSM, the Netherlands), a commercial test kit for antibiotic residue in food, to detect the presence of furazolidone in cooked chicken tissue based on the bioactivity. However, the Delvotest ® can only test the presence/absence of active antibiotics but not the actual concentration of antibiotic residues. As the degradation products of antibiotics may be bioactive, biological tests may not always reflect the real concentration of the parent compound (Traub and Leonhard, 1995; McCracken and Kennedy, 1997). The utilization of techniques based on chromatography or a combination of microbial methods with chromatography allowed for more accurate results (Franje et al., 2010; Hsieh et al., 2011).

As the use of chromatographic analysis became more widespread, liquid chromatography (HPLC and more recently UHPLC) coupled to a range of detectors started to become the method of choice for the quantification of antibiotic concentrations in food. LC-based analysis first requires the extraction of the target analyte from the food matrix. Liquid food matrices such as milk or food juice can be extracted using solid-phase extraction (SPE) (Sun et al., 2010). Solid food matrices are usually extracted with solvent and/or buffer mixtures optimized to obtain good extraction recoveries (Ridgway, Lalljie and Smith, 2007). For example, the standard extraction solvent utilized in tetracycline analysis is a McIlvaine-EDTA buffer system (Anderson, Rupp et al. 2005), although other solvents such as methanol, citrate buffer, and trichloroacetic acid are sometimes used. Eventually, target antibiotics in the extract are quantified using HPLC coupled to detectors such as UV-Vis detectors (including diode array detectors), fluorescence detectors or mass spectrometry (Joshi, 2002). Many studies reported the use of HPLC coupled with UV-Vis or fluorescence detection. While these instruments allow

for the quantification of the parent antibiotic in food extracts, they seldom allow for the unambiguous identification of the degradation products.

In the recent years, liquid chromatography coupled with mass spectrometry, notably tandem mass spectrometry, became the gold standard for antibiotic residue analysis, allowing in some cases for the identification of degradation products (Bogialli and Di Corcia, 2009). Thus, McCracken and Kennedy (1997) identified the metabolite of furazolidone residue (AOZ) in pig meat by using HPLC-thermospray mass spectrometry. Grunwald and Petz (2003) explored the degradation of four penicillins in milk by using LC-UV and LC connected to a Thermo Finnigan TSQ 7000 triple quadrupole mass spectrometer. Such an approach helped determine that penicillin G degrades into penillic acid and that cloxacillin degrades into penillic, penilloic and penicilloic acids. It was the first paper that gave a brief explanation of the degradation products of antibiotic residue during milk processing. Later, Junza et al. (2014) identified the thermal transformation products of quinolones in cow's milk by utilizing LC-LTQ-Orbitrap-MS/MS and LC-ToF-MS/MS. These tools helped in identifying the chemical

structure, molecular mass and reaction type (decarboxylation, reductive defluorination, etc.) of both the thermal degradation products and the intermediary transformation products (only exist during the heating procedure) (Junza et al., 2014).

3.2 Thermal kinetics of antibiotics

Studies investigating the effects of thermal treatment on antibiotic residues most commonly present their findings in terms of the degradation percentage of the residues following the treatment. From the available studies, it can be concluded that, in general, thermal treatment leads to the degradation of antibiotic residues and consequently a reduction of the residue concentration or bioactivity in the food product. That being said, the values reported in the literature vary widely depending on the type of treatment used, the matrix, the pH, and the temperature. Some researchers applied the degradation rate constant k to study the thermal kinetic of antibiotic, which achieved a good regression coefficient. Later, the kinetics of some antibiotics were proved by calculating the Ea (minimum energy required to start the chemical reaction), lnA (collision frequency) and ΔG^0 (the standard molar Gibbs free energy of activation)

by some authors (detailed information was shown in section 3.2.3, section 3.2.6, section 3.2.7 and section 3.3.1).

The range of degradation percentage reported in the literature for antibiotics is summarized in Table 1. The complete detailed information is reported in Table S1 of the Supplementary Information. Sections 3.2.1 to 3.2.8 below discuss the results for each specific family of compounds.

3.2.1 β-Lactams antibiotics

β-Lactam antibiotics (such as penicillin and cephalosporins) are effective veterinary drugs widely used in animal production, which may result in β-lactam antibiotic residues in food, particularly in milk (Yamaki et al., 2004).

According to reported literature, the degradation percentages of β-lactams residues in food during cooking range from 0.1% to 100% (Table 1). The first tests on β-lactams using MIC tests indicated that most of these antibiotics are unstable during heat treatment (Traub and Leonhard,

1995). The degradation of β-lactams antibiotics was later shown to follow the first-order kinetic model (Fuliaş et al., 2010; Roca et al., 2011), and the degradation percentage was reported to be temperature-dependent (Roca et al., 2011). Grunwald and Petz (2003) noted that, in the case of penicillin in milk, the concentrations influenced the thermal stability, and relatively higher degradation percentages were recorded for lower initial concentrations.

A classic sterilization procedure (120°C for 15-20 min) induced significant decrease of β-lactams antibiotics in milk and water (Hsieh et al., 2011; Roca et al., 2011). When heated in tissue, high degradation of ampicillin was also found by long-time roasting (O'Brien et al., 1981).

The low stability of β-lactams under heating is reported mainly due to the high ring strain of the small β-lactone ring, which makes it susceptible to hydrolysis (Baertschi and Alsante, 2005). Ester bonds of cephapirin and cephuroxime are unstable in biological media, which make them more susceptible to heating than other β-lactams antibiotics, even at relatively low temperatures e.g. 60-80°C (Roca et al., 2011).

3.2.2 Tetracyclines

Tetracyclines (TCs) are a class of broad-spectrum antibiotics including tetracycline, oxytetracycline (OTC), doxycycline (DOC), and chlortetracycline (CTC) and residues have commonly been reported in food (Myllyniemi et al., 1999).

Under heat treatments, the degradation percentages of tetracyclines range from 2% to 100%. Studies have demonstrated that DOC is the most heat stable of the four compounds, while OTC is the least heat stable both in a chicken matrix (Abou-Raya et al., 2013) and in a buffer system (Hassani et al., 2008). Oxytetracycline (OTC) appears to be very heat-labile, as it can be almost completely degraded during boiling for half an hour in water (Rose et al., 1996). However, when heated in oil at a high temperature, the degradation was lesser than that obtained in water (Rose et al., 1996). The author indicated that this might be due to the hydrolysis of OTC in water.

Hsieh et al. (2011) demonstrated that in water, OTC was found to degrade more at 100°C than at 121°C. Kitts et al. (1992) found the thermal kinetics of OTC to be pseudo-first-order under 100°C, and first-order at higher temperatures (110-140°C) (Hassani et al., 2008). This may

explain why there was more degradation of OTC at 100°C than at 121°C. For other TCs, the thermal stability was temperature-dependent, and higher degradation was detected under higher temperature (121°C).

Studies investigating TC degradation in chicken and pig demonstrated that the type of food matrix and cooking method affects degradation (Nguyen et al., 2015; Gratacós-Cubarsí et al., 2007), and the results of one study indicated that the presence of fat as a matrix component might lead to decreased reduction in residues following thermal treatment (Gratacós-Cubarsí et al., 2007). Both studies showed a larger reduction in OTC residues in pig in comparison to chicken, and a larger reduction with microwave treatment in comparison to boiling treatment. Further studies are needed to explore the effect of specific matrix components on degradation.

Kitts et al. (1992) investigated the degradation kinetics of OTC in salmon muscle as well as buffer systems of varying pH. In both the salmon muscle and the buffer systems (pH 3 and pH 6.9), the degradation rate increased at higher temperatures. This was presented as a decrease in the D value. D values were higher at pH 3 indicating slower degradation. This is supported by

the findings of Xuan et al., which indicate that OTC hydrolysis in a pH neutral solution is faster than in acidic or alkaline solutions (Xuan et al., 2009).

Kühne et al. (2001) investigated the degradation of TC and 4-epitetracycline (4eTC) in animal feed along with the corresponding formation of anhydrotetracycline (ATC) and 4-epianhydrotetracycline (4eATC), known degradation products of the compounds. While there was an initial increase in TC and 4eTC concentrations, higher temperature and longer treatment time resulted in an average of 50% decrease in residues. At these same treatment conditions, there were large increases in concentration of the degradation products ATC and 4eATC: 941% and 200%, respectively. Whereas most studies inaccurately conclude that a reduction in the parent compound means the product is safer to consume, these results highlight the importance of investigating the fate of the residues as they may degrade into biologically active and sometimes toxic degradation products. Degradation products of TCs are discussed in section 3.4.

3.2.3 Macrolides

Macrolides are a class of antibiotics with a 12--16-atom lactone ring in their structure. The thermal degradation percentages of macrolides were about 0-93%. Erythromycin is the most widely used antibiotic in the macrolide family, often used as an alternative to penicillin (Reeves, 2012). Erythromycin is also the most susceptible antibiotic to heat treatment in the macrolide family (Zorraquino et al., 2011). The activation energy Ea of erythromycin, the minimum energy required to start the chemical reaction, was lower than those for other macrolides, which also indicated erythromycin was more sensitive to heating than other macrolides (Li, 2010). This was proven by real heating treatment--heating in milk at 120°C for 20 min induced more than 90% reduction of residues of erythromycin, while the figure was much lower for other macrolides (Zorraquino et al., 2011). What needs to be mentioned is that the result of Zorraquino et al. (2011) was measured by the change of antimicrobial activity, which cannot be taken to indicate the structural degradation of the compound.

3.2.4 Aminoglycosides

Aminoglycosides are a class of antibiotics with an aminocylitol ring linked to one or more amino sugars by a glycosidic linkage (Zorraquino et al., 2009). Due to the significant post-antibiotic effect of aminoglycosides, many aminoglycosides are banned in food-producing animals (Reeves, 2012). However, some aminoglycosides are permitted for use in dairy cows (Reeves, 2012). Thus, numerous studies have been done to investigate the degradation of aminoglycosides in milk. Almost all the aminoglycosides are heat-labile in milk. Heating at 120°C for 20min in milk led to the reduction in residues by more than 95% (Konecny, 1978; Zorraquino et al., 2009). Interestingly, when heating in water at 121°C, all the aminoglycosides showed only slight changes in bioactivity (Traub and Leonhard, 1995).

3.2.5 Amphenicols

Amphenicols are a class of broad-spectrum antibiotics including chloramphenicol, florfenicol and thiamphenicol. In water, all amphenicols are relatively stable during heating. Indeed, boiling (30-60min) and microwave heating (5min) were shown to result in less than 10% degradation for these three chemicals (Franje et al., 2010). Even when the treatment time was prolonged to 2

hours, degradation only increased slightly (Franje et al., 2010). However, when heated in meat tissue, chloramphenicol is much less stable and degradation is almost 5 times greater (O'Brien et al., 1981; Shakila et al., 2006). The bioactivity of chloramphenicol in beef was also shown to decrease by 70% after roasting for 2h according to the MIC test (O'Brien et al., 1981). Higher degradation of chloramphenicol in meat has been suggested to be a result of its lipophilic nature (Reeves, 2012). Franje et al. (2010) proposed that the greater degradation might result from the low water binding capacity of meat after heating, as Clarke et al. (1987) ever reported that low water binding capacity could increase the degradation of antibiotics. Franje et al. (2010) investigated the changes in the parent compound peak and the appearance of new peaks during the heating of three amphenicols in different media, and results varied amongst food matrices. In water, boiling induced more new peaks for florfenicol than the other two drugs, which indicated that florfenicol had more kinds of degradation products than the other two amphenicols. This could be explained by the structural differences of the three amphenicols, as the active fluorine group is more susceptible to nucleophilic substitution than the hydroxyl group (Franje et al.,

2010). However, the total new peak area of florfenicol was lower than the other two drugs, which indicated that only a small percentage of florfenicol degraded during heating, while the other two amphenicols suffered high degradation in quantity.

3.2.6 Quinolones

The degradation of quinolones was proven to obey the first-order kinetics and was temperature-dependent (Roca et al., 2010). Both the MIC test and the chromatographic analysis indicated that quinolones were heat-stable in water and milk (Traub and Leonhard, 1995; Roca et al., 2010). Roca et al. (2011) also compared the activation energy (Ea) and collision frequency (lnA) for quinolones with β-lactam antibiotics. Low Ea and lnA values indicated a lower degradation rate for quinolones than for β-lactam antibiotics. The same author also reported that the quinolone ring was more stable than the covalent bounds, which may explain why quinolones are stable during heating. Ciprofloxacin and norfloxacin were slightly less heat-stable than flumequine, oxolinic acid and enrofloxacin in water and milk (Roca et al., 2010). However, oxolinic acid showed more degradation when heated in black tiger shrimp tissue than in milk

(Uno et al., 2006b; Roca et al., 2010). Furthermore, Junza et al. (2014) found that enrofloxacin was less stable than ciprofloxacin, as enrofloxacin could degrade into ciprofloxacin during heating. Also, Junza et al. (2014) reported that one degradation product of ciprofloxacin combined with lactose in milk when heated at 120°C for 60min. The conclusions of Uno et al. (2006b), Roca et al. (2010) and Junza et al. (2014) indicated that the heating media was important to the degradation of quinolones.

3.2.7 Sulfonamides

Sulfonamides were proven to obey the first-order kinetics by Zhao et al. (2011) with high coefficient of regression (0.933-0.990) and then later proved by Roca et al. (2013). The degradation percentages range from zero to 99%.

Zhao et al. (2011) heated the hen eggs with six spiked sulfonamides (0.1mg/Kg) using a water bath, and the results showed that high degradation of all six sulfonamides happened under high temperature with long heating time (100°C, 20min). Sulfadiazine and sulfadimethoxine showed

the shortest and the longest half-life respectively among the six compounds (Zhao, Wu and Zhang, 2011).

Roca et al. (2013) conducted the experiment in milk and they also found that the thermal degradation of sulfonamides was time-dependent. The same author calculated the standard molar enthalpy and entropy of activation of sulfonamides. The entropy of activation was found to be negative, which indicated sulfonamides were not very susceptible to thermal treatments. High Ea and lnA indicated that the molecules needed high temperature to achieve activation energy, and when heated at high temperature, the high collisions of molecules would have enough energy to induce reaction (Roca et al., 2013). This theory explained the real degradation of sulfamerazine, sulfamethazine, sulfadiazine and sulfaquinoxaline in milk---the reaction rate was slow under low temperature and quickly increased under high temperature. Then, the high collisions between molecules had enough energy to break the pre-existed bound, which induced high degradation (Roca et al., 2013). In contrast, sulfadimethoxine and sulfathiazole showed low collision frequency, which was indicated by the low rate of reaction and low degradation. This was also

confirmed by the heating experiment in milk (Roca et al., 2013). Furthermore, Roca et al. (2013) compared the k of sulfonamides with the other antibiotics in their former studies. The values for sulfonamides were similar to those for penicillins but higher than those for quinolones. This conclusion was also proven by the heating experiments in milk (Roca et al., 2010; 2011; 2013).

3.2.8 Other antibiotics (teicoplanin, polymixin B, vancomycin, oxfendazole and lasalocid)

Other antibiotics reported in literatures are partly heat-labile or stable. For example, the MIC test indicated that polypeptide antibiotics such as teicoplanin and polymixin B were partially heat-stable, but vancomycin was remarkably heat-stable (Traub and Leonhard, 1995). Oxfendazole was almost heat-stable in water, but heating in oil at high temperature thoroughly destroyed the residue (Rose et al. 1997a). Lasalocid was stable in neutral and acid matrix but when the pH increased to 10, heating in oil at 100°C completely broke down the residues (Rose et al. 1997b). Unfortunately, there was no literature available for the kinetic studies of these antibiotics.

3.2.9 Discussion on the influence of the family of antibiotics

Zorraquino et al. (2011) observed that, although compounds within a class of antibiotics may share similarities in terms of chemical structure and bioactivity, their thermal stability within their group might greatly differ (Zorraquino et al., 2011). The present review also confirms that, based on the current literature, antibiotics cannot be ranked for thermal stability merely based on their class, as the heating environment namely the matrix and pH also has great impact on the degradation of antibiotics. In a specific condition, antibiotics in the same family have similar thermal property. Quantitative relationships describing the effect of heat on the residues are yet to be established.

3.3 Degradation kinetics

To date, only a few studies have attempted to model degradation kinetics of antibiotics during thermal food processing. Amongst these studies, the first-order model is dominant and has been validated for 28 antibiotic compounds (See section 3.2). Degradation rates, k, are scarce in the literature, and are mostly limited to the studies by Roca et al. (2010, 2011, 2013) on penicillin, sulfonamides and quinolones. In the present study, degradation rates were computed from other

studies to allow for some critical discussion. Therefore, when available, experimental time series were computed as described in the section 2.2 to derive k values. To date, there is no report available for the degradation kinetics of amphenicols, macrolides and aminoglycosides in food. We hypothesized that their degradation also follows first-order kinetics, but this should be further confirmed in the future. The resulting k values are summarized in Table S1 (Supplementary Information).

3.3.1 Influence of time on the degradation

To date, first-order-kinetics have been applied by several researchers to model experimental datasets (Hassani et al., 2008; Fuliaş et al., 2010; Roca et al., 2010; 2011; 2013). Good regression coefficients, ranging from 0.703 to 1.0 for β-lactams, quinolones, sulfonamides and tetracyclines (Fuliaş et al., 2010; Roca et al., 2010; 2011; 2013; Hassani, Lázaro et al. 2008), which indicates that this model is relatively suitable to study thermal kinetics for these compounds. The existing literature does not contain sufficient data to validate the applicability

of this model to the degradation of other antibiotics including macrolides, aminoglycosides, amphenicols and lincomycin.

3.3.2 Influence of temperature on the degradation

Temperature has been demonstrated to have an effect on the degradation rate of antibiotics. For example, comparing the k value for β-lactams and sulfonamides in milk at 60-65°C with those at 120°C, k values at 120°C are significantly higher than those at 60-65°C (P = 0.0013 for β-lactams ; P = 0.008 for sulfonamides) (Figure 1 and Figure 2). Although the degradation percentage and k values for most sulfonamides in this review are lower than other antibiotics, the k values for sulfonamides increase with the temperature significantly. Thus, the thermal degradation of β-lactams and sulfonamides is confirmed to be temperature-depended. Comparing the figure for macrolides, k values also showed an up-trend when temperature increased (P = 0.124), but it is not as significant as β-lactams and sulfonamides. Lincomycin only degraded under high temperature in milk, as the k value is zero at about 60°C (Figure 1 and Figure 2). Comparing the k value for antibiotics in water at 100°C, the figure for β-lactams

and tetracyclines are higher than the other antibiotics, while k value for levamisole is the lowest one (Figure 3). Temperature has been demonstrated to have an effect on the degradation rate of tetracyclines. In model buffer systems of varying pH, the degradation rate increased as the temperature was increased from 60-100°C at both pH 3.0 and pH 6.9 (Kitts, Yu et al. 1992). Similarly, in a system of pH 9.06, the degradation rate increased as the temperature increased from 25-60°C (Xuan, Arisi et al. 2009). Tetracycline residues in animal feed were shown to increase within the first 30 minutes at 100°C, but decreased by approximately 50% at 133°C (Kühne, Hamscher et al. 2001).

3.3.3 Influence of matrix on the degradation

Although the exact effect is still unclear, it has been demonstrated that the food matrix does indeed have an effect on the degradation of antibiotic residues. For example, it has been reported that high fat content meat increased the efficiency of microwave heating which result in the higher degradation of antibiotics than low-fat meat (Gratacós-Cubarsí et al., 2007). Shahani et al. (1956) and Grunwald and Petz (2003) pointed out that penicillins degraded more

in water than in milk under thermal treatment. Nonetheless, an assessment of the k values for penicillin G in water and in milk at 120°C revealed they are similar. In another study on amphenicols (Franje et al., 2010), k values in soybean sauce were found to be about 4 times greater than those in water at 100°C ($P < 0.01$). The effect of the food matrix on the degradation of tetracyclines also remains unclear as studies have yet to investigate the effect of specific food matrix components that may influence degradation. Xuan et al. (2009) tested the effect of Ca^{2+} on the degradation of OTC and found that it leads to a slower rate of hydrolysis, as well as a deviation from the simple first-order kinetic model. This is likely a result of the interaction of tetracyclines with divalent metal ions (Samanidou, Nikolaidou et al. 2007).

The pH may also affect the degradation rate. For example, OTC degraded more rapidly under neutral environment than acid one when heated at 60°C to 100°C (Kitts et al., 1992). Similarly, TC and doxycycline were reported to show higher degradation at 130°C under neutral environment than at pH 4.0 (Hassani et al., 2008).

Compared with solid matrix, the liquid matrix itself can affect the degradation rate, as solvent shows higher ion strength than solid matrix (Mollica et al., 1978). Thus, we can conclude that the physical and chemical properties of the food matrix can affect the thermal degradation of antibiotic residue. Finally, Fedeniuk et al. (1997) demonstrated that the thermal degradation of antibiotics can also be influenced by food additives.

Due to the insufficient data, it is difficult to determine whether thermal degradations of other antibiotics, not mentioned in the present section, are affected by the food matrix. Thus, exploring the influence of matrix on degradation is highly recommended in future studies for the purpose of risk assessment.

3.3.4 Influence of cooking methods on the degradation

Ibrahim and Moats (1994) studied the influences of different cooking methods on OTC in lamb meat. Boiling in a plastic bag for 30 min and microwave heating (98-102°C) for 8 min could destroy 95% and 60.5% of residue, respectively. Frying was less effective, which reduced only 3.6% (frying 4 min) and 17.3% (frying 8 min). However, frying was efficient in reducing more

than half of OTC in seafood. Frying at 100°C for 15 min broke down 60% of OTC in salmon muscle (Kitts et al., 1992). Similar result was found in channel catfish. Indeed, Du et al. (1997) measured the effect of three common cooking methods on OTC in channel catfish and the result was influenced by the treatment dose. When OTC was heated in a chicken and pig matrix, there was a larger reduction in residues with microwave treatment in comparison to boiling treatment (Nguyen et al., 2015; Gratacós-Cubarsí, Fernandez-García et al. 2007). Comparing the data for milk processing, long-time boiling is better than short-time ultra-high temperature treatment in breaking the antibiotic residues in milk (Grunwald and Petz, 2003; Zorraquino et al., 2009; Zorraquino et al., 2011; Roca et al., 2010; Roca et al., 2011; Roca et al., 2013).

The cooking method can also affect the k values as Franje et al. (2010) reported that microwave heating induced faster degradation of amphenicols than boiling. Furthermore, industrial processing such as canning can achieve relatively higher temperature than common home cooking, which in turn induces higher degradation. Cooking in a water bath at 100°C for 30min only broke down 29% of chloramphenicol in shrimp (Shakila et al., 2006). However, Epstein et

al. (1998) reported that canning could completely destroy the chloramphenicol residues. Hsu and Epstein (1993) concluded that the degradation of levamisole only occurred under severe heating but not during domestic cooking. Rose et al. (1995a) also found similar results. All these studies indicated that cooking methods affect the degradation of antibiotics. Unfortunately, it is not possible to do a correlation analysis of the influence of cooking methods on degradation, due to the limitation of reports. Further investigation on whether the influence of cooking methods on the degradation is due to the temperature difference is also highly recommended.

Other food processing methods such as fermentation and cold storage can also affect the percentage of antibiotic residues in food. Epstein et al. (1988) found that sulfamethazine was partially degraded during the procedure of sausage emulsion, even though sulfamethazine was reported to be stable during cooking. Alfredsson and Ohlsson (1998) reported that long-term cold storage (at -20°C for more than 1 month) induced significant decrease (about 35%-55%) of

five sulfonamides. However, the storage at -20°C for one week did not change the drug level significantly.

3.4 Identification of degradation products

To date, few studies have successfully identified the degradation by-products of antibiotics in food induced by thermal or food processing. Junza et al. (2014) reported that enrofloxacin and difloxacin degraded to ciprofloxacin and sarafloxacin, two common antibiotics, respectively. The degradation products of antibiotics may represent a potential threat to human health. For example, penicilloic acid, a degradation product of penicillin, may induce allergic reaction in sensitive populations (Sullivan et al., 1981). There is more information in the literature on the formation and identity of the degradation products of tetracyclines than those of other antibiotic families (Hsieh et al., 2011).

Thus, tetracycline can degrade into their 4-epimers and anhydro products under certain conditions. 4eTC is biologically active, albeit at a much lower level than the parent compound,

and it can also convert back to the parent compound (Fritz and Zuo, 2007). The antimicrobial activity of tetracycline is thought to be dependent on certain structural requirements, and the reduction in biological activity is thus attributed to structural changes at important carbon positions (Halling-Sørensen et al., 2002；Blasco et al., 2009). TCs follow different degradation pathways depending on the pH of the medium (Loftin et al., 2008). A dilute acid medium favours the formation of 4eTCs and anhydro-TCs (Samanidou et al., 2007; Xuan, Arisi et al. 2009)., while a strong acid medium favours the formation of anhydro-TCs which can undergo cleavage and lactonization to produce apo-derivatives (Samanidou et al., 2007). The formation of two unidentified compounds from TC and eTC, was reported following thermal treatment of chicken and pork residues (Gratacós-Cubarsí et al., 2007). The formation of unidentified compounds from TC and OTC following treatment at 100°C was reported (Hsieh et al., 2011). It is not clear whether they correspond to degradation products observed in previous studies (4-epimers, anhydro-TCs, apo-TCs) or to new products not previously identified. Further investigation into the degradation products is thus required.

Thus far, information regarding the toxicity of TC degradation products is limited. Anhydrotetracyclines are known to be toxic, exhibiting renal toxicity, which is reported to be reversible (Frimpter et al., 1963; Benitz and Diermeier 1964). Using the Ames test, heated CTC was found to induce mutagenicity in comparison to the control, while heated DOC was not (Hsieh et al., 2011). Nguyen et al. studied the effects of OTC degradation products α-apo-OTC and β-apo-OTC on male rats following oral exposure for 90 days. No adverse effects were observed for α-apo-OTC, while for β-apo-OTC toxic effects including liver and kidney damage, hepatocyte degeneration, and hepatocyte necrosis was observed (Nguyen et al., 2015). Due to the limited information on the profile of TC degradation products under different treatment conditions, there are uncertainties as to whether toxic degradation products are formed in significant amounts under typical domestic cooking procedures. Concerning the possible adverse effect of degradation products, there is thus a need to define these profiles, as well as any unidentified degradation products, and to further investigate the possibility of acute or chronic toxicity. It is urgent to have a good understanding of the degradation products of

antibiotic residues in food, because even if the parent compound was shown to be destroyed completely by using quantitative analysis, the degradation products may combine with the food matrix (e.g. one thermal degradation product of ciprofloxacin combined with lactose of milk during heating) or even react with the human body (e.g. penicilloic acid, one of the major degradation products of penicillin), could cause delayed contact allergy (Levine, 1960).

4. Conclusions

The present literature review explored the fate of antibiotic residues in food during thermal processing. To quantify degradation, two major techniques have been applied: liquid chromatography-based methods and microbiological tests. As the degradation products may also display some antimicrobial activity, microbiological tests cannot be considered accurate analytical methods for quantifying antibiotic residues' degradation. Coupling advanced mass spectrometry techniques to liquid chromatography separation can provide insight about the identity of the degradation by-products.

The various studies presented in the literature confirm that the thermal degradation of β-lactams, quinolones, sulfonamides and tetracyclines can be described using a first-order kinetic model. Degradation rates, k, derived for this model for liquid matrix (water) at 100°C, followed the general trend amongst antibiotic classes: β-lactams = tetracyclines (most heat-labile) > lincomycin > amphenicols > sulfonamides > oxfendazole > levamisole (most heat-stable).

The thermal degradation of β-lactams, quinolones, sulfonamides, macrolides, tetracyclines and aminoglycosides are temperature-dependent, and under certain temperatures, prolonged heating time helps to induce more degradation. Furthermore, the food matrix composition and physico-chemistry, (e.g. pH, fat content), the cooking methods, and the presence of food additives were shown to be parameters possibly influencing the degradation of antibiotics. Further studies are needed in this field to systematically understand the impact of these parameters and the profile of the degradation products throughout heating procedures.

According to the *Codex Alimentarius* commission (2010), dietary intake assessments for contaminants should account for the effect of food processing and cooking. Thermal processing usually results in a decrease in the concentration of parent antibiotic residues in food, but degradation by-products have not been properly characterized to date. As some of these products were shown to be hazardous, further investigation is needed to determine their impact on food safety and human health. It is therefore currently difficult to definitively conclude whether or not antibiotic degradation during food processing is necessarily beneficial in terms of food safety, thus only partially satisfying *Codex Alimentarius*' requirements.

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Table 1: Summary of thermal degradation range reported in literatures

Antibiotic Famil	Compound(s)	Processing Methods	Matrix	DP Range	Reference
β-lactams	Amoxycillin/Ampicillin/Penicillin G/Oxacillin/Dicloxacillin/Cloxacillin	Boiling	Water	8-78 %	Hsieh et al., 2011
	Oxacillin/Dicloxacillin/Cloxacillin/	Boiling	Water/ Milk	8-64 %	Grunwald and Petz, 2003
	Nafcillin				
	Penicillin G	Boiling	Milk	32%	Konecny 1978
	Ampicillin	Grilling/Roasting	Meat	2.3-100 %	O'Brien et al., 1981
	Penicillin G	Boiling	Water/	80-9	Rose et al.

			Oil	0%	1997c
	Cefuroxime/Cefquinome/Cephalexin/	Boiling	Milk	0.1-100 %	Roca et al., 2011
	Cephalonium/Cephapirin/Cefoperazoe/Amoxycillin/Ampicillin/PenicillinG/				
	Cloxacillin				
	Penicillin G	Boiling	Meat	8.2-59.7 %	Shahani et al. 1956
Tetracyclines	Tetracycline/Oxytetracycline/	Boiling/Roasting/	Meat	42-100%	Abou-Raya et al., 2013
	Doxycycline/Chlortetracycline	Microwave			
	Oxytetracycline	Frying/Baking/	Seafood	25-93%	Du et al.,

		Smoking			1997
	Tetracycline	Boiling/Microwave	Meat	56-81.8 %	Gratacós-Cubarsí et al., 2007
	Doxycycline/Oxytetracycline/	Boiling	Water	8-99 %	Hsieh et al., 2011
	Tetracycline/Chlortetracycline				
	Oxytetracycline	Boiling/Microwave/Frying	Meat	2-95 %	Ibrahim and Moats, 1994
	Doxycycline	Boiling/Microwave/Roasting	Meat	35-100%	Javadi, 2011
	Oxytetracycline	Frying/Water/Oil Bath	Seafood/Buffer	60%	Kitts et al., 1992

	Oxytetracycline	Boiling/Microwave	Meat	50-59%	Nguyen et al., 2015
	Oxytetracycline	Grilling/Roasting	Meat	4.3-74%	O'Brien et al., 1981
	Oxytetracycline	Boiling/Microwave/Roasting/Frying/ Grilling/Braising	Water/Oil/Meat	25-99%	Rose et al. 1996
	Oxytetracycline	Canning	Meat	100%	Scheibner, 1972a
	Oxytetracycline	Boiling/Baking/ Frying	Seafood	17-80%	Uno et al., 2006a
Macrolides	Erythromycin/Spiramycin/Tylosin	Boiling	Milk	0-93%	Zorraquino et al.,

					2011
	Ivermectin	Boiling	Milk	1.1-1.5 %	Imperiale et al., 2009
	Ivermectin	Boiling/Frying	Meat	45-50%	Slanina et al., 1989
Aminoglycosides	Gentamicin/Kanamycin/Neomycin/Streptomycin	Boiling	Milk	17-98%	Zorraquino et al., 2009
Amphenicols	Chloramphenicol/Florfenicol/Thiamphenicol	Boiling/Microwave	Water/ Seafood/Meat	2-80 %	Franje et al., 2010
	Chloramphenicol	Canning	Meat	100 %	Epstein et al., 1988

	Chloramphenicol	Grilling/Roasting	Meat	0-100%	O'Brien et al., 1981
	Chloramphenicol	Boiling	Seafood	6-29%	Shakila et al., 2006
Quinolones	Ciprofloxacin/Norfloxacin/Flumequine/Oxolinic acid/Enrofloxacin	Boiling	Milk	0.01-12.71%	Roca et al., 2010
	Oxolinic acid	Boiling/Baking/	Seafood	20-50%	Uno et al., 2006b
		Frying			
	Enrofloxacin	Boiling/Microwave/Roasting/Frying/	Meat	77.04%	Lolo et al., 2006
		Grilling			
Lincosamide	Lincomycin	Boiling	Water	0-15	Hsieh et al.,

s				%	2011
	Lincomycin	Boiling	Milk	0-5 %	Zorraquino et al., 2011
Sulfonamides	Sulfamethoxazole/Sulfadiazine/	Boiling/Microwave/Roasting	Meat	2-61 %	Furusawa and Hanabusa, 2002
	Sulfaquinoxaline/Sulfamonomethoxine				
	Sulfamethazine	Canning	Meat	50%	Epstein et al., 1988
	Sulfamethoxazole/Sulfamethazine	Boiling	Water	3-10 %	Hsieh et al., 2011
	Sulphadimidine	Grilling/Roasting	Meat	0-7.6%	O'Brien et al., 1981

	Sulfamethazine/Sulfachloropyridazine/Sulfadiazine/Sulfadimethoxine/Sulfam-erazine/Sulfapyridine/Sulfathiazole/	Boiling	Milk	0-85.1%	Roca et al., 2013
	Sulfaquinoxaline				
	Sulfamethazine	Boiling/Frying	Water/ Oil	3-99 %	Rose et al. 1995b
	Sulfadimethoxine	Frying/Baking/	Seafood	7.5-63.5 %	Xu et al., 1996
		Smoking			
Nitrofurans	Furazolidone	Frying	Meat	0	Shit et al., 2008
Others	Dimetridazole (DMZ and its metabolite RNZ)	Boiling/Frying	Water/ Oil	0-60 %	Rose et al. 1998

	Levamisole	Boiling/Microwave/Frying/Grilling	Water/Oil/Meat	13%-99%	Rose et al. 1995a
	Levamisole	Frying	Meat	11-42%	Cooper et al., 2011
	Oxfendazole	Boiling/Microwave/Frying/Braising	Water/Oil/Meat	5-100%	Rose et al. 1997a

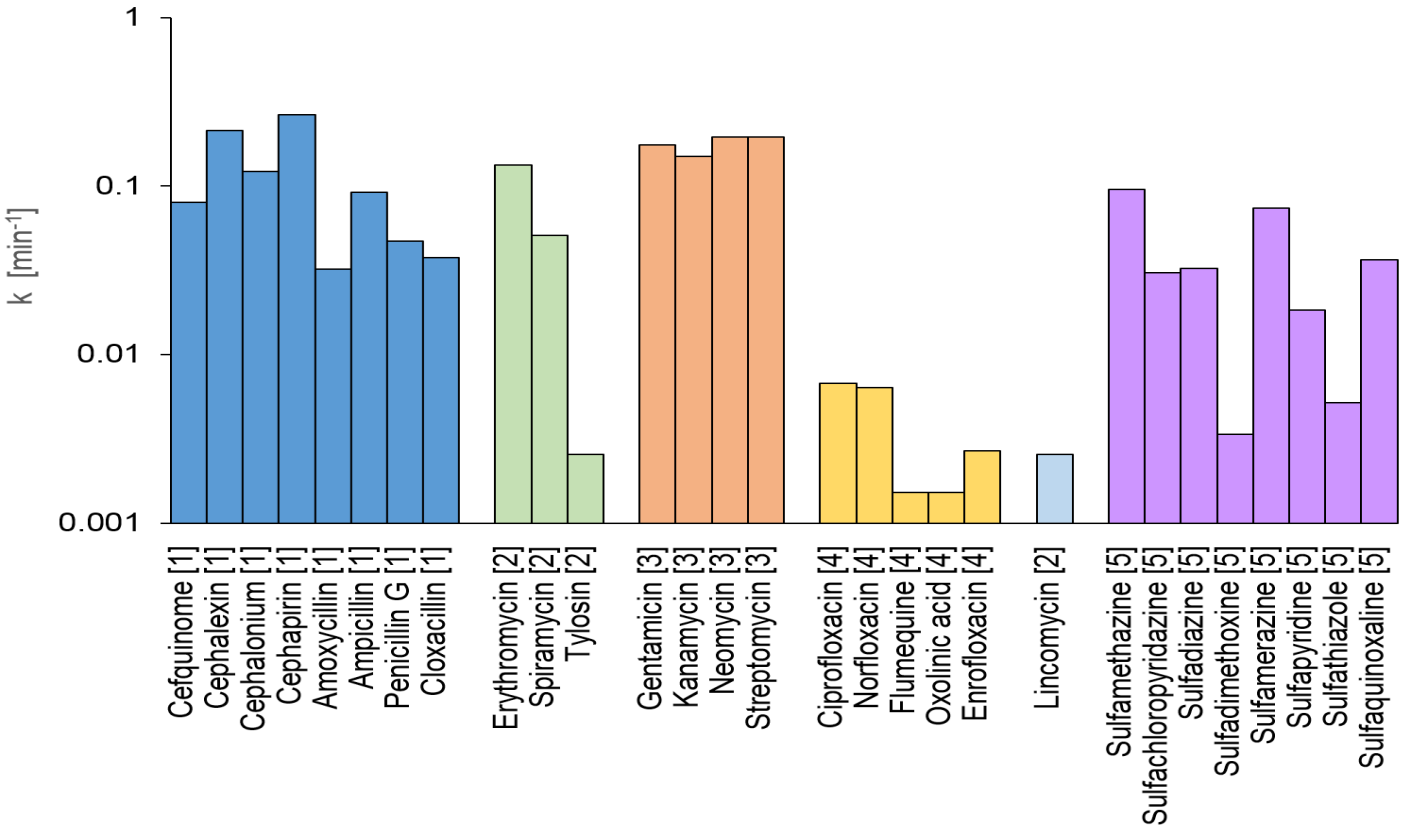

Figure 1. k values for selected antibiotics at 120°C derived from literature data on milk. [1] Roca et al., 2011; [2] Zorraquino et al., 2011; [3] Zorraquino et al., 2009; [4] Roca et al., 2010; [5] Roca et al., 2013

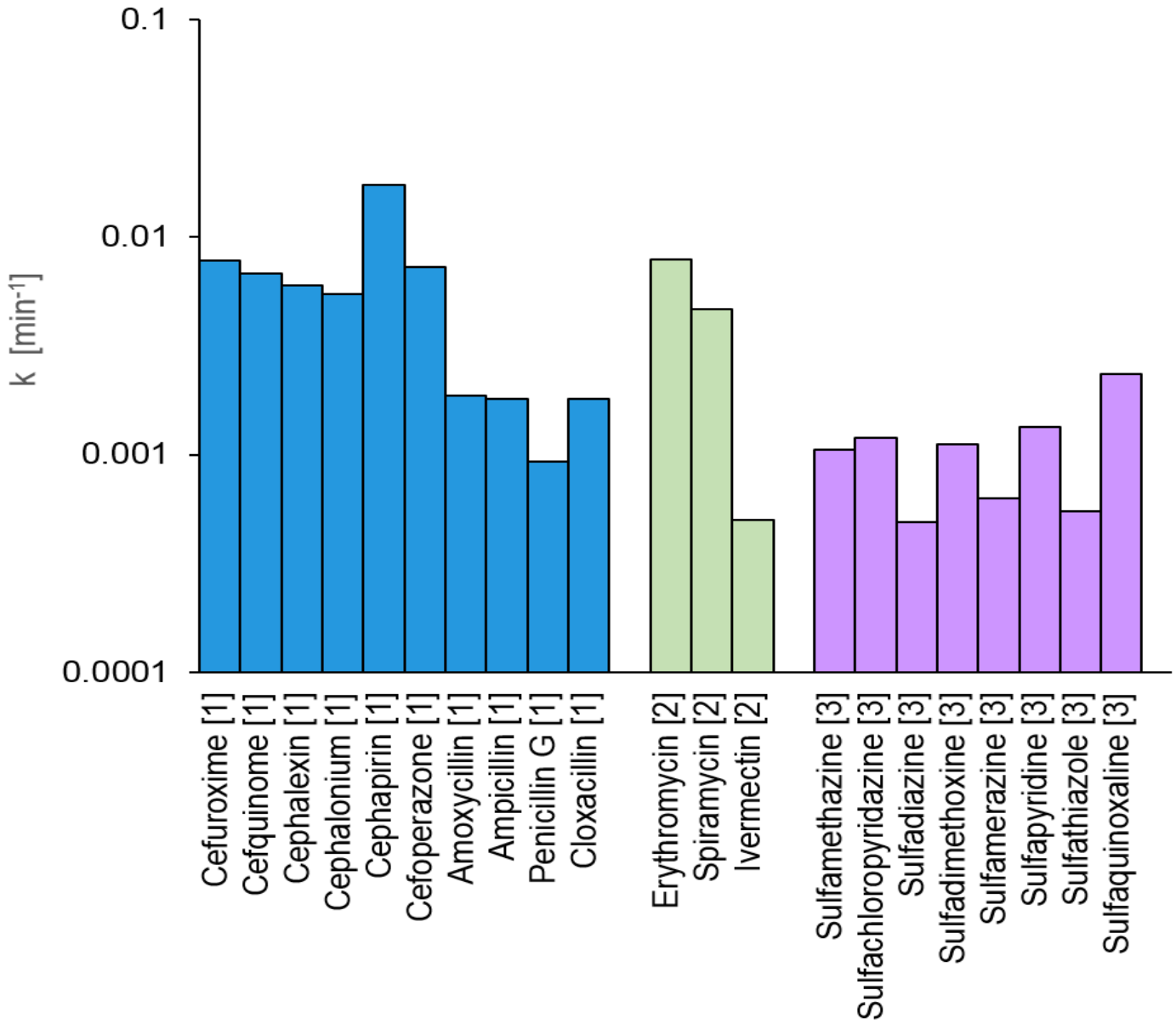

Figure 2. k values for selected antibiotics at 60-65°C derived from literature data on milk. [1] Roca et al., 201; [2] Zorraquino et al., 2011; [3] Roca et al., 2013.

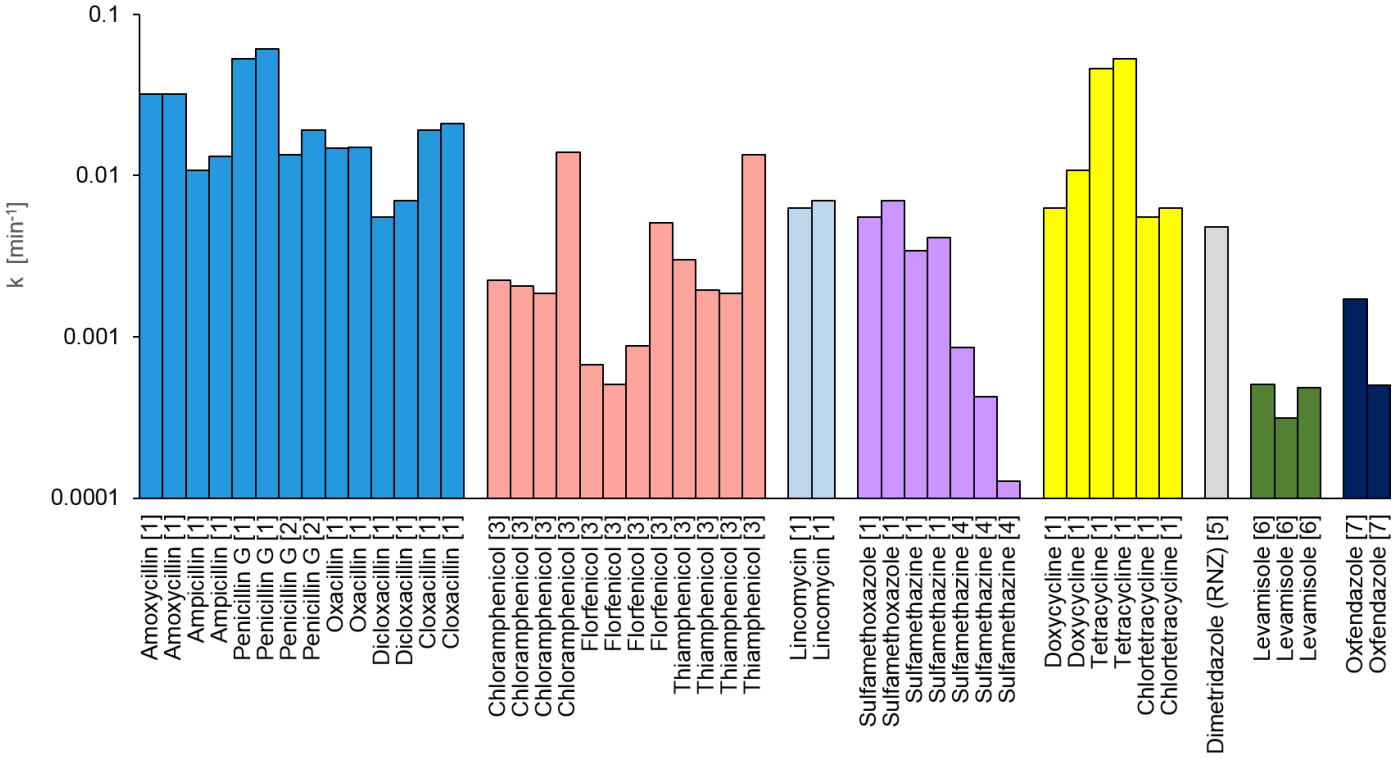

Figure 3. k values for selected antibiotics at 100°C derived from literature data on water. [1] Hsieh et al., 2011; [2] Rose et al. 1997c; [3] Franje et al., 2010; [4] Rose et al. 1995b; [5] Rose et al. 1998; [6] Rose et al. 1995a; [7] Rose et al. 1997a