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Inactivation of *Salmonella* Enteritidis on Raw poultry Using Microwave Heating

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ABSTRACT

The effect of microwave heating on Salmonella *Enteritidis inoculated on fresh chicken was investigated using a microwave oven (800 w) to determine the destruction of* Salmonella *Enteritidis isolated from chicken carcasses, in relation to the time of heating at two power settings: high (power level 10) and medium (power level 6); The relationship between heating time and temperature was also been studied. The destruction was 6.4 log cycles at time 95 sec for the high power level, and 5 log cycles at time 140 sec for medium power setting. After 110 sec for higher power level, no survival of* Salmonella *Enteritidis was detected in samples (100g), but at 140 sec for medium power level, these food pathogens were still present.*

Key words: *Microwave, Salmonella enteritidis, destruction, chicken thighs*

INTRODUCTION

The use of microwave heating to cook and process food has become remarkably popular in commercial and home cooking (Giese, 1992). In a microwave oven the heating of food results from molecular friction between water molecules under an oscillating electric field of specific frequency. There are many parameters that can affect the microwave heating in food like wattage, cavity size, feeding system, use of turntables, age of the magnetron, browning elements, or microwave-convection systems. Food mass, moisture and ionic content, density, shape, thermal conductivity and specific heat also affect microwave heating. (Schiffmann, 1987, 1990).

There have been some attempts (Cunningham, 1980; Culkin and Fung, 1975) to ascertain if microwaves have a non-thermal inactivation on the microorganisms, and predominant theories have been used to explain this effect (Kozempel et al., 1998). Regardless of the mode of action, microwave radiation has been reported to be feasible for achieving longer shelf lives of several food products. (Cunningham and Albright, 1977; Cunningham, 1978).

The main concern about the efficiency of microwave in the inactivation process relates to an uneven distribution of heat on product surface cooked in microwave oven, resulting in the formation of hot and cold spots in food (Mudgett, 1989; Datta and Davidson, 2001). Temperature uniformity may be improved by using wave stirrers, turntables, or by heating at lower power levels for longer periods of time.

Several studies have reported incomplete inactivation of microorganisms in inoculated food cooked, or reheated in microwave oven (Fung and

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Cunningham, 1980; Linsay et al., 1986); Heddleson et al. 1994; Farber 1998; Doyle and Mazzotta, 2000). In the case of *Salmonella*, it has been demonstrated that only a few cells may cause a person to become ill (D`Aoust 1985, 1989), and the dose depends upon the strains used, the age and physical conditions of the person, and can therefore show wide variations (Kothary and Babu, 2001).

In Argentina, *Salmonella typhimurium* was predominant in outbreaks of gastroenteritis until 1987; at present *Salmonella* Enteritidis ranks first. The use of microwave oven is increasing particularly in Argentina, and considering that *Salmonella* inactivation could be a problem; it results important to know the effect of microwave heating in these pathogens. This effect has not been reported in the literature.

The objective of this investigation was to examine the destruction of *Salmonella* Enteritidis isolated from chicken carcasses, heated by home microwaves. Chicken thighs were used as a model system, and the relation of time of heating at two power levels in the inactivation was considered. In addition, relationship between time of heating and temperature was also studied.

MATERIALS AND METHODS

Bacterial strain

Salmonella Enteritidis strain (Nº4225), which was the predominant serotype isolated from local chicken carcasses in our laboratory, was selected for this study (Benassi et al., 1995). It was maintained in tryptic soy agar (TSA; Difco) at 5ºC (Tessi et al., 1992). Cultures were activated at 37ºC for 24 h in a tryptic soy broth (TSB; Difco). Cultures were wireloop transferred at 24 h intervals (10^8 CFU/mL). (Zhuang et al., 1995).

Microwave source

Irradiation was performed in a home use oven (23L capacity) with rotating plate (30.5 cm in diameter) at a speed of 5 rpm (800 watts, 570*420*368mm, with grill, 9 automatic programs, European design SUGGAR-23GD).

Inoculation on chicken thighs

Salmonella Enteritidis inoculum was added into a beaker with 100 mL of buffered 0.1% peptone water to give a viable cell population of ≈ 10^7 CFU/mL. Each sample consisting in a chicken thigh (≈ 100 g) was transferred into the beaker and agitated for two minutes, then left to rest 60 minutes at room temperature to allow the cells to attach.

Chickens

Twenty whole chickens prepackaged in plastic bags, with a purchase weight of approximately 1.5-2 kg, and 9 to 12 weeks old, were obtained from commercial processor and local supermarkets and stored at 4ºC for 12 h or less.

In order to determine the amount and type of natural contamination, the chickens were placed in sterilized polyethylene bags (30 x 40 cm) with 300 mL of sterile peptone water (0.1%) and agitated for 2 minutes. The chicken was aseptically transferred to a sterile container and the peptone water was transferred to sterilized Erlenmeyer flask (1000 cc). This nonselective enrichment solution was incubated at 37ºC for 24 h and 1 mL was transferred to 10 mL of the following selective enrichment media: tetrathionate (TT), Selenite-Cystine (SC) and Rappapport-Vasilliades (RV) (BAM, 1992). Selective enrichments were incubated at 35ºC (SC and TT), or at 43ºC (RV). After 24 h incubation, a loopful from each selective enrichment broth culture was streaked in duplicate on the following selective agar plates: bismuth-sulfite (BS) and xylose lysine desoxycholate (XLD). The plates were examined after 24-48 h at 35ºC. Suspected colonies of *Salmonella* were tested into triple sugar iron (TSI) and lysine iron (LIA) agar. Colonies exhibiting typical reactions on TSI and LIA were purified and further characterized using the urease, oxidase, FDA-BAM tests.

Preparation of chicken thighs

Forty thighs were aseptically separated from the whole chickens. These were checked for the presence of *Salmonella* with the procedure described below. Seven different time-temperature combinations were studied in duplicate using a microwave oven to determine the relationship between time of heating, temperature and resulting bacterial destruction (logN/No). The heating time varied from 10 to 140 sec. Determinations of total destruction of inoculated *Salmonella* Enteritidis on chicken

thigh were made using existing power setting on the microwave of 10 (high) and 6 (medium). Three trials were performed for each level, and the total "magnetron-on" time and power in watts were also determined.

Internal temperatures were taken with multimeter thermometers, which had been soaked in chlorine solution and rinsed with sterile water and then were inserted into each thigh at two specific locations. One thermometer (T_1) was located just under the skin of the thigh and the other (T_2) was located 1.5 cm inside the thigh.

Each heated thigh chicken was aseptically transferred to sterile 1-L beaker, completely covered with 100 mL of peptone water and was cooled into an ice bath to around 25ºC. Beakers were covered with sterile foil and incubated at 25ºC for 18-20 minutes in order to repair the injured cells. The chicken thigh and the liquid were transferred to sterile plastic bag and hardly agitated for 3 minutes. The chicken thigh was aseptically removed from the bag and the liquid was transferred into an Erlenmeyer flask. Decimal dilutions were performed serially. Aliquots of 0.1 mL were then plated in triplicate onto BS and XLD. Presumptive colonies *Salmonella* Enteritidis that developed after incubation for 48 h at 35ºC were counted to get the *final concentration* (N). Three colonies per plate were randomly selected and confirmed by appropriate biochemical tests. The *initial concentration* (N_0) was counted from the buffered peptone water after inoculating the chickens; then serially diluted samples aliquots of 0.1 mL were surface plated in BS and XLD, and the colonies after the incubation for 48 h at 35ºC, were counted.

The microbiological destruction was calculated as log (initial) – log (final). The mean microbiological destruction was calculated in each trial. Amount of the Energy was determined using 200 mL destilled deionized water in six different times. Q = Mass * specific heat * Temp. rise.; where the initial temperature was 25ºC, and the final temperature was taken after stirring for 5 sec to ensure uniform temperature.

Data Analysis

Analysis of Variance (ANOVA) was employed with trials to determine differences in microbiological destruction after heating. Fisher Probable Least Significant Different (PLSD) multiple comparison test (p=0.05) was used to calculate means and analyze microbiological destruction, using Statgraphics Plus (Version 7 FOR DOS) Statgraphics User Manual Manugistics, Inc. Rockville, Maryland. USA. 1993.

RESULTS AND DISCUSSION

The temperature evolution at two positions in the sample is shown in Figs. 1 and 2 for high and medium power levels, respectively. The positions in the samples were under the skin and inside the thigh respectively. For both power levels the temperature increased with time in a non-smooth way, reaching maximum around 90ºC for high power and 70ºC for medium power at both positions in the sample. The heating rates for high and medium power were 0.56 and 0.32 ºC/sec, respectively. On comparing the temperature evolution for the two power levels, following apparent anomalous behavior were observed. In the first 20 sec the temperature at medium power level was a little higher than for high power level. This behavior could be a result of the intrinsic mode of operation of the microwave oven in on and off fashion, which was not effective until many cycles were produced.

There was temperature difference between the two positions in the chicken thigh. At both power levels the temperature under the skin was practically always lower than the temperature inside the thigh. The difference increased until a maximum value of around 11ºC was reached after 80 seconds. After this, the difference started to decrease. This effect could be due to intrinsic heating mechanism in microwave oven as opposed to convection oven, producing a more even heating. At longer heating times, there could be a small effect of cooling from the surface and uneven heating, which inverted the temperature relation resulting in the skin lower values than in the bulk of the sample. However, in both cases, it was concluded that the more uniform heating mode of microwave oven for short periods of time was beneficial and more advantageous than heating with conventional convection oven. In the first case, heating occurs by radiation whereas in the second heating occurs from the outside to the inside of food and mainly by conduction.

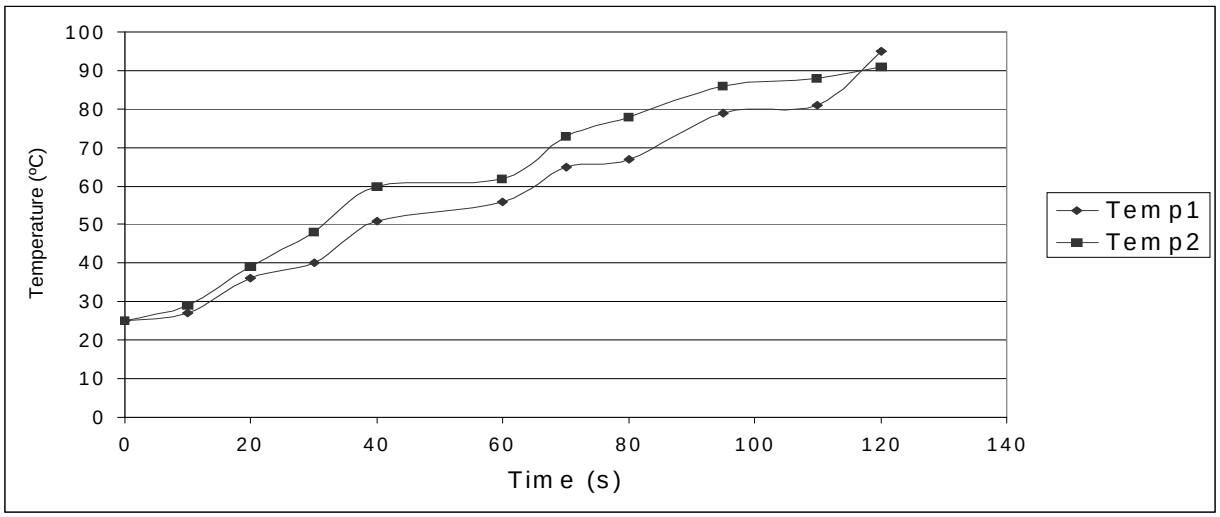

Figure 1 - Temperature evolution of the samples (Temp 1: under the skin, Temp 2: 1.5cm inside the thigh) as function of time for Power level 10(high).

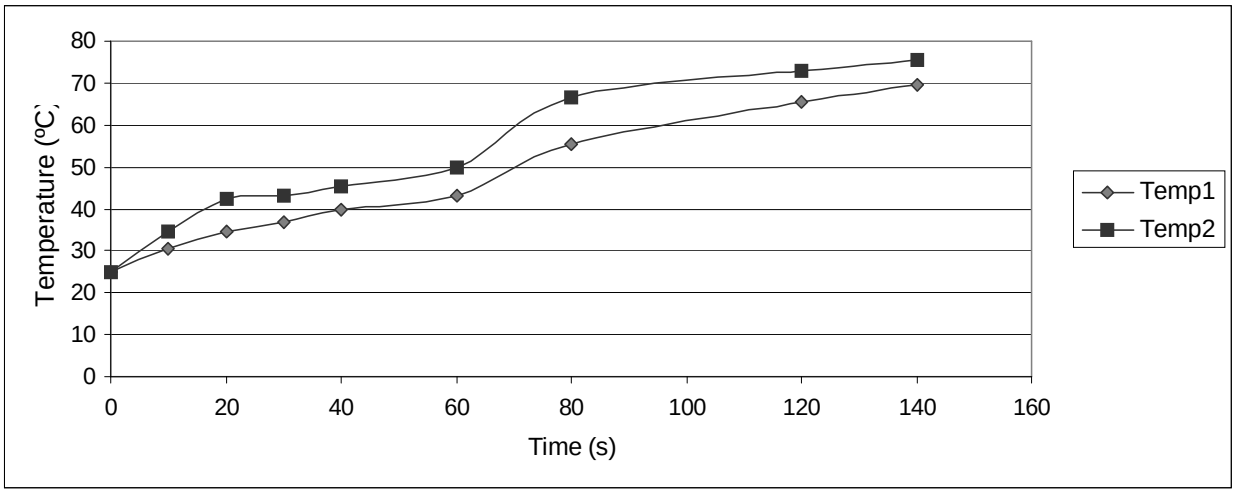

Figure 2 - Temperature evolution of the samples (Temp 1: under the skin, Temp 2: 1.5cm inside the thigh) as function of time for Power level 6 (medium).

Results of destruction of *Salmonella* Enteritidis in chicken thighs versus heating time are illustrated in Fig. 3 for power level 10 and in Fig. 4 for power level 6. In each figure the two curves corresponded to BSA and XLD as described above. The destruction curve, which showed typical results of the present investigation, did not present significant differences for both culture media ($p < 0.05$).

The *Salmonella* destruction at high power level as a function of time showed three distinctive regions, one at low time up to about 40 sec, a second region from 40 to 80 seconds with a sharp slope which indicated a higher destruction rate and finally, a third region from 80 to 120 sec where the slope in the log scale leveled off again to rates similar to the first region. The destruction in the regions were correspondingly different; in

the first there was a destruction of 1 log cycle, in the second 4 log cycles and in the third 1.4 log cycles.

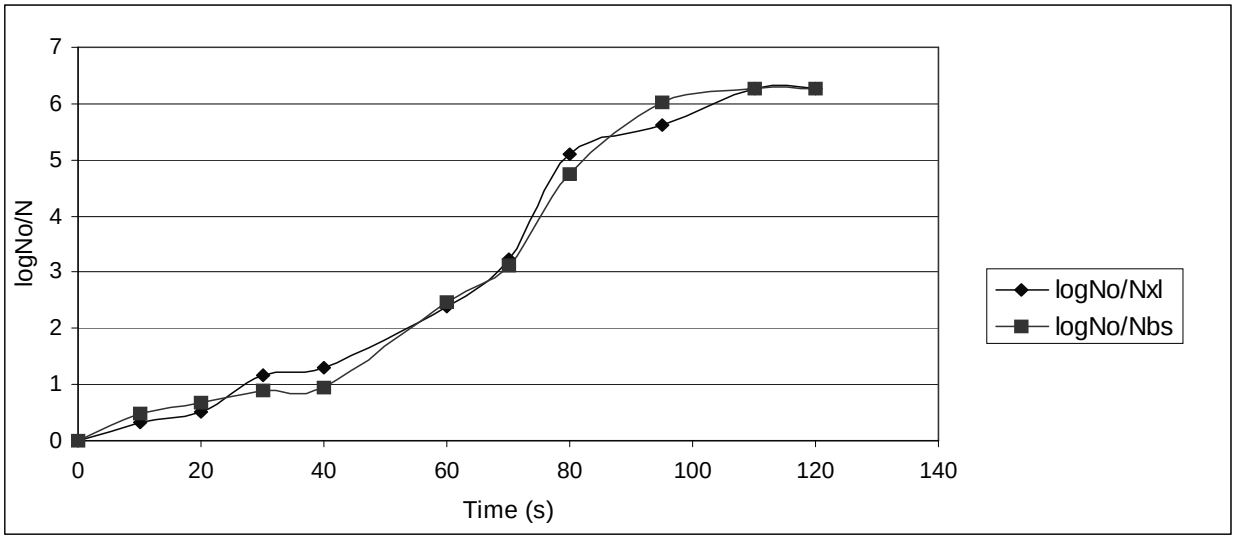

Figure 3 - Microbiological Destruction Curve vs. Time for Power level 10 (high) for two medium XLD agar (logNo/Nxld) and BS agar (logNo/Nbs)

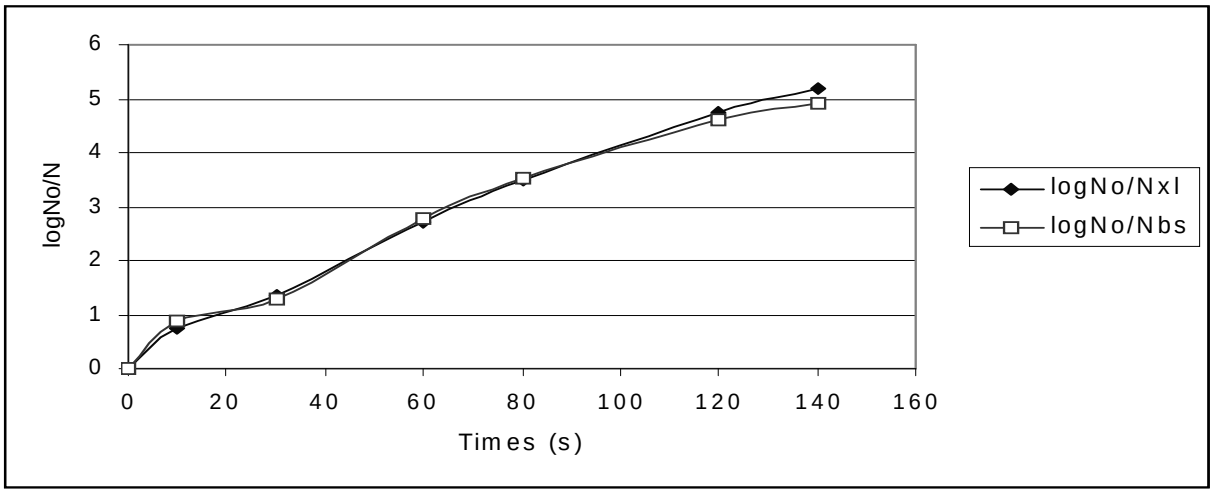

Figure 4 - Microbiological Destruction Curve vs. Time for Power level 6 (medium) for two medium: XLD agar (log No/Nxld) and BS agar (log No/Nb).

When the described evolution was compared with the temperature of the sample at the two positions, it was observed that the main destruction in region 2 occurred at a temperature of about 60 ºC. After 110 sec, there was no detection of *Salmonella*.

The *Salmonella* destruction at medium power level showed a uniform destruction rate, compared to the non- uniform rate described for the case of high power level. In this particular case, during the first 10 sec of the heating process, there was a higher destruction than at high power level. This was a result of the energy

delivered by the microwave oven as discussed below. However, the final destruction at power level medium was less than at power level high, since at 140 sec there was a destruction of 5 log cycles, level which was reached after only 80 sec of processing at high power level. In addition, the heating process at medium power level was much less efficient from the food safety point of view, since at 140 sec there were still cells of *Salmonella* Enteritidis. Analysis of un-inoculated controls revealed no *Salmonella* present in the chicken thighs used in this study.

Influence of amounts of the Energy (Q)

There were differences in the energy output of the microwave ovens used between the Power levels 10 and 6 (high and medium) according to the Fig. 5. At 10 sec, the energy-time relationship was higher at Power level 6 than at Power level 10. Total power output of a microwave oven (cal/s or watts) determines the amount of energy absorbed by the food. Some microwave oven manufactures have adopted recommendations given by the International Microwave Power Institute (IMPMI), IEC (1998) and Schiffman (1987), and use a standardized test to measure power output.

When the chicken thigh was heated at high setting during 10 sec, lower numbers of *Salmonella* Enteritidis were destroyed more significantly than at medium setting. The oven used in this experiment gave more energy at medium setting than at high setting at time of 10 sec (Fig.5), and the temperature reached was higher too. Power level is a source of variability between ovens, because the pulsing time for a given power level may vary with the oven type (Schiffman 1990). Different power levels are achieved by varying magnetron pulse on and off times.

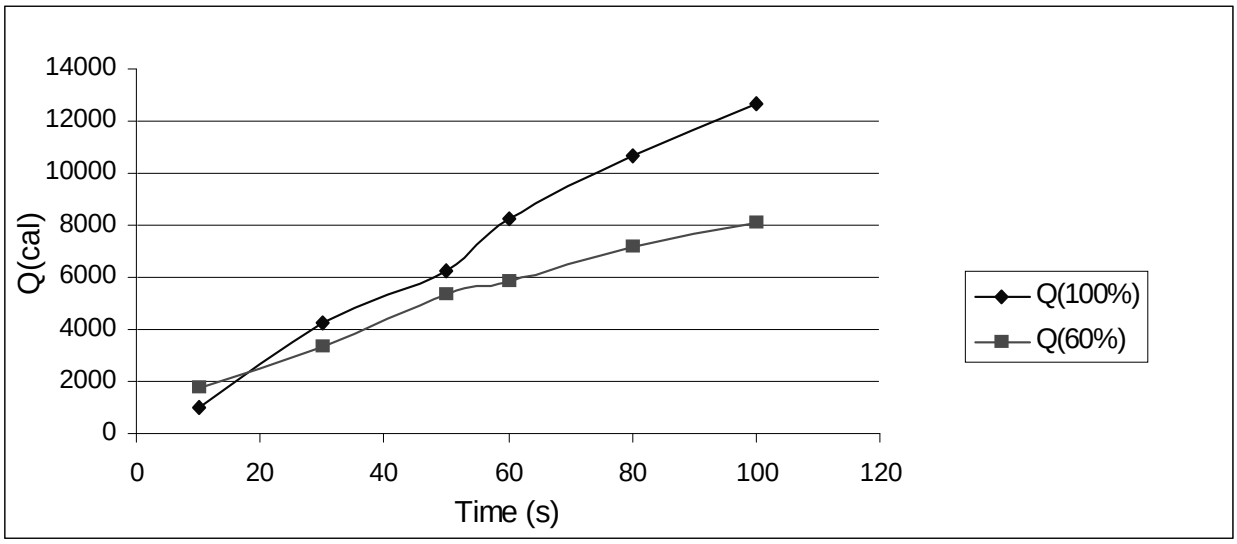

Figure 5 - Amount of Energy (Q) as function of Time.

CONCLUSIONS

The results of this investigation showed that the heating with microwave oven produced non uniform temperatures increase with time with average heating rate of 0.56ºC/sec and 0.32ºC/sec for power level high and medium respectively. The temperature difference between outside and inside of the chicken samples varied from 3ºC to 11ºC and this maximum value occurred at time 80 sec for both power settings. The results of the *Salmonella* Enteritidis counts were the same for both culture media employed; BSA and XLD.

Microwave heating showed to be very efficient on destruction of *Salmonella* Enteritidis depending on heating conditions: time and power level. Cell destruction was more efficient at high power level than at medium power level. At high power level no bacteria was detected after 110 sec but at

medium power level, bacterial cells were still detected after 140 sec.

RESUMO

Foi investigado o efeito do aquecimento por microondas sobre *Salmonella* Enteritidis inoculada em frangos frescos usando um forno de microondas doméstico (800 W) para determinar a destruição da *Salmonella* Enteritidis isolada a partir de carcaças de frangos, em relação com o tempo de aquecimento a dois níveis de potência: alta (nível 10) e média (nível 6); a relação entre tempo de aquecimento e temperatura também foi estudada. A destruição foi de 6 log em 95 s de tempo para o nível alto e 5 log em 140 s de tempo para o nível médio de potência. Depois de 110 s no nível de potência alta, não foi detectada sobrevivência de *Salmonella* Enteritidis em amostras de 100g de peso, porém, depois de 140 s a potência média, esse patôgeno nos alimentos ainda permanecia.

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