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THE EFFECT OF ACETIC ACID ON *SALMONELLA* SPP. IN MICROBIOLOGICAL MEDIA AND IN TURKEY CARCASSES

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K e y w o r d s: *Salmonella*, acetic acid, turkey carcasses, microbiological media.

A b s t r a c t

The studies aimed at determining the influence of acetic acid concentrations on *Salmonella* spp. in microbiological media and on turkey carcasses. The average number of bacteria in control samples without the supplement of acetic acid was for *S.* Enteritidis $1.3 \cdot 10^8$, *S.* Anatum $1.9 \cdot 10^8$ and *S.* Typhimurium $2.3 \cdot 10^8$. Acetic acid in agar medium at 0.1% concentration inhibited growth of studies *Salmonella* strains entirely. In case of acetic acid concentration of 0.05% the number of bacteria compared to the controls decreased by 6 logarithmic cycles. In case of 0.03% concentration the number of *S.* Anatum decreased by 5 logarithmic cycles while *S.* Enteritidis and *S.* Typhimurium by 4 logarithmic cycles. In the presence of 0.02% acetic acid *S.* Enteritidis and *S.* Typhimurium grew in numbers that were within the same logarithmic range, only *S.* Anatum decreased in number by one logarithmic cycle as compared to the controls. The results of studies obtained after immersing elements of turkey carcasses in acetic acid indicate that the recovery of *Salmonella* spp. from the samples depends on the inoculum of those bacteria in poultry carcass surface. In case of contamination with 10 colony forming units (cfu) of *Salmonella* spp. on the surface of a turkey carcass element and immersing it for 15 minutes in 1%, 1.5% and 2% acetic acid solutions a decrease in the number of samples from which those microorganisms were recovered as compared to the number of control samples was recorded. In case of contamination with 10^2 cfu on the turkey carcass surface and immersing it in tested 1%, 1.5% and 2% solutions of acetic acid for 15 minutes no influence on detection of *Salmonella* spp. was recorded. The inhibitory influence of acetic acid on *Salmonella* spp. was much more pronounced in case of the microbiological medium than in case of poultry carcasses on which satisfactory elimination of *Salmonella* spp. was not achieved.

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WPŁYW KWASU OCTOWEGO NA PAŁECZKI *SALMONELLA* W PODŁOŻACH MIKROBIOLOGICZNYCH I W TUSZKACH INDYCZYCH

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Słowa kluczowe: *Salmonella*, kwas octowy, tuszki indycze, podłoża mikrobiologiczne.

A b s t r a c t

Celem badań było określenie wpływu stężeń kwasu octowego na pałeczki *Salmonella* w podłożach mikrobiologicznych i na tuszkach drobiowych. Średnie liczby bakterii w próbkach kontrolnych bez dodatku kwasu octowego wynosiły dla *S.* Enteritidis – $1{,}3 \cdot 10^8$, *S.* Anatum – $1{,}9 \cdot 10^8$, *S.* Typhimurium – $2{,}3 \cdot 10^8$. Kwas octowy w podłożu agarowym o stężeniu 0,1% całkowicie hamował wzrost wszystkich badanych szczepów *Salmonella*. Gdy stężenie wynosiło 0,05%, liczba bakterii w porównaniu z kontrolą zmniejszyła się o 6 cykli logarytmicznych. Dla stężenia 0,03% liczba *S.* Anatum zmniejszyła się z kolei o 5 cykli logarytmicznych, a w przypadku *S.* Enteritidis i *S.* Typhimurium – o 4 cykle logarytmiczne. W obecności 0,02% kwasu octowego *S.* Enteritidis i *S.* Typhimurium rosły w liczbach mieszczących się w tym samym przedziale logarytmicznym, jedynie liczba *S.* Anatum w porównaniu z kontrolą zmniejszyła się o jeden cykl logarytmiczny. Wyniki badań uzyskane po zanurzeniu elementów tuszek indyczych w kwasie octowym wskazują, że wykrycie pałeczek *Salmonella* w próbkach zależy od inoculum tych bakterii na powierzchni tuszki drobiowej. Po kontaminacji 10 jednostek tworzących kolonie (jtk) pałeczek *Salmonella* na powierzchni elementu tuszki indyczej i po zanurzeniu jej na 15 minut w wodnych roztworach 1%, 1,5% i 2% kwasu octowego zaobserwowano zmniejszanie się liczby próbek, w których wykryto te drobnoustroje, w stosunku do liczby próbek kontrolnych. Po kontaminacji 10^2 jtk na powierzchni tuszki indyczej i zanurzeniu jej na 15 minut w badanych, wodnych 1-, 1,5- i 2-procentowych roztworach kwasu octowego nie stwierdzono ich wpływu na wykrywalność pałeczek *Salmonella.* Unieszkodliwiające działanie kwasu octowego względem bakterii *Salmonella* było zdecydowanie silniejsze w podłożach bakteryjnych niż w tuszkach drobiowych, na których nie uzyskano skutecznej eliminacji pałeczek *Salmonella.*

Introduction

During the recent years a minor decrease in the number of cases of bacterial food poisoning and infection has been recorded in Poland. In 2004 – 19.872 cases of that type were recorded, in 2005 – 20.065 while in 2006 – 17.264 and in 2007 – 15.241 cases. Nevertheless, cases of food poisoning caused by *Salmonella* spp. still represent the largest group among bacterial food poisoning cases. During the years 2004–2007 they represented in average 77.75% of the total number of bacterial food poisoning cases (*Sytuacja epidemiologiczna...* 2007).

Human and animal infections and contamination of food with *Salmonella* spp. result in high economic losses. They are the consequence of eliminating large numbers of people from work for the duration of the disease and additionally

costs of treatment of people, the costs of treatment of animals and destroying the contaminated batches of foods.

Control of *Salmonella* spp. in the environment is a standing problem for people dealing with prevention and treatment of people as well as animal and food production. Numerous methods concerning, e.g. slaughter, immunisation and application of bactericides have been developed and implemented. None of those methods, however, guarantied full success although all of them improved the hygienic conditions and food health safety.

Elimination of *Salmonella* spp. was tested on various experimental models using a diversity of chemicals, e.g. hexadecylpyridinium chloride (BREEN et al. 1997, WANG et al. 1997, RADKOWSKI and MIKOŁAJCZYK 2004), trisodium phosphate (WANG et al. 1997, XIONG et al. 1998), organic acids (DORSA et al. 1997, TAMBLYN and CONNER 1997, TSAI and INGHAM 1997, SMULDERS and UPMANN 2000, MIKOŁAJCZYK and RADKOWSKI 2002), hydrogen peroxide and sodium bicarbonate (RUSSELL et al. 1993). Not all of those methods proved effective. The medium is considered effective if under its influence the number of specific microorganisms decreases by 2 log (JETTON et al. 1992).

The possibility of applying additional substances in food processing are limited as a consequence of their negative influence on human body as well as difficulties with solubility and possibility of direct application. Care for quality and health standards of food forces application of only those chemicals in food technology that are classified as safe.

Organic acids and their salts, commonly considered safe, are among the preferred additives destroying the bacterial flora on carcasses (NETTON VAN et al. 1994, IZAT et al. 1989).

In Polish food industry application of the following organic acids and their salts is allowed: ascorbic, citric, lactic, acetic and tartaric (*Rozporządzenie Ministra Zdrowia...* 2008).

Acetic acid E 260 (at 4–7% concentration) is defined in food as an additive substance the acceptable daily intake (ADI) of which, according to the opinion of FAO/WHO experts on Food Additives does not require limiting, which means that it does not pose a hazard for consumer health, of course on condition that it satisfies specified purity criteria for that acid. As a consequence, it can be applied in food processing as an additive substance in quantities „quantum satis". Not determining any maximum level of that acid application in food production (including some products for infants and small children), acetic acid as an additive is applied according to the good manufacturing practice in doses not exceeding those necessary for achieving the intended goal on condition that the consumer is not mislead. According to the provisions of the regulation by the Minister of Health of the 18th of September 2008 on allowed additive substances, application of acetic acid is allowed

in some food products as acidity control or stabiliser, e.g. in Feta and Mozzarella cheeses, emulsified sauces, mustards, fish products, fruit and vegetable products excluding products of mushrooms and marinades, cured meat products, bakery products and meal concentrates (*Rozporządzenie Ministra Zdrowia*... 2008). Differentiating between the terms of "acetic acid" and "vinegar", knowing that vinegar is not an additive, manufacturers of food products containing acetic acid are required to mark their products according to the European Union legislation, including the Polish one, i.e. providing the basic information on presence of the above additive substance, specifying its technological function as well as the name and number E 260.

Considering that the acetic acid is legally allowed for application in food industry and understanding the complexity of the problem of search for methods of eliminating *Salmonella* spp. bacteria it was decided to embark on studies aiming at determination of different concentrations of that acid on *Salmonella* spp. in microbiological media and on poultry carcasses.

Materials and Methods

During stage one of the study, the influence of acetic acid in microbiological media on *Salmonella* spp.

The following concentrations of analytically pure acetic acid ($C_2H_4O_2$) in nutritive agar were applied: 0.01%, 0.02%, 0.03%, 0.05%, 0.1%, 0.25%, 0.5%, 1%, 1.5%, 2.0%. Strains: *Salmonella* Enteritidis no. 33/66, *Salmonella* Anatum no. 30/93 and *Salmonella* Typhimurium no. 227/84, obtained from the Museum of bacterial strains of the National Veterinary Research Institute in Puławy were the object of the study.

The acetic acid was sterilised using the Millipore filter (Millex 9P, 022 μ, Bedford), and next added at appropriate concentrations to the medium at the temperature of 50°C.

The studied strains were inoculated into 9 ml of nutritive agar and after 24 hours of incubation at 37°C the starter culture for further studies was obtained. Next ten times dilution of the culture was made and each strain from each dilution was inoculated on the nutritive agar without the chemical (control) and on the nutritive agar supplemented with different quantities of acetic acid. Surface inoculation was applied. Plates were incubated at 37°C for 24 to 48 hours. Tests for each strain was done in ten repetitions and averages for all repetitions were computed.

The second stage of the studies concerned analysis of acetic acid influence on *Salmonella* Enteritidis present in elements of turkey carcasses.

Tests were conducted on 236 samples of turkey breast purchased from

poultry processing plants. After delivery to the laboratory the material was kept in a refrigerator at 4°C, and next used for preparation of 25 g samples for further tests. In the tests for checking the natural presence of *Salmonella* spp. conducted on random samples (20% of all samples prepared for tests from each turkey breast purchased) no *Salmonella* spp. was detected. The other samples were contaminated purposefully. The strain of *Salmonella* Enteritidis no. 33/66 was used for the tests. That strain was first inoculated on nutritive broth and incubated at 37°C for 24 hours and then every sample was inoculated with 0.05 ml of 24-hour broth culture of *S.* Enteritidis diluted to from 10^{-4} to 10^{-8}. The initial inoculum of test samples was determined for each test series. The bacterial suspension was delicately spread with a special wide loop over the widest area possible. After inoculation with the bacteria, each sample was kept for 20 minutes in a refrigerator at 4°C aiming at full drying of the suspension. Next, each sample was transferred to sterile beaker with 250 ml of 1%, 1.5% and 2% solution of acetic acid for 15 minutes. From among the methods recommended for detection of *Salmonella* spp. on poultry carcasses, pluck and products the method given in the regulations (*Microbiology...* ISO 6579 1993, *Mikrobiologia...* PN-ISO 6579 1998) was applied.

Following 15 minutes in acetic acid solution, each sample was moved to a sterile beaker and covered with 225 ml of buffered peptone water (BPW, CM 509, Oxoid Basingstoke Hampshire, UK), and incubated at 37°C for 20 hours. Selective growth was achieved on the SC medium (SC, 0 687-17-1, Difco Laboratories Detroit MI, USA), Müller-Kauffman medium (MK, CM 343, Oxoid Basingstoke Hampshire, UK) and Rappaport-Vassiliadis medium (RV, CM 669, Oxoid Basingstoke Hampshire, UK) while the further culturing was done on brilliantine green and phenol red agar (BGA, CM 329, Oxoid Basingstoke Hampshire, UK) on BSA medium (BSA, 00 73-01-1, Difco Laboratories Detroit MI, USA) and on XLD agar (XLD, CM 469 Oxoid Basingstoke Hampshire, UK). Colonies typical and suspect of belonging to *Salmonella* spp. were identified by serological and biochemical methods. Biochemical characteristics of *Salmonella* spp. were determined using API Test 20 E. Serological types were determined on the basis of the Kauffmann-White classification scheme as proposed by Popoff and Le Minor using the sera produced by the National Salmonella Centre.

Turkey breast samples contaminated with *Salmonella* spp. immersed in sterile water for 15 minutes were the controls.

Each variant of the experiment was done in ten repetitions.

The data collected from the experiment was processed statistically using T – Student test and correlation analysis. Correlation analysis was conducted on log values.

Results and Discussion

The results of studies obtained are presented in Table 1, Table 2 and Figure 1.

Assessment of acetic acid influence on *Salmonella* spp. in microbiological medium is presented in Table 1 and Figure 1.

Table 1

Growth of *Salmonella* spp. on agar medium supplemented with acetic acid ($n=10$)

Salmonella spp. type	Acetic acid concentration (%)										
	0.00	0.01	0.02	0.03	0.05	0.1	0.25	0.5	1	1.5	2
	number of colonies (cfu/ml)										
S. Anatum	$1.9 \cdot 10^8$	$1.6 \cdot 10^8$	$8.0 \cdot 10^7$	$9.3 \cdot 10^3$	$1.0 \cdot 10^2$	0	0	0	0	0	0
S. Enteritidis	$1.3 \cdot 10^8$	$1.6 \cdot 10^8$	$1.7 \cdot 10^8$	$1.0 \cdot 10^4$	$1.0 \cdot 10^2$	0	0	0	0	0	0
S. Typhimurium	$2.3 \cdot 10^8$	$2.3 \cdot 10^8$	$2.0 \cdot 10^8$	$1.0 \cdot 10^4$	$1.0 \cdot 10^2$	0	0	0	0	0	0

Table 2

Number of samples from elements of turkey carcasses treated with acetic acid solution, in which *Salmonella* Enteritidis was detected ($n=10$)

Concentration (%)	Treatment time (minutes)	*Salmonella* Enteritidis no 33/66				
		dilution (inoculum)				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
		number of positive results				
0	15	10	10	10	10	0
1	15	10	10	10	8	0
1.5	15	10	10	10	3	0
2	15	10	10	10	3	0

The data presented in Table 1 indicates that the average numbers of cfu in control samples without acetic acid addition were as follows for *S.* Enteritidis $1.3 \cdot 10^8$, *S.* Anatum $1.9 \cdot 10^8$ and *S.* Typhimurium $2.3 \cdot 10^8$.

Acetic acid in agar medium at the concentration of 0.1% inhibited entirely the growth of all tested strains of *Salmonella*. At the concentration of 0.05% the cfu number decreased by 6 log cycles as compared to the controls. At the concentration of 0.03% the number of *S.* Anatum cfu decreased by 5 log cycles and in case of *S.* Enteritidis and *S.* Typhimurium by 4 log cycles. In presence of 0.02% of acetic acid *S.* Enteritidis and *S.* Typhimurium grew in numbers that were within the same logarithmic range, only the number of *S.* Anatum cfu decreased by 1 log cycle as compared to the controls.

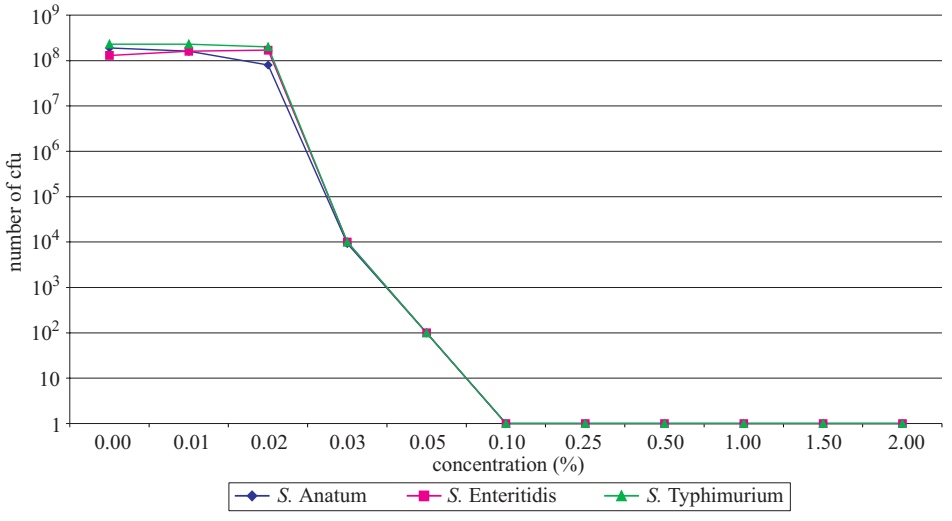

Fig. 1. *Salmonella* spp growth on agar medium supplemented by different acetic acid concentrations

The results of studies on the effect of acetic acid on *Salmonella* Enteritidis on elements of turkey carcasses are presented in Table 2.

Analysing the results of studies obtained after immersing elements of turkey carcasses in acetic acid it is easy to notice that detection of *Salmonella* spp. from samples depends in the inoculum of the bacteria no poultry carcass surface. In case of contamination with 10^1 cfu of *Salmonella* spp. on the turkey carcass surface and immersing it for 15 minutes in 1%, 1,5% and 2% water solutions of acetic acid a decrease in the number of samples in which that microorganism was detected as compared to the controls was observed.

In case of contamination with 10^2 cfu on the turkey carcass surface and immersing it for 15 minutes in tested 1%, 1,5% and 2% water solutions of acetic acid no influence on detection of *Salmonella* spp. was recorded.

The results of own studies show that the effectiveness of anti-bacterial influence of acetic acid at different concentrations on *Salmonella* spp. in microbiological media was high. Unfortunately, in case of applying the tested concentrations of acetic acid against *Salmonella* spp. present on turkey carcasses no effective elimination of *Salmonella* spp. was obtained. Only the number of samples in which the microorganism was detected decreased as compared to the controls.

Obtained results of own studies (Table 2) are not consistent with the data published by other authors (CONNER et al. 1997, DORSA et al. 1997, OKREND et

al. 1986, TAMBLYN and CONNER 1997). Acetic acid at tested concentration showed poor eliminating influence on *Salmonella* spp. present in poultry carcasses.

So far conducted studies (DICKSON et al. 1994, DORSA et al. 1997, LILLARD 1994, OKREND et al. 1986, TAMBLYN and CONNER 1997) on elimination of *Salmonella* spp. from poultry carcasses by chemical means aimed, during the first stage, at liquidating the accompanying microflora, e.g. by means of ultraviolet radiation, and only then contaminating them with *Salmonella* spp. As a result it was possible to apply non-selective media that allowed detection of *Salmonella* spp. damaged by chemical substances (CONNER and BILGILI 1994). All the referred studies show that the experimental model applied in them was far from the actual contamination of carcasses with *Salmonella* spp. In own studies the influence of 1%, 1,5% and 2% of acetic acid on *Salmonella* spp. under conditions closest to the natural, i.e. on carcasses originating directly from processing plants not subjected in the laboratory to any processes aiming at liquidation of accompanying microflora was studied.

In the available literature there is no detailed information on the influence of acetic acid on different initial numbers of *Salmonella* spp. on turkey carcasses (TAMBLYN and CONNER 1997, HWANG and BEUCHAT 1995).

The data obtained from own studies indicate that anti-bacterial effectiveness of acetic acid is diversified and depends on the initial number of *Salmonella* spp. on turkey carcasses.

The *Salmonella* spp. eliminating mechanism in case of acetic acid involves the presence of dissociated particles and low pH of acids (CONNER and BILGILI 1994).

Works by numerous authors (DICKSON 1992, DICKSON and ANDERSON 1992, TAMBLYN and CONNER 1997, SAWAYA et al. 1995) indicate that the higher the concentration of organic acids the higher their effectiveness against *Salmonella* spp. In case of high concentrations of acids in disinfecting solutions organoleptic changes in the carcasses appear (KOTULA and THELAPPURATE 1994). TAMBLYN and CONNER (1997) noticed changes of that type in case of test concentrations of acids equal to or exceeding 2%.

Conclusions

1. Eliminating influence of acetic acid on *Salmonella* spp. was definitely stronger in microbiological media than in poultry carcasses.

2. The inhibitory influence of acetic acid on *Salmonella* spp. in microbiological media was high. The acid in agar medium at 0.1% concentration inhibited growth of all tested strains of *Salmonella* entirely.

3. *Salmonella* spp. present on turkey carcasses showed a relatively low sensitivity to the influence of 1%, 1,5% and 2% concentrations of acetic acid and no effective elimination of those microorganisms was obtained.

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