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UNIVERSITY OF PADOVA
DEPARTMENT OF ENVIRONMENTAL MEDICINE AND PUBLIC HEALTH
Laboratory of Environmental Epidemiology
Via Loredan, 18 – 35131 Padova - Italy
Tel.: +(39) 049 827 5399 – Fax: +(39) 049 827 5392

Padova, 19 February 2004

Sital Klima
Via L. da Vinci, 26
31021 Mogliano Veneto (TV)

REPORT ON THE MICROBICIDAL ACTION OF AIR IONISERS AND MEASUREMENT OF OZONE EMISSION

A number of the company's products were tested to assess their microbicidal action and measure the quantity of ozone emitted.

The air ionisers had the following characteristics:

to measure ozone production, model C was used, with two tubes of 2 W each (total power 4 W), power supply 230-250 V.

for assessing microbicidal activity, models F + C were used, 1 + 1 tubes (power tube F = 7 W, power tube C = 2 W, total power = 9 W); power supply 230-250 V.

EXPERIMENTAL METHOD

Microbicidal action

To assess the microbicidal action of the ionisers, their efficacy on the following strains of microbe was tested:

- *Staphylococcus aureus* ATCC 29213
- *Escherichia coli* ATCC 25922
- *Saccharomyces cerevisiae*

The appliance was placed in a temporarily unused room in the microbiology laboratory in the Hygiene building of the Padua University Department of Environmental Medicine and Public Health. The room measured about 75 m^3 and contained all the usual laboratory furniture and fittings.

The ioniser was hung upside down, about 1.5 m from the surface of a table. On the table, the open Petri plates containing a culture terrain on which the microorganisms had been placed were exposed.

The microorganisms were exposed to ionisation for various periods of time - 3 hours, 8 hours and 24 hours.

Each test included a control Petri plate which was obviously not exposed to ionisation, but kept in the same environmental conditions and for the same period of time as the experiments in a nearby room, covered and resting on a table.

Ten Petri plates were set up for each test and each microorganism, plus 10 for the control.

The experiments followed the schedule below:

First cycle

F model ioniser with pulse knob on "minimum" and windows closed.

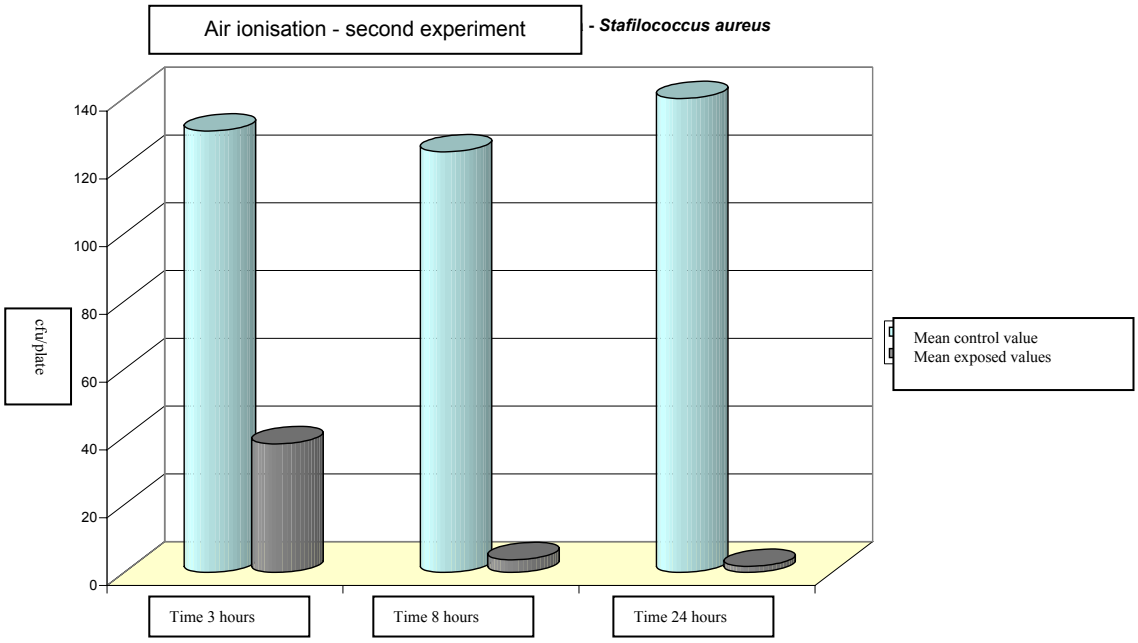
The table gives the mean values obtained during the first experiment. As a result of the incorrect dimension of the ioniser, there was no detectable effect.

Staphylococcus aureus	**Mean control value**	**Mean exposed value**	**Reduction %**
Time 0	1.3	---	
Time 3 hrs.	0.8	1.4	-75.00
Time 8 hrs.	1.3	1.8	-38.46
Time 24 hrs.	0.7	1.7	-142.86
Escherichia coli	**Mean control value**	**Mean exposed value**	**Reduction %**
Time 0	3.4	---	
Time 3 hrs.	3.9	3.9	0
Time 8 hrs.	4.8	4	16.67
Time 24 hrs.	2.9	2.3	20.69
Saccaromices cerevisiae	**Mean control value**	**Mean exposed value**	**Reduction %**
Time 0	186.7	---	
Time 3 hrs.	98.5	113.5	-15.23
Time 8 hrs.	175.2	179.4	-2.40
Time 24 hrs.	110.2	109.1	1.00

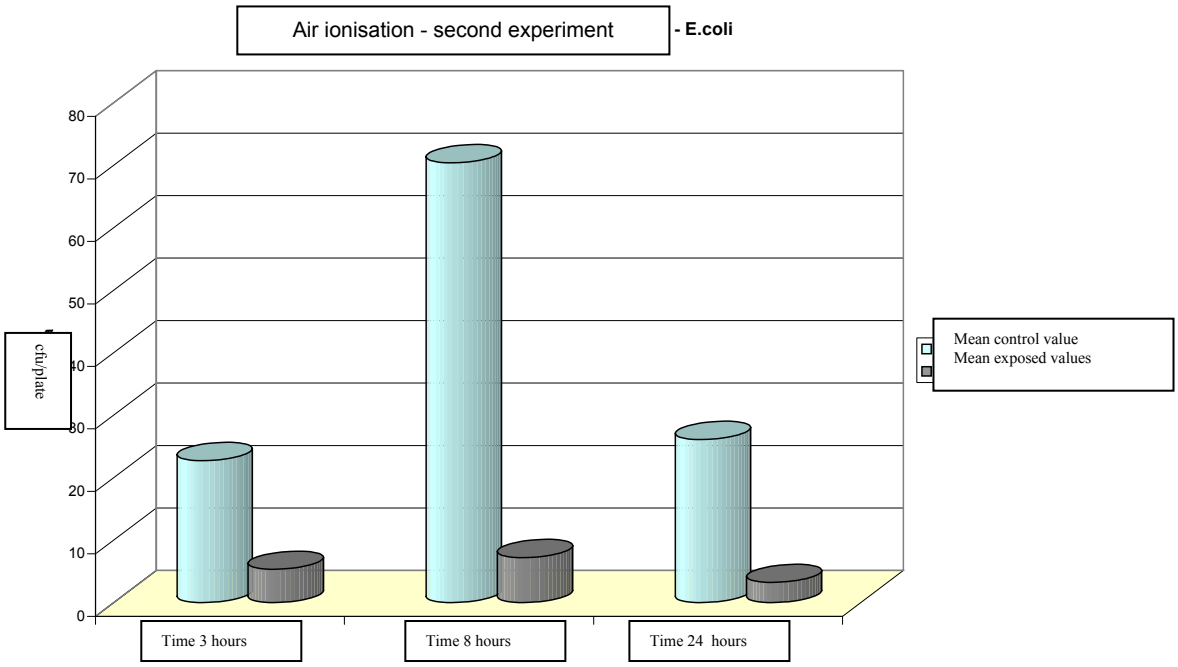
Second cycle

The F model ioniser was supplemented by a second C model appliance. A window was kept partly open. The data reported represent the mean values obtained after the second test with the addition of a second appliance to supplement the model used in the first test. In this case, a considerable reduction was obtained with all three strains considered.

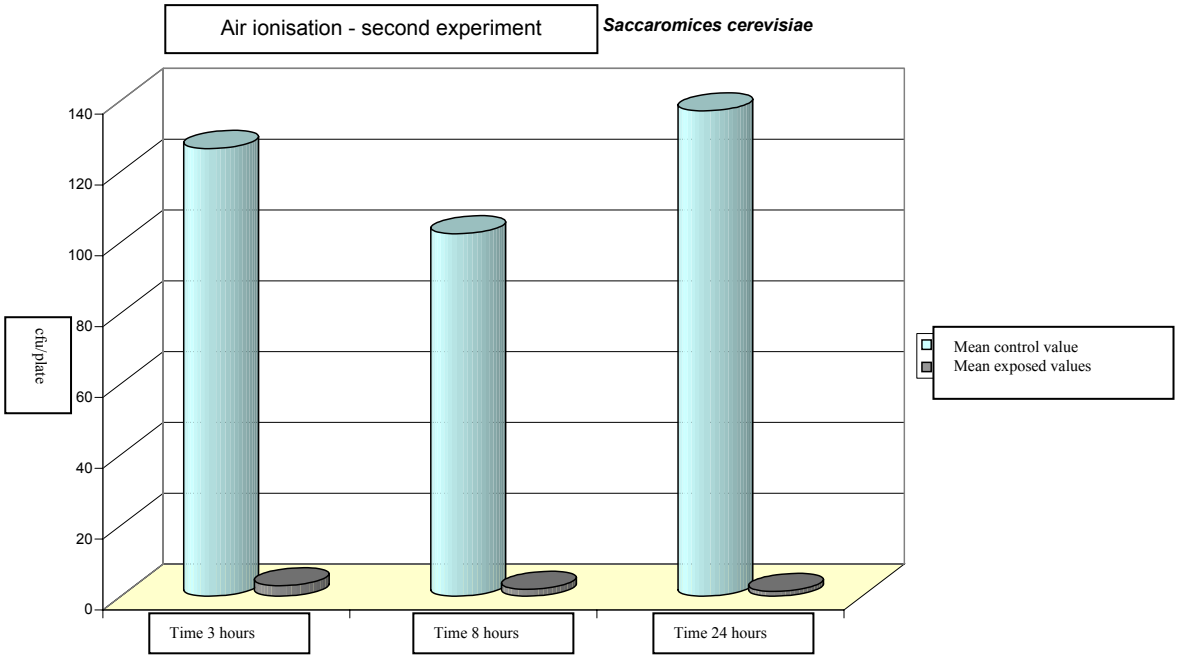
Staphylococcus aureus	**Mean control value**	**Mean exposed value**	**Reduction %**
Time 0	122.4	---	
Time 3 hrs.	130.2	37.9	70.89
Time 8 hrs.	124.1	3.7	97.02
Time 24 hrs.	139.8	1.7	98.78



Escherichia coli	Mean control value	Mean exposed value	Reduction %
Time 0	16.8	---	
Time 3 hrs.	22.7	5.3	76.65
Time 8 hrs.	70.4	7.2	89.77
Time 24 hrs.	26.1	3.2	87.74



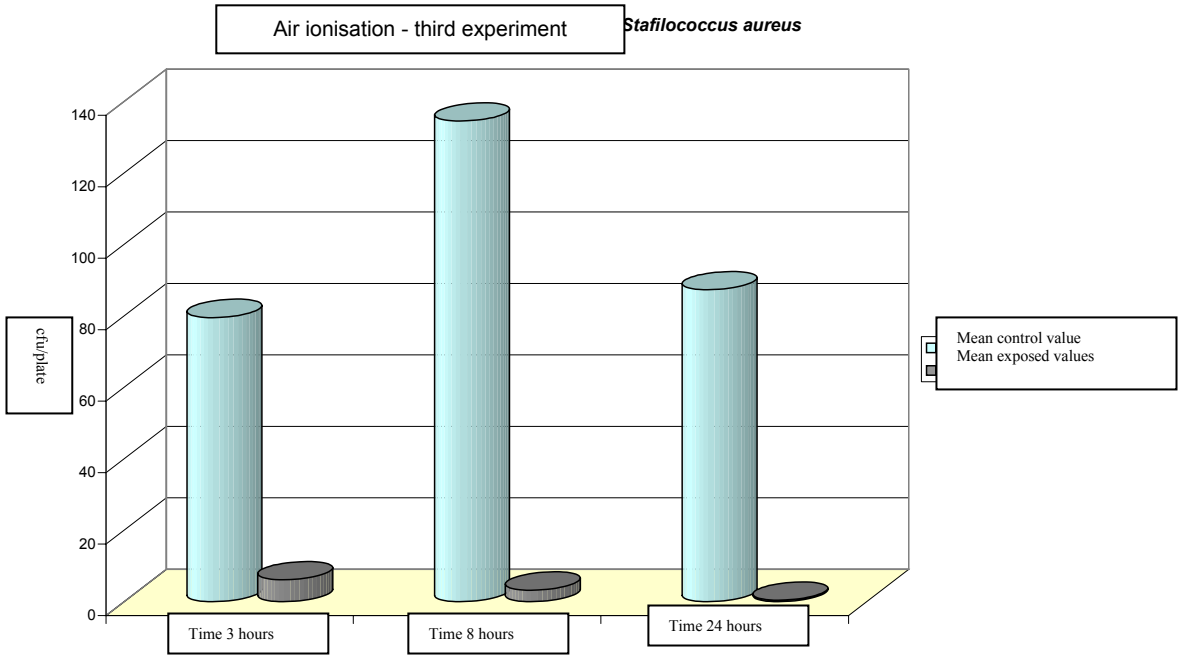
Saccaromices cerevisiae	Mean control value	Mean exposed value	Reduction %
Time 0	116.2	---	
Time 3 ore	126.4	2.9	97.71
Time 8 ore	102.3	1.9	98.14
Time 24 ore	137	1.3	99.05



Third cycle

The same ionisation conditions as the second cycle, but with the windows closed. The third test was performed using *Staphylococcus aureus* only to verify the second test which had shown a reduction after 3 hours in line with the other strains of microbes. A considerable reduction after just 3 hours was confirmed.

Staphylococcus aureus	**Mean control value**	**Mean exposed value**	**Reduction %**
Time 0	75.1	---	
Time 3 hrs.	79.5	6.1	92.33
Time 8 hrs.	134.5	3.2	97.62
Time 24 hrs.	87.3	0.4	99.54



Culture broths were produced to seed the microorganisms - Tryptic Soy Broth for the *Staphylococcus aureus* and *Escherichia coli* and Sabouraud Broth for *Saccharomyces cerevisiae*. The surface of the Petri plates containing terrain suitable for the growth of the microorganisms concerned was seeded with 0.1 ml of the appropriately diluted specific broth.

PCA (Plate Count Agar) was used for *Staphylococcus aureus* and *Escherichia coli* and Sabouraud Agar for *Saccharomyces cerevisiae*.

At the end of each experiment, the plates were incubated at an appropriate thermostatically controlled temperature - for *Staphylococcus aureus* and *Escherichia coli,* 37°C, for *Saccharomyces cerevisiae* 30°C.

RESULTS

The accompanying tables summarise the results obtained after exposure of the Petri plates contaminated with controlled quantities of microorganisms to the ionising action.

In particular, Table 1 gives the results obtained for the first cycle of experiments involving *Stapylococcus aureus* (page 1), *Escherichia coli* (page 2) and *Saccharomices cerevisiae* (page 3).

With reference to the second cycle of experiments, Table 2 gives the data for *Stapylococcus aureus* (page 1), *Escherichia coli* (pages 2 and 3) and *Saccharomices cerevisiae* (pages 4 and 5).

Finally, Table 3 gives the results obtained with *Stapylococcus aureus* in the third cycle of experiments.

DISCUSSION

First cycle

Before starting the experiments, Petri plates containing Sabouraud Agar were exposed in order to assess the presence of microbes in the environment, with particular attention to moulds. The results of this exposure showed a mean presence of moulds of about 20 CFU per plate including moulds and bacterial colonies.

Subsequently the ionisation experiments were carried out as described above.

In the first cycle of experiments, the ioniser had no appreciable microbicidal action.

The colony count for the three microorganisms tested showed no quantitative reduction between the controls and the exposed plates. In some cases there was, in fact, an increase (although not substantial) in the microbial forms on the exposed plates.

The exposed plates also contained species of mould and bacteria other than those tested. This could indicate poor efficacy not just on the microbial forms considered, but also on those in the environment.

Second cycle

For the second cycle of experiments, the conditions were modified, increasing the ionising power by adding a second appliance, giving a total power of 9 W. A window was also left partially open to permit an appreciable change of air.

Unlike the first cycle, the ionisation in these experiments was shown to have a considerable microbicidal action.

In fact, as shown in Table 2 (pages 1, 2, 3, 4, 5), the reduction in all three microorganisms tested was consistent and in some cases more than 90%.

It must, however, be noted that during these experiments, the exposed plates were also contaminated by microorganisms from the environment, thus increasing the number of microbial forms present.

However, even taking this new contamination into consideration, the quantitative difference between the control plates and exposed plates remained consistently in favour of the latter.

Third cycle

The third cycle of experiments considered the ionising action on *Staphylococcus aureus* in conditions identical to those used for the second cycle of experiments, except that the window was closed.

The results obtained confirm those of the second cycle.

With the window closed, the contamination by exogenous microbial forms was noticeably less, as can be seen in Table 3.

Measuring ozone

EXPERIMENTAL METHOD

For the first experiment, the Model C appliance was set up in a room with a volume of about 200 m³ used as a study and with the door open. For the second experiment, it was set up in a smaller room (about 60 m³) with a closed door.

The ozone was determined by three samplings of the environment using Radiello chemical absorbent cartridges (Fondazione Maugeri) and subsequent spectrophotometric reading.

The experiments were preceded by sampling without the ioniser in operation in order to assess the baseline level. Two experiments were carried out, one for each room, each lasting 24 hours.

The ozone was then measured with the appliance in operation in the first room for an exposure period of 20 hours. It is important to note that the door was left open for just four hours. As a result of the odour produced by the appliance, the door was then closed.

The second experiment was carried out in a smaller closed room and lasted 24 hours.

RESULTS

The results obtained, expressed as means for the exposure time, are given in the following table:

Room	Exposure (hours)	Ozone µg/m^3
Room 1 (baseline level) m^3 200	24 open door	Not detectable
Room 1	20	60.8
Room 2 (baseline level) m^3 60	24 close door	Not detectable
Room 2	24	94.2

DISCUSSION

They have been tested oversized equipments in order to check that, also in extreme conditions, the ozone production was compatible with the presence of people.

The experiments carried out to measure the ozone produced by the appliance indicate that production is within the legal limits.

CONCLUSIONS

In the experiments performed after the first cycle, the appliances tested were shown to be efficacious in reducing microbial loads.

This difference is probably due to the increase in ionisation power, resulting in a stronger action against the microorganisms.

OFFICER IN CHARGE OF ANALYSES
(Prof. Giorgio Moretti)

OFFICER IN CHARGE OF LABORATORY
(Prof. Giuseppe Rausa)

UNIVERSITA' DI PADOVA

Department of Environmental Medicine and Public Health
Hygiene Building

Via L. Loredan, 18 - 35131 PADOVA - Tel. **+39 049 8275388** - Fax **+39 049 8275392**

Padova, 24 March 2004

Sital Klima
Via L. da Vinci, 26
31021 Mogliano Veneto (TV)

Re: - Experiments to identify moulds in the air
- Experiments to evaluate the efficacy of the ioniser on *Legionella*.

1. Experiments to identify moulds in the air

The quantity of airborne moulds in the rooms where the ionisation experiments would be carried out was measured.

Six open Petri plates containing Sabouraud Destrose Agar were placed in room A where the ioniser (on continuously) had been positioned. The side-hinged window was left open to allow a regular flow of air. After three hours of exposure, three of the exposed plates were taken, closed and placed in a thermostatically controlled temperature of 30°C, while the other three were removed after an exposure time of 24 hours.

The same procedure was performed in room B, with the only difference that there was no ioniser.

Table 1 gives the results obtained.

Room 1 with ioniser		**Room B without ioniser**	
3h	24h	3h	24h
3m + 4b	20m	15m + 4b	17m + 4 b
9m	9m	16m + 3b	7m + 4b
1b	6m	11m + 4b	18m + 3b

m = moulds b = bacteria

Table 1

The results showed that moulds were also present in the room containing the functioning ioniser, but the quantities were lower than in room B without ioniser.

2 Experiments to evaluate the efficacy of the ioniser on *Legionella*.

An experiment was set up to evaluate the efficacy of the ioniser against *Legionella pneumophila* in aqueous suspension.

A litre of water was contaminated with *Legionella pneumophila ssp pneumophila* ATCC 33152 in a quantity of 1,920.000 CFU/l.

The ioniser supplied by SitalKlima was placed in this water and turned on. Three series of 0.1 ml samples were taken from the water after 5, 15, 30 and 60 minutes and then examined for the presence of *Legionella* by seeding in BCYE α Agar. The colonies were counted after incubation for 7 days at 37°C in the presence of 2.5% CO_2.

Before inoculating the *Legionella* in the water, the absence of this microorganism in the water itself was verified.

The results are summarised in Table 2.

	CFU/0.1 ml	**CFU/L**
Negative control	0 – 0 - 0	0
Positive control	191 – 217 168	1.920.000
After 5'	180 – 164 -161	1.680.000
After 15'	3 – 5 - 5	43.000
After 30'	0 – 0 – 0	
After 60'	0 – 0 – 0	

The results show a considerable and rapid reduction in microbial load. After just 30 minutes *Legionella* was no longer detected in the samples examined.

OFFICER IN CHARGE OF ANALYSES
(Prof. Giorgio Moretti)

OFFICER IN CHARGE OF LABORATORY
(Prof. Giuseppe Rausa)

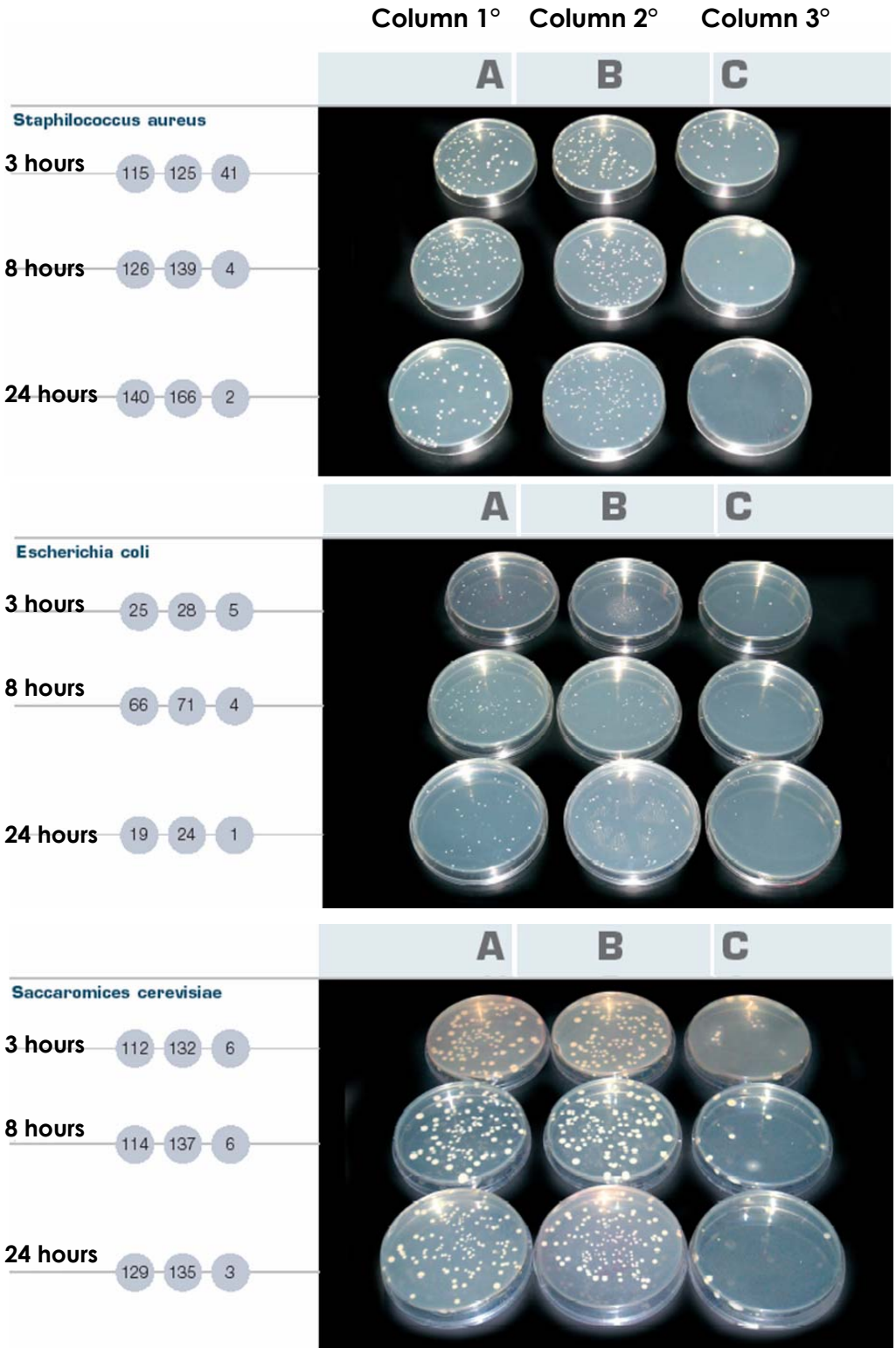
Experimental method adopted

Photograph of the reduction in microbal content inoculated in plates exposed to the effect of a BIOXIGEN air ioniser.

A Zero plate with CFU inoculated

B Control plate not exposed to treatment

C Plate exposed to BIOXIGEN treatment



Università degli Studi di Udine

Use of an ioniser to reduce the surface contamination of structures and equipment used in the food industry.

Giuseppe Comi, Milena Osualdini, Marisa Manzano, Andrea Lovo[1], Nadia Bortolussi[1], Alessandro Berton[2] and Giacomina Bustreo[2]

Dipartimento Scienze degli Alimenti, Università degli Studi di Udine, via Marangoni 97, 33100 Udine.
[1]Alimentaria srl, Via Rizzardi, 95, 30175 Venezia-Marghera (Venice).
[2]Sistema Bioxigen, SitalKlima srl, Via L. da Vinci, 26, 31021 Mogliano Veneto (Treviso).

SUMMARY
The aim of this study was to reduce the microbial load on surfaces in food storage environments and equipment used to store and handle foods by means of static and dynamic ionisers (Bioxigen system, SitalKlima) The treatment involved ionising the air in a room containing deliberately contaminated panels, glasses and plates simulating the surfaces of environments and equipment used in the food industry. The tests were performed using ***Escherichia coli***, ***Listeria monocytogenes*** and ***Saccharomyces cerevisiae***. The results showed that the treatment produced a mean percentage reduction of greater than 99% in the microbial populations inoculated. The post-treatment counts enabled a decontamination level of less than or equal to 3-4 log units to be achieved. The residual contamination, consistently less than 1 log unit/$cm^{2,}$ is considered optimum for surfaces in production facilities or in contact with food.

INTRODUCTION
Sanitation of the production environment and equipment is one of the most effective ways of preventing and reducing the risk of diseases produced by food. Traditional methods involve cleaning and sanitation techniques based on the use of detergents and disinfectants. In particular, sanitisers are sprayed on the surfaces of the production equipment and environment and left in contact for a length of time varying according to the type of microbe involved. After cleaning and rinsing in drinking water, equipment may be completely immersed in disinfectant. In both cases, the period of contact depends on the concentration and type of disinfectants used. After contact with the disinfectant, the surfaces are rinsed in drinking water to eliminate residual disinfectant which could be absorbed by the food and eaten by man, causing allergic symptoms.
Although efficient in eliminating microorganisms and pathogens from production environments, these techniques may have limits due to factors intrinsic in the surfaces to be sanified or the microorganisms. The efficacy of sanitation is, in fact, often correlated to the composition and type of surface (smooth, rough, engraved or with abrasions) and the microorganisms which may either be "resting" on the surfaces or attached to them by protein structures such as capsules, fimbriae or pili, or by production of glycocalyx or biofilm (30). Pathogenic microorganisms such as *L. monocytogenes*, *Salmonella* spp. or *E. coli* O157H7 may be present and develop in cracks, abrasions or the rough surfaces of equipment or structures in food processing facilities. Sometimes the sanitisers distributed in aqueous phase on surfaces with these characteristics may fail to reach and inactivate the pathogens located there. The surface may therefore be responsible for transmitting dangerous germs to the food. In recent years, it has become common practice to apply sanitisers in the form of aerosol sprays.
An aerosol spray disperses a liquid or solution in the air in the form of very small drops. The technique was initially used for therapeutic purposes to combat *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus fumigatus* (18,28,35,38) and to disinfectant environments (10,19). An aerosol spray is also commonly used to disinfect food production environments or animal rearing establishments. In these cases, its use has enabled food-associated disease to be reduced and the productivity of animal rearing establishments to be increased (10,34,37). Various authors (10,20) have amply demonstrated that application of lactic acid solutions by aerosol spray in animal rearing establishments enables infectious diseases of poultry to be controlled. The efficacy is apparently explained by the aerosol technique itself. It enables the antimicrobial agent to be distributed in the form of small drops (< 2 μm) which reach all parts of the substrate to be treated, including the most hidden and difficult to reach even with spray systems. Using a mixture of acetic acid and hydrogen peroxide applied by aerosol spray, Oh et al., (30) managed to reduce populations of pathogenic microorganisms deliberately inoculated on surfaces simulating food production environments by a number of log units. In particular, they showed that distributing both antimicrobial agents via an aerosol for about one hour onto the surfaces reduced the population of *Bacillus cereus* by about 3.09 log units, *Listeria innocua* by 7.69 log units, *Staphylococcus aureus* by 6.93 log units and *Salmonella typhimurium* by 8.18 log units. The authors chose these antimicrobial agents as they are not corrosive, have a wide spectrum of action and

rapidly break down into acetic acid, oxygen and water. More importantly, they also demonstrated that the effect of these antimicrobial agents was enhanced by use of an aerosol appliance which dispersed drops of about 2 µm in size.
Gaseous sanitisers are another powerful means of decontaminating air and surfaces. This technique is widely used and effective (14,15,16), although it does have disadvantages such as the need for sophisticated equipment to generate the gas and the small number of molecules which can be used. Ozone is an innovative molecule used to decontaminate pathogenic or altering microorganisms from contaminated surfaces or environments. Recently, a number of authors have developed methods involving the use of ozone to inactivate plant life or spores of various microorganisms of interest to the food sector (17,21,22,23,24,25). Finally, ionisation may be considered a valid system to decontaminate the surfaces of food or food production environments. Recently, an ioniser was used to reduce the airborne microbial load in salting, pre-curing and curing environments used to produce San Daniele prosciutto (6) . The treatment involved ionising the air in each room for 24 hours with a Maia static ioniser (Bioxigen system SitalKlima). The experiment showed that the treatment produced a definite reduction in the airborne fungi and bacterial populations in the environments examined. The decrease in microbial load depended on the initial level and the environment considered, but the mean reduction was in any case 47-52% of the initial load. The post-treatment counts enabled contamination level of less than 200 CFU/m3 to be reached, a level considered as optimum for these production environments.

Principal phases in the production of San Daniele prosciutto

Operation	Processing rooms		Mean length of process in days	Mean cumulative length in days
	Temperature	Temperature		
Trimming, weighing and chilling	2/3°C	90-95%	1	1
Salting	2/3°C	90-95%	15	16
Pressing	4/5°C	70%	2	18
Pre-resting	4/6°C	70-75% 80-85%	21	39
Resting (cleaning, refreshing and washing)	from 4/6°C		54	93
	to 8/10°C			
Drying	20/27°C	90%	8	101
Pre-curing	12/14°C	85-80%	37.5	138.5
	14/19°C	75-70%		
Curing and filling	15/22°C	70-80%	240	378.5

Percentage of microorganisms rendered inactive by treatment with the Bioxigen ionisation system

Room	Moulds/Yeasts	Bacteria
Salting	49.0	50.0
Pre-curing	49.5	48.0
Curing	47.5	53.2

On the basis of these figures, we wanted to assess the potential of this type of treatment against microbial populations on the surface of food production environments and equipment. In particular, the aim was to reduce or completely inactivate strains of ***Listeria monocytogenes***, ***Escherichia coli*** and ***Saccharomyces cerevisiae*** deliberately inoculated on surfaces simulating food production equipment or environments. Study of the effects of ionisation on *Listeria monocytogenes* was particularly important as for some time the research group which I coordinate has been trying to produce and optimise effective, innocuous methods permitted by Italian and the EEC legislation to eradicate this microorganism from food production environments and equipment. In particular, we have focused on the development of methods to eradicate *Listeria monocytogenes* from the surface of San Daniele prosciutto and the environments and equipment used to produce it. The aim was and is to achieve the "0" tolerance required by legislation in the USA (9,11) and EC (including Italy) first by EEC regulation no. 2073/2005 (15/11/05) GUCE L338/1 of 22/12/05. Now in Europe, this regulation brings the limit to 100 *Listeria monocytogenes*/g at the end of a food product's shelf life. Prosciutto is not a listeriosis risk. During the production process, the slow and constant reduction of aw prevents the development of microorganisms such as *L. monocytogenes* which may be accidentally present on the surface of the product. As ageing continues, these microorganisms may be inactivated (7,13). However, *L. monocytogenes* may re-contaminate prosciutto during the phases preceding selling. In particular, if performed in environments or with equipment which are inadequately sanified, deboning or slicing techniques may encourage this recontamination. However, due to its low aw (< 0.90 ± 0.01), neither sliced nor deboned prosciutto can support the development of pathogens including *L. monocytogenes* (7, 13).
To prevent this contamination, the food industry has implemented severe systems such as HACCP (Hazard Analysis Critical Control Point), GMP (Good Manufacturing Practise) and SSOP (Standard Sanification Organisation

Program) and this has led to a definite reduction in the presence of *L. monocytogenes* in production environments, particularly those where prosciutto is produced (5, 9,11). The efficiency and efficacy of HACCP and SSOP plans are demonstrated by the fact that the percentage of *L. monocytogenes* isolated in prosciutto has dropped over the years and the risk of producing disease in consumers has therefore decreased. However, much still remains to be done before it is completely eradicated (4,9,11,13). The work environment and equipment are still one of the main sources of food contamination by *L. monocytogenes* and other pathogens. As a result, producers are always asking for research into innovative and more effective sanitation techniques. These techniques must, however, be productive, they must be inexpensive, of low environmental impact and compatible with the presence of operatives. Ionisation seems to respect these prerequisites.

To date, it has been widely used to ionise the air in private homes, institutions and industrial environments. The negative ions produced eliminate particulate, pollens, microorganisms and pollution in general (1,2,12,26,29,32,36). They therefore have a positive effect on the prevention of diseases such as asthma and allergies (2,3,27,29,31,33,39). They can also affect the mood of people. It is known that depression, nausea, insomnia, irritability, tiredness, migraine and asthma attacks can be caused by the excessive presence of positive ions in the atmosphere. In fact, meteopathic people often change mood following a storm due to the presence of hot dry winds which carry the positive ions in the air (27,31,35). According to doctors and hygienists, negative ions improve the immune system, increase the body's capacity to use the oxygen present in the air, improve the lung's capacity to eliminate pollutants, make you breathe more easily, improve sleep, reduce stress, increase attention and reduce blood platelet aggregation. As a result, they suggest living in a pollutant-free environment or using an ioniser to decontaminate the air. Over and above these considerations and suggestions to improve human life, reducing airborne microbial contamination is of vital importance for the food industry. This can be achieved by using closed rooms, known as clean rooms, where the air introduced is previously sterilised by filtration or if the space is exposed to frequent contact with the outside, by physical means such as steam, ionisation, ionising radiation, UV rays or infrared radiation (26). In particular Corry and Mead (11) suggest that ionising the air in cold storage rooms has a positive influence on the microbial load of meat. They quote figures showing that the microbial load of meat kept in cold storage rooms with ionised air is less than that of meat kept in cold storage rooms without any treatment. On the basis of these considerations, we therefore wanted to verify whether the use of an air ioniser (Bioxigen from Sital Klima) could reduce the microbial load on the surfaces of equipment and environments involved in food production.

MATERIALS AND METHODS

A) Experimental ionisation of a panel simulating the wall of a food production environment. The test was repeated 3 times (3 experiments).

A panel measuring approximately 1500 cm^2 (30 x 50 cm) was prepared. It was divided into 15 100 cm^2 squares numbered from 1 to 15 (Fig. 1). Squares 1 to 5 were inoculated with a suspension of *Listeria monocytogenes*, from 6 to 10 with a suspension of *E. coli* and from 11 to 15 with a suspension of *Saccharomyces cerevisiae*.

Preparation of the suspensions:

Listeria monocytogenes: a drop on a loop was taken from a culture of *L. monocytogenes* cultivated for 18 hours on BHIagar (Oxoid, Italy) and diluted in peptone water. After homogenisation, 1 ml of a decimal dilution of the suspension was inoculated on the surface of squares 1 to 5.

The final value was 64,000 CFU/cm^2.

E. coli: a drop on a loop was taken from a culture of *E. coli* cultivated for 18 hours on BHIagar (Oxoid, Italy) and diluted in peptone water. After homogenisation, 1 ml of a decimal dilution of the suspension was inoculated on the surface of squares 6 to 10. The final value was 120,000 CFU/cm2.

Saccharomyces cerevisiae: A drop on a loop was taken from a culture of *Saccharomyces cerevisiae* cultivated for 18 hours on BHIagar (Oxoid, Italy) and diluted in peptone water. After homogenisation, 1 ml of a decimal dilution of the suspension was inoculated on the surface of squares 11 to 15. The final value was 150,000 CFU/cm2.

After inoculation, the panel was placed in a 10 m^3 storage room at a temperature of 12°C and treated for 24 hours with a Sfera model ioniser with fan positioned about 1 m from the panel itself.

The inoculated microorganisms were recovered at Time 0 using sterile swabs and after 3, 17.5, 21 and 24 hours exposure using 24 cm^2 contact plates and the number of cells surviving ionisation was estimated. To estimate the surviving cells, Plate Count Agar (PCA, Oxoid, Italy) incubated at 30°C for 24 hours was used for the bacteria and Agar Malto (Oxoid, Italy) incubated at room temperature for 3-5 days was used for *S. cerevisiae*. In particular, squares 1, 6 and 11 were analysed at time 0; squares 2, 7 and 12 were analysed after 3 hours; squares 3, 8 and 13 after 17.5 hours, squares 4, 9 and 14 after 21 hours; and squares 5, 10 and 15 after 24 hours. A control was also performed, consisting of a panel of the same composition as above inoculated with the same quantities of

microorganisms (100 cm² for each microorganism). After inoculation, the panel was closed in a sealed container to block the ionising treatment and positioned in the same room.

Hours of exposure	*Listeria monocytogenes*	*Escherichia coli*	*Saccaromyces cerevisiae*
Time **0**	1	6	11
Time **3**	2	7	12
T. **17.30**	3	8	13
Time **21**	4	9	14
Time **24**	5	10	15

Figure 1

B) Treatment of substrates simulating surfaces in direct contact with foods

This experiment considered the effects of ionisation on *Listeria monocytogenes*, held to be the most typical of the pathogenic food microorganisms deriving from environmental contamination.

Plastic glasses and plates and Tetrapaks were used. First the total bacterial count on each substrate (3 samples of each type) was estimated on a generic PCA terrain to obtain an idea of the baseline contamination and evaluate the positive negative influence of this on retrieval of the cells of the pathogen being studied. The natural bacterial load was less than 10 CFU/cm² and therefore could not influence the experiment.

1) Plastic glasses and plates: 6 plastic plates and 6 plastic glasses were inoculated with a suspension of 10^6 cells/ml of *L. monocytogenes* prepared as above. They were then placed in a portable refrigerator thoroughly cleaned with ethanol. A static ioniser was placed in the refrigerator.

The ioniser was turned on and the refrigerator was hermetically sealed. The ionisation lasted about 24 hours. A control was again performed involving 3 glasses and 3 plates inoculated with the same suspension, closed in a portable refrigerator similar to the previous model and left for 24 hours in the same conditions as the treated samples. The aim was to compare the results of cell retrieval with and without treatment. Survived cells were retrieved using gauze moistened with 10 ml of physiological solution passed a number of times over the inoculated surface. The gauze was handled using sterile forceps. Each piece of gauze was then introduced into a Stomaker bag (for the glasses) or test tube (for the plates) containing 10 ml of sterile peptone water (the same as used to wet the gauze). The content of the test tubes was vortexed, while the content of the bags was hand homogenised to make sure all the microorganisms present on the gauze entered the solution. One millilitre of the suspension thus obtained was diluted in 9 ml of peptone water and 0.1 ml was spread with a spatula on selective terrain (Palcam agar, Oxoid). Three dilutions were performed for each sample. The plates were incubated at 37°C for 48 hours. The colonies which developed were counted and the result expressed in CFU/ml.

2) Tetrapaks. The same experiment was repeated inoculating and sampling 6 Tetrapaks. Each sample was inoculated with a suspension of 10^6 CFU/ml of *Listeria monocytogenes.* The samples were treated as before, closed in a portable refrigerator and exposed to ionisation for 24 hours. Once again, a control was performed consisting of 6 Tetrapaks inoculated with 10^6 CFU/ml di *L. monocytogenes*, placed in a portable refrigerator and left without ionising treatment for 24 hours. Unlike the previous experiments, the Tetrapaks were not inoculated by spreading with a spatula, but simply by depositing the suspension in the form of small drops.

After the established period, the Tetrapaks were placed in Stomaker bags and sterile peptone water was added. To recover the maximum number of cells, the diluted sample was homogenised for about 5 minutes. The survived cells of *L. monocytogenes* were counted following inoculation of about 0.5 mm of the homogenised solution directly on 15 cm diameter plates containing Palcam agar and incubated at 37°C for 48 hours. Use of these plates enabled the detectability threshold of the method to be lowered (5 CFU/ml peptone water).

RESULTS AND DISCUSSION

The results of the experiments are given in figures 4-5-6 and tables 1-2-3. As can be seen in all experiments, the ionising treatment produced a clear reduction in the inoculated microorganisms on both the surfaces simulating the walls of food storage rooms and those used to simulate equipment in direct contact with food.

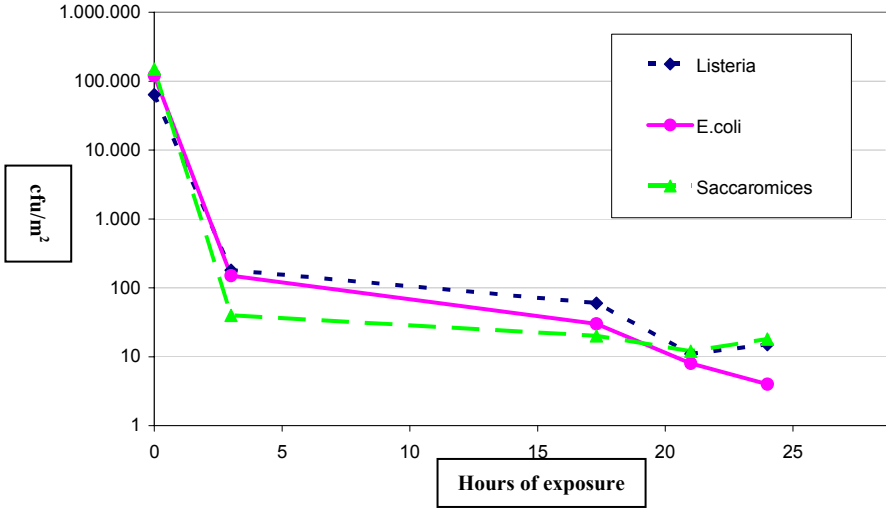
A) Experimental ionisation of a panel simulating the wall of a food production environment.

The 24 hours of ionisation produced a loss of more than 99% of all microorganisms inoculated on the panels simulating the walls of the food storage room. The efficacy of the ioniser was not apparently dependent on the microorganisms tested. The repeats performed (3 different tests) all produced more or less similar results, demonstrating that the efficacy of the treatment is repeatable. The peak of inactivation was observed as early as 3 hours from the beginning of treatment. It then slowed down to achieve a mean reduction of 3-4 log units/cm² depending on the inoculated microorganisms. Vice versa, in the samples taken from the untreated panels, there was a reduction of 22-30% with respect to the inoculated microorganisms, compared with a 99% reduction on the treated panels. In all the repeats, *Listeria monocytogenes* was reduced by 2 log units/cm² within 3 hours from the beginning of treatment. After 3 hours and until the end of the experiment, the decrease continued at a constant 3 log units/cm². *E. coli* dropped by 3-4 log units/cm² within 3 hours. The decrease then continued until it reached 4-5 log units/cm² at the end of treatment (24 hours). *Saccharomyces cerevisiae* dropped by 4 log units within 3 hours from the beginning of treatment and a constant reduction in load was then noted. However, at the end of treatment, the mean reduction in all repeats was 4 log units.

Tab. 1 - TESTS ON SURFACES			a	b	c	d	e	Reduction %
TEST	Hours of exposure	units	0	3	17,30	21	24	
1	Listeria	cfu/cm2	64000	110	28	18	20	-99,83
	E.coli	cfu/cm2	120000	15	2	0	1	-99,99
	Saccaromices	cfu/cm2	150000	80	10	6	2	-99,95
2	Listeria	cfu/cm2	64000	180	60	11	15	-99,72
	E.coli	cfu/cm2	120000	150	30	8	4	-99,88
	Saccaromices	cfu/cm2	150000	40	20	12	18	-99,97
3	Listeria	cfu/cm2	64000	110	28	18	20	-99,83
	E.coli	cfu/cm2	120000	15	18	6	4	-99,99
	Saccaromices	cfu/cm2	150000	80	15	12	6	-99,95

NOTES: Test carried out in the same room inoculating 3 different surfaces with 0.1 ml of solution per 100 cm²; The initial bacterial suspension was titrated (see column a); The control was assessed on a surface not exposed to ionised air after 24 hours. The surface was in the same room, but closed in a sealed 1 m³ container. The whole inoculated surface (100 m²) was sampled with a swab. Experiment a) was performed with a swab to collect over 100 cm²; experiments b, c and d were performed using 24 cm ² contact plates.

Fig. 3 - **Assessment of the efficacy of the Bioxigen ionisation system**



CONTROL	CFU/cm2
Listeria ivanovii	68.000
E.coli	110.000
Saccaromices spp	131.000

10 m³ room, room temperature (about 12°C), no air exchange.

Ioniser used: Sfera model, placed near the surface. The model has a fan and is recommended for rooms with 30/75 m3 of air.

B) Treatment of substrates simulating surfaces in direct contact with foods:

1) Plastic glasses and plates

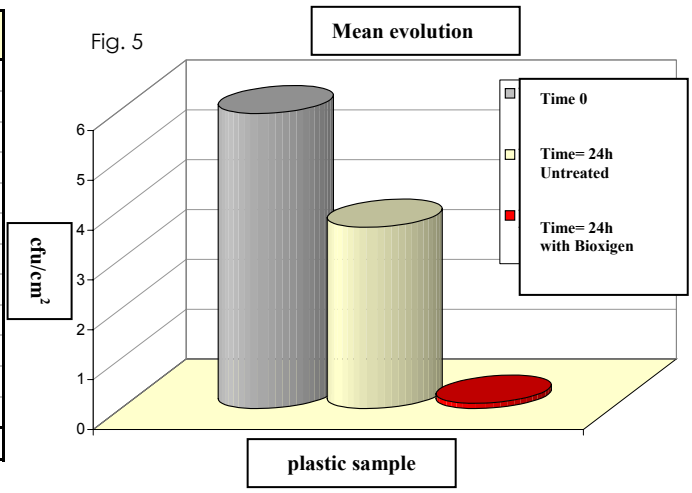
The treatment was performed using *Listeria monocytogenes* inoculated on the plastic surfaces, bearing in mind the fact that it is able to create a biofilm and adhere to the most diverse of surfaces. There was again a difference of 2 units between the CFUs obtained from the treated and untreated samples, as described in table 2. In fact, as can be seen, there was also a mean loss of about 2 log units/total product surface in the untreated samples (Tab. 2). This loss may be due either to natural inactivation of the microorganism over time, although not observed in the previous experiment, or to problems in retrieving the microorganisms from the substrate. This appears to be the main reason for the lack of retrieval of the majority of cells inoculated from the substrate. We believe, in fact, that the technique used is not able to ensure total retrieval of the *Listeria monocytogenes* cells from the substrate. It is known that use of gauze or sponges moistened with peptone water does not allow maximum retrieval of the microorganisms from the substrate, irrespective of the fact that this technique is considered one of the best and proposed in numerous cases of legislation aimed at counting the microorganisms on the surfaces of equipment, production environments and food (animal carcasses). In any case, table 2 shows that the treatment produced a reduction of about 1-2 log units/total surface of the samples tested. As can be seen, the data are highly repeatable. In all samples, the number of cells retrieved was below the limit of detectability.

Table 2: Evolution of *L. monocytogenes* inoculated on plastic surfaces treated with ionised air

Sample	Time=0	Time=24 h Untreated	Time=24 h with Bioxigen
Glass 1	5.914	4	<2
Glass 2	5.914	4.477	<2
Glass 3	5.914	4.301	<2
Glass 4	5.914	3.342	<2
Glass 5	5.914	3.415	<2
Glass 6	5.914	3.398	<2
plate 1	5.914	3.204	<2
plate 2	5.914	3.301	<2
plate 3	5.914	3.415	<2
plate 4	5.914	3.505	<2
plate 5	5.914	3.477	<2
plate 6	5.914	3.778	<2
Mean	**5.914**	**3.635**	**<2**

Data: log CFU/total sample surface

Fig. 5



2) Tetrapaks

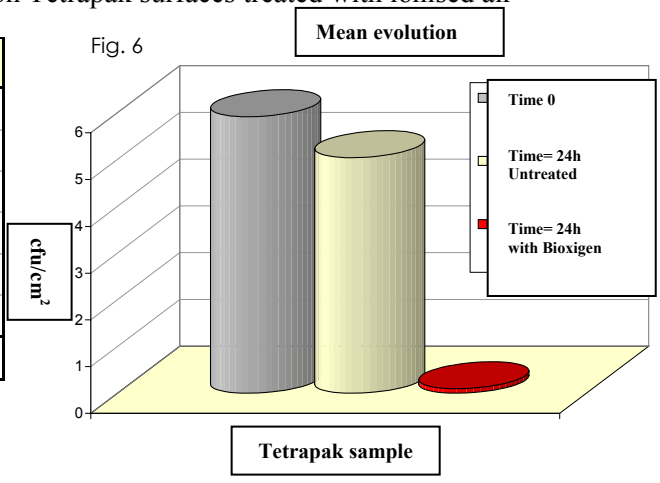
Tetrapak surfaces were also used for the experiment. The inoculation method was modified. Instead of spreading with a spatula, inoculation was performed by depositing the suspension in the form of small drops distributed at random over the entire surface so as to reduce the level of dehydration to which the cells are exposed. Use of this technique made it possible to achieve a reduction in inoculated load of more than 4 log units (Tab. 3). As for the other surfaces treated, the number of cells recovered was below 4 log units in the first series of tests and 3 log units in the second series of tests.

Table 3: Evolution of *L. monocytogenes* inoculated on Tetrapak surfaces treated with ionised air

Sample	Time=0	Time=24 h Untreated	Time=24 h with Bioxigen
Tetrapak 1	5.914	5.892	<1
Tetrapak 2	5.914	6.176	<1
Tetrapak 3	5.914	5.869	<1
Tetrapak 4	5.914	4.301	<1
Tetrapak 5	5.914	4	<1
Tetrapak 6	5.914	4	<1
MEDIA	**5.914**	**5.04**	**<1**

Data: log CFU/total sample surface

Fig. 6



CONCLUSIONS

This study assessed the efficacy of ionisers ***(Bioxigen system)*** to decontaminate the surfaces of substrates simulating the walls of food storage rooms and substrate in indirect contact with foods. Microbial contamination of the surfaces may derive from the food itself, from man, from insects or from the air.

In particular, airborne microorganisms are present in food processing environments as "passengers" associated with solid dust particles and the skin, hair and clothes of operatives working in the environments. The microorganisms may also derive from drops caused by mists formed by cleaning equipment, condensate from air conditioners or installations regulating the temperature and RH in the environments. As a result, good hygiene practices applied to work environments and equipment in close or direct contact with the food enables the risk of contamination to be eliminated, improving the healthiness of the food. Sanitation involves the use of detergents/disinfectants. Although extensively used, these methods do not always produce satisfactory results. The antimicrobial solutions used, in fact, sometimes fail to reach the areas, rough zones or cracks where the microorganisms are located. Often to eradicate particularly "hidden" microorganisms, spray or aerosol systems able to distribute the sanitiser in every crack in the surface must be used. These systems are still highly valued and easy to apply although they have the disadvantage of using active ingredients looked on with suspicion by SSOP operatives. In particular, the use of chemical sanitisers has always been considered "a necessary evil". The molecules used in aqueous solutions, although recognised as innocuous, have always been a source of concern as their long term use may in any case cause environmental pollution. Hygienists are therefore always looking for alternative methods with low environmental impact. The treatment considered based on ionisation of the air or surfaces satisfies the requisites. The fundamental advantage of this treatment with respect to traditional methods of cleaning and sanitation lies in the fact that the active principle at room temperature is a gas, disappearing in time without leaving a trace and penetrating everywhere, even in small cracks or rough areas (which should be few in the food industry) which detergents and disinfectants penetrate with difficulty. It is known that production of negative ions and in particular a correct ratio between negative and positive ions results in a reduction in contamination by particles and microbes and of odours in the air in the room. The results showed that treatment with the ions used resulted in a clear reduction in the population of *Escherichia coli*, *Listeria monocytogenes* and *Saccharomyces cerevisiae* on surfaces simulating food storage rooms or surfaces in direct contact with food. The decrease in microbial load depended on the initial level and the environment considered, but the mean reduction was in any case 99% of the initial inoculated microorganisms.

The post-treatment counts enabled a contamination level of less than or equal to 1 log unit/cm² to be achieved, a level considered as optimum for these surfaces. The presence of a low microbial load on the walls of the food storage rooms or equipment in direct contact with food may have a positive influence on the hygiene and sanitary quality of the product itself. As good hygiene and production practice considers the surfaces of rooms used for food production and processing to be one of the principal vehicles for the diffusion of microorganisms, we suggest the use of ionising equipment in the Bioxigen system (Sital Klima) as a means of achieving acceptable levels of contamination. To optimise the treatment, the ioniser must be positioned inside the room to be sanified and left turned on to allow the constant release of negative ions. Once liberated, these act on the mood of people and inactivate the majority of microorganisms present in the air and on the surfaces. The ioniser may, therefore, be used to reduce the microbial load as an alternative to disinfectants or to reinforce their action. It cannot be an alternative to cleaning. This is necessary in any case to eliminate surface biofilm or organic residues which could encourage microbial growth. The ioniser may be used in parallel with or subsequent to this treatment.

The flow diagram of the method suggested should include cleaning the surfaces with detergent, rinsing and ionisation. To fully exploit the potential of the technique, ionisers should be positioned in all work environments which should be treated continuously.

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Università degli Studi di Udine

Use of an ioniser to purify air in rooms used in the production of San Daniele prosciutto

Giuseppe Comi, Andrea Lovo[1], Nadia Bortolussi[1], Mario Paiani[2] , Alessandro Berton[3] e Giacomina Bustreo[3]

Dipartimento Scienze degli Alimenti, Università degli Studi di Udine, via Marangoni 97, 33100 Udine.
[1]Alimentaria srl, Via Rizzardi, 95, 30175 Venezia-Marghera (Venezia).
[2]ASL 4, Medio Friuli.
[3] Sistema Bioxigen, Sital Klima srl, Via L. da Vinci, 26, 31021 Mogliano Veneto (Treviso).

SUMMARY
The aim of this study was to reduce the airborne microbial load in salting, pre-curing and curing rooms used in the production of San Daniele prosciutto using an ioniser. The treatment involved ionising the air in each room for 24 hours using a Maia static ioniser (Bioxigen system SitalKlima). The results showed that the ionising treatment produced a definite reduction in the fungi and microbial populations in the air contained in the rooms examined. The decrease in microbial load depended on the initial level and the environment considered, but the mean reduction was always 47-52% of the initial load. The post-treatment counts enabled a contamination level of less than 200 CFU/m^3 to be reached, a level considered as optimum for these production environments.

INTRODUCTION
Prosciutto is a cured meat made from pork (haunch) salted and cured for the time necessary to acquire the correct aroma and flavour. Together with the sausage, it is the oldest meat product still eaten today, as it was prepared and consumed in China and Europe as long as 2500 years ago. The name has evolved from the Latin (classical) *prae exuctus* meaning very dry, dried, through the Vulgar Latin *perexuctus*, into *prosciutto* in Tuscan and Italian. It represents a genuine historical, cultural and commercial common denominator throughout the European continent, with production established from the Iberian peninsula to Italy, France and the whole of central and Western Europe.
San Daniele prosciutto is a typical Italian cured meat, universally considered as a delicacy for its specific and unique aroma and flavour. Its popularity in Europe and the world is constantly increasing. The Italian State has promoted safeguarding of this product since 1970 and in 1990 it approved a new protection law, Law No. 30 of 14 February 1990.
With EEC regulation No. 1017/96 of 12 June 1996, the European Union attributed San Daniele prosciutto with PDO (Protected Designation of Origin) status in accordance with EEC regulation No. 2081/92 (establishing Community protection for food and agricultural products with designation of origin). The production phases are set out in Law No. 30 of 14/02/1990 and Ministerial Decree No. 298 of 16 February 1993. They derive from the process used traditionally. Modern industry has merely attempted to give a clearer form to the various phases in order to emphasise the operations which characterise San Daniele prosciutto. The stages of the process consist in meat selection, chilling, trimming, massaging, salting, pressing, pre-resting, resting, cleaning, softening and washing, drying, pre-curing, filling and greasing and curing (3,8,9,28).
Fresh haunches of "Suino Pesante" (an Italian breed of pig) coming exclusively from the regions of Friuli Venezia Giulia, Veneto, Lombardy, Piedmont, Emilia Romagna, Umbria, Tuscany, Marche, Abruzzo and Lazio are delivered directly to the factory complete with foot or trotter which will be left on the finished product and represents a characteristic of San Daniele prosciutto (8,9). On arrival, the haunches undergo veterinary examination by the Consortium's personnel who mark those considered suitable with a brand bearing the letters DOT and the complete date on which the production process begins. The various production stages are described below.
Meat selection: this involves visual inspection of the product and verification of the accompanying certification, microbial load, temperature and pH and weight of the haunches, which must not be less than 11 kg. The visual inspection of the haunches is necessary to ensure they have not been damaged during transport or soiled with earth or other forms of dirt, to check for the presence of bruising, abnormal coloration or excessive intramuscular fat and to assess the commercial quality. The total number of germs inside the haunches must be less than 100 CFU/g. Stringent controls are performed to detect the presence of *Enterobacteriaceae psicrotrofe*, which could lead to loss during production. Such a low load can only be obtained in haunches from pigs slaughtered in conditions of optimum hygiene (8,9,10,28).

Massaging: this is done by passing the haunches through rollers which massage the product, exerting a light pressure in order to drain the blood vessels of residual blood which might otherwise encourage the development of microorganisms.
Salting: a sufficient quantity of salt is added to bring the salt concentration to no more than 6%. The "golden rule" handed down by tradition specifies that the haunch should remain "under salt" for one day for every kilogram of weight.
Pressing: this helps eliminate fluids from the femoral artery and associated veins and from the most heavily draining parts of the haunch (around the small shell shaped bone known as the *anchetta*). It also gives the haunch its typical guitar shape.
Pre-resting and resting: it is crucial that pre-resting is carried out at a temperature of between 4 and 6°C with an RH of between 70-75% or 80-85% depending on the size of the haunches. It lasts an average of 21 days and continues the dehydration process initiated with salting. Resting is carried out at a temperature of from 4-8°C and RH of 70-75% or 80-85% for 60 days, the length of time necessary to achieve an internal salt concentration of 4.0-4.5%. Control of temperature and RH is important during these stages as if they are excessively high, surface mould may form (3,7,8, 27).
Cleaning: this is done manually with the help of electrical equipment or knives. The aim is to remove and trim the protruding part of the small shell shaped bone known as the *anchetta*, trim the area around the head of the femur and stimulate moisture elimination.
Softening and washing: during this phase, the haunches are sprinkled with an atomised mixture of water and air (120 Atm, 50°C) for 2/3 hours. The aim is to remove the surface film (the "*molliga*") formed by purging of the meat and soften the exterior.
Drying: this is done at 20-27°C, RH 90%, for 7 days, keeping the most heavily draining points dry to encourage dehydration.
Pre-curing: this lasts 35-40 days. The temperatures used depend on the size of the haunches and range from 12 to 19°C, with an RH of between 75 and 90%.
Filling and greasing: this is performed about seven months after salting and involves spreading a mixture of lard and pig fat mixed with sodium chloride, pepper, paprika and cereal flours onto the flat surface of the haunch, in the cracks and on the exposed muscle tissue. Two compounds are used, *sugna* and *stucco*, differing only in the fat content which is higher in the former. These mixtures serve to soften the exposed muscle surface in order to stimulate osmosis between this and the external atmosphere.
Curing: this is a delicate phase which stimulates ageing and helps the product acquire the correct aroma by means of a technological process involving variations in humidity, temperature and air exchange. Lasting from 8-16 months, it is performed in rooms which are either ventilated naturally or equipped with air-conditioning systems.
Deboning: this eliminates the "bone" of the prosciutto and prepares it for slicing. After deboning it is vacuum packed (8,9,10).
Packaging and Packing: prosciutto is sold either whole or vacuum packed following deboning, or sliced and packed in a modified atmosphere.
The quality of the product therefore depends on the composition, choice and hygiene-sanitary characteristics of the raw materials, production technology, production equipment and environment and, finally, the conditions under which the product is stored (7,8,9,10,28). Any weak link in the chain may affect efficiency. It is therefore extremely important that each phase of the production process is performed correctly and, above all, that a high standard of hygiene is maintained to prevent marketing products with poor organoleptic qualities and hygiene.
The raw materials are an excellent substrate for the development of germs as their chemical composition, redox potential, pH, aw and moisture content do not in any way prevent bacterial proliferation.
Anything which comes into contact with the food during processing may represent a source of contamination, particularly production equipment, surfaces and implements, the air contained in the room and its humidity, the water used and the production operatives (3,9,10,16). The air contained in the processing rooms has a high influence on the microbial load of the product, whether in the form of raw materials, semifinished or finished products. Certain alterations such as acid or putrid fermentation are, in fact, caused by defective technology and massive contamination of the meat or derivatives from the environment.
During salting, pre-curing and curing in particular, the equipment, weighing scales, gratings the prosciutto rests on during ageing, walls and air contained in the room transfer pathogenic altering microorganisms to the product where their development is closely linked to temperature, salt concentration and aw level.
Moulds coming from the air and environment in the rooms could develop in the products during pre-curing or curing, producing the so-called "phenic acid" defects (5,7,17,27) or mite infestations. One of the microorganisms

deriving from the air is *Listeria monocytogenes*. This does not develop on raw prosciutto due to the presence of salt and the aw level which is less than or equal to 0.92. Pig haunches, the raw material of prosciutto, are in fact salted, dehydrated and cured for more than a year until the aw level is equal to or less than 0.92. The slow and constant reduction in aw prevents the development of contaminating microorganisms and may kill them, preventing pathogens such as *L. monocytogenes* from developing. In fact, over time they become inactive (10,16). However, *L. monocytogenes* may recontaminate prosciutto during the phases preceding sale. In particular, if performed in rooms or with equipment which are inadequately sanitised, deboning or slicing may encourage this recontamination. However, due to its low aw ($< 0.90 \pm 0.01$), neither sliced nor deboned prosciutto can support the development of pathogens including *L. monocytogenes* (10,16).

In many countries, including Italy and the USA, the tolerance for this microorganism in prosciutto is "0", despite the fact that a minimum infectious dose has not been established (6,10,13,14,16) and that to date, although sold for years on a vast scale, ready-to-eat food products have produced only a very small number of cases of listeriosis (16).

To prevent this contamination, prosciutto factories have implemented severe systems such as HACCP (Hazard Analysis Critical Control Point), GMP (Good Manufacturing Practise) and SSOP (Standard Sanification Organisation Program) and this has led to a definite reduction in the presence of *L. monocytogenes* in production environments, particularly those where prosciutto is produced. The efficiency and efficacy of HACCP and SSOP plans are demonstrated by the fact that the percentage of *L. monocytogenes* isolation in prosciutto (whole, in pieces, deboned or sliced) has for years been less than < 0.3%. This has made it possible to further reduce the probability of the risk of consumers falling ill, as is further confirmed by the absence of reports of listeriosis following consumption of this product. However, much still remains to be done before it is completely eradicated (9).

As air is one of the sources of contamination of the prosciutto by *L. monocytogenes* and other moulds and microorganisms, this study set out to assess use of an ioniser in salting, pre-curing and curing rooms to reduce the microbial load in the air contained in the rooms. Ionisation of air has been widely used in private homes, institutions and industries for many years. The negative ions produced eliminate particulate, pollens, microorganisms and pollution in general (1,15,18,21,23). They therefore have a positive effect on the prevention of diseases such as asthma and allergies (4,19,21,22,24,29). They can also affect peoples' mood. Depression, nausea, insomnia, irritability, tiredness, migraine and asthma attacks are known to be caused by the excessive presence of positive ions in the atmosphere. In fact, meteopathic people often change mood following a storm due to the presence of hot dry winds which carry the positive ions in the air (19,22,24). According to doctors and hygienists, negative ions improve the immune system, increase the body's capacity to use the oxygen present in the air, improve the lung's capacity to eliminate pollutants, make you breathe more easily, improve sleep, reduce stress, increase attention and reduce blood platelet aggregation.

They therefore recommend living in a pollutant-free environment or using an ioniser to purify the air. Over and above these considerations and suggestions to improve human life, reducing airborne microbial contamination is of vital importance for the food industry. This can be achieved by using closed rooms, known as clean rooms, where the air introduced is previously sterilised by filtration or if the space is exposed to frequent contact with the outside, by physical means such as steam, ionisation, ionising radiation, UV rays or infrared radiation (26). In particular Corry and Mead (11) suggest that ionising the air in cold rooms has a positive influence on the microbial load of meat. They quote figures showing that the microbial load of meat kept in cold rooms with ionised air is less than that of meat kept in cold rooms without any treatment.

On the basis of these considerations, we therefore wanted to verify whether the use of an air ioniser (Bioxigen from Sital Klima) could reduce the airborne microbial load in rooms used to produce San Daniele prosciutto.

MATERIALS AND METHODS

The air was sampled before and after treatment in three rooms: the salting room (C1), the pre-curing room (C2) and the curing room (C3) in a small prosciutto factory in San Daniele. The first room (C1) measured about 120m³, the second room (C2) about 150m³ and the third room (C3) about 180m³. The air in each room was sampled at five different points. Four of the sampling points were about 1 m from each corner, while the fifth sampling point was at the centre of the room.

The air samples were taken using an SAS sampler (SES - Super 100 - PBI International) set with an inflow of 100 l/min.

The sampling times were Time 0 and Time 24 hours. Between the sampling at Time 0 and after 24 hours, the air in the room was treated with a Maia ioniser (Bioxigen-Sital-Klima). After the first sampling, the ioniser was

positioned at the centre of the room. It was turned on and left in operation with the room closed for 24 hours. After this period, the air in the room was sampled again.
The microbiological analyses included an estimate of the total bacterial and mould loads. The total count was performed in Plate Count Agar (Oxoid, Italy) incubated at 30°C for 24 hours. Mould and yeast detection was performed in Malt Agar (Oxoid, Italy) incubated at room temperature for 3-5 days. Fifty five millimetre contact plates were used, with two different selective terrains suitable for use with the SAS Super 100 instrument (PBI). The results were defined in CFU/m^3 of air. One-way variance analysis (ANOVA) was used to process the data. The means and standard deviations were calculated for the data observed and the significant differences (evaluated for $p < 0.05$) were determined using Tukey's Honest Significant Difference Test (HSD test).
The Maia ioniser and the Mistral model (Bioxigen-Sital, Klima) can be correctly dimensioned according to the volume of the rooms, the microbial content of the environment, the quantity and type of stored goods, the people entering the room during the day, air change and desired microbial abatement. The equipment used (Maia ioniser) was under dimensioned with respect to the normal standard.

RESULTS AND CONSIDERATIONS

The results of the experiment are given in graphs 1,2,3,4,5,6. As can be seen, in all the rooms considered, the load of moulds, yeasts and bacteria in the air clearly decreased after ionising treatment. The reduction in both microbial groups was between 47% and 53% (table 2). The figures show that the concentrations of the various microbial populations present before treatment differ significantly from those found after treatment ($p< 0.05$), demonstrating that use of an ioniser (Bioxigen system) is one of the most effective methods of purifying the air of atmospheric dust, particles and microorganisms.
The air was sampled in five points at the centre and corners of each room considered. The salting room was static, while the other two were dynamic. Despite this, the data were comparable, in both the concentrations of microbial populations observed at the five air sampling points and the approximately similar percentage reduction.
The salting room (C1) had a mean initial fungal load of 188 CFU/m³, dropping to about 95 CFU/m^3 after treatment. There did not appear to be a major difference between the loads observed at the different points considered, demonstrating that the microorganisms were evenly distributed in the environment. In each instance, the ionising treatment resulted in a reduction of 49% in the initial fungal flora. The same is true for the bacterial load in this room. The air contained an average of about 113 bacteria/m³. After treatment this mean load dropped to 56 CFU/ m^3. The reduction was about 50% of the initial total (table 2).
The air in the salting room is an important factor in contamination of the products kept there. The lower the contamination, the less probable the product will be contaminated. Salting of the haunches is, in fact, a delicate phase as the product is fresh and therefore has an aw of more than 0.96 units. It can thus support development of microorganisms coming from the raw material itself or the environment where the salting takes place. The phase is, in fact, controlled by the use of temperatures between 2°C and 4°C and by the presence of salt on the exposed muscle tissue and partly on the skin of the haunch. The low temperature encourages slow and even penetration of the salt and blocks all bacterial activity. However, contamination by microbes from the atmosphere must be prevented during this phase as once established on the surface and adapted to living in the presence of salt and at low-temperatures, they could develop during subsequent phases. In particular, during the phases downstream of salting (known as "out of salt") and resting when the temperature begins to be raised above 8°C, this microbial population could lead to the onset of abnormal fermentation or produce surface films.
The pre-curing room (C2) has a mean fungal and bacterial load of, respectively, 213 and 116 CFU/m³, dropping after ionising treatment. After the air in this room has been treated for 24 hours with the Maia ioniser, the residual fungal load amounts to 108 CFU/m³, and the bacterial load to 60 CFU/m³. There is therefore a reduction of 49% in the fungal load and 48% in the bacterial load (table 2). Once again, good hygiene and production practices suggest that contamination of the prosciutto should be avoided.
The prosciutto is now salted and has an aw of equal to or less than 0.94 and can therefore no longer support microbial growth. However, in this room, the temperatures of between 14 and 19°C and the RH of from 70 to 80% are optimum for surface growth of salt-tolerant moulds, yeasts and bacteria. This growth could be encouraged by poor temperature and RH control. It is known that during pre-curing and curing, surface films may develop on the prosciutto caused by the uncontrolled development of moulds. This causes defects such as phenic acid defect (27) and, in prosciutto factories with structures still made from wood, the presence of mites which eat the mould mycelia. Control of the airborne load in the pre-curing rooms is also therefore vitally important to the success of the prosciutto ageing process.

As was expected, the air in the curing room (C3) was the most contaminated of the environments considered. The mean initial fungal load was about 332 CFU/m^3, while the bacterial load was about 155 CFU/m³. Ionising treatment produced a 47% reduction in fungal load and 48% in bacterial load.
In particular, after treatment the mean load of yeasts and moulds was 174 CFU/m^3 and of bacteria, 72 CFU/m^3. At this point, the prosciutto has an aw value of equal to or less than 0.93 and as a result does not support the development of microorganisms. However, poor control of temperature and, above all, RH in the atmosphere could encourage the surface development of salt-tolerant microorganisms and mould, with the consequences described above. In fact, the temperature (15-22°C) and RH (70-80%) of this room is optimum for microbial development. If these parameters are not controlled correctly and the RH rises to more than 80%, condensate could form on the skin, encouraging the uncontrolled growth of moulds coming from the environment. As a result, although this may be stating the obvious, it is important that the air in this room has a fungal and bacterial load of less than 200 CFU/m^3, a load obtained after treatment.
All the rooms considered started out with an acceptable microbial population, below that usually observed in meat and meat processing factories. As confirmed by the figures, the highest fungal load was, in fact, equal to 350 CFU/m^3, while the highest bacterial load was 180 CFU/m^3. Both were observed, as was to be expected, in the curing room and were 10 times lower than those found in meat production/processing laboratories by various authors (12, 20). These note that air contamination can vary from room to room and may be between 2 and 3 log/m^3 units, depending on the type of production plant considered. This variability in load may be attributed to the food produced, type and design of the plant, cleaning procedures, human activities and season considered (2, 25).
In particular, in the summer, microorganisms are known to multiply in all the damp niches of the plant and from there can enter and contaminate the air contained through evaporation of the moisture and production of dust. There may therefore be differences of 0.5 or 1.0 log/m^3 units between the load in the air during the cold and hot seasons. In this study, the experiment was performed during the winter/spring and therefore the low microbial load found could not be attributed to the season as the rooms considered were air-conditioned.
The climate or external temperature did not, apparently, have any influence on contamination of the air contained in the rooms. Probably the low initial level of contamination in the air in the rooms considered can be attributed to the structures which are of recent construction, to the maintenance and, above all, to the low presence in them of operatives during the day.

CONCLUSIONS

The efficacy of an ioniser *(Bioxigen system)* to purify the air contained in the processing rooms of a San Daniele prosciutto factory was assessed. The airborne microorganisms are present in the processing rooms as "passengers" associated with solid dust particles and the skin, hair and clothes of operators working in the rooms.
They may be present in drops caused by mists formed by cleaning equipment, condensate from air conditioners or installations regulating the temperature and RH in the rooms. The salting room, pre-curing room and curing room were considered. The treatment involved ionising the air in each room for 24 hours with a Maia static ioniser (Bioxigen system from SitalKlima).
Production of negative ions and, in particular, a correct ratio between negative and positive ions is known to lead to a reduction in contamination by particles and microbes and in odours in the air contained in a room. The results showed that ionising treatment produced a definite reduction in the airborne fungi and microbial populations in the rooms examined. The decrease in microbial load depended on the initial level and the environment considered, but the mean reduction was always in the order of 47-52% of the initial load.
The post-treatment counts enabled a contamination level of less than 200 CFU/m3 to be reached, a level considered as optimum for these production environments.
The presence of the low microbial load in the air in the rooms may have a positive influence on the hygiene/sanitary characteristics of the product and may avoid massive microbial contamination of the surface of the prosciutto which could lead to alterations and anomalous odours and flavours in the prosciutto itself.
As good hygiene and production practices consider the air in a food product production and processing room as one of the principal vehicles for the diffusion of microorganisms, we suggest the use of ionising equipment in the Bioxigen system (Sital Klima) as a means of achieving acceptable levels of contamination.

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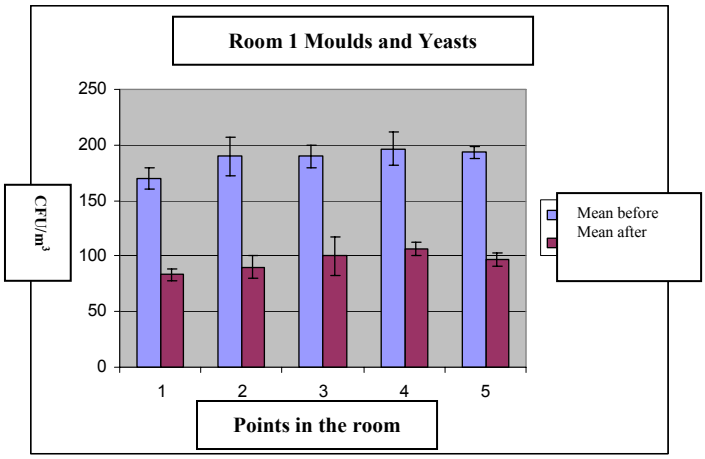
Principal phases in the production of San Daniele prosciutto

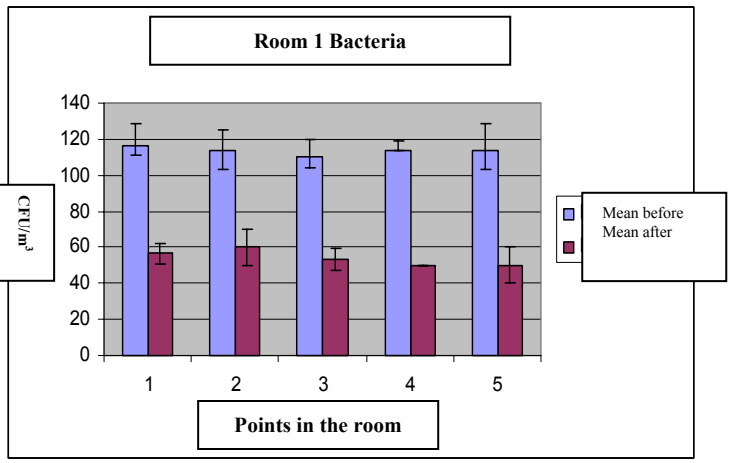
Operation	Processing rooms		Mean length of process in days	Mean cumulative length in days
	Temperature	RH		
Trimming, weighing and chilling	2/3°C	90-95%	1	1
Salting	2/3°C	90-95%	15	16
Pressing	4/5°C	70%	2	18
Pre-resting	4/6°C	70-75% 80-85%	21	39
Resting (cleaning, refreshing and washing)	from 4/6°C to 8/10°C		54	93
Drying	20/27°C	90%	8	101
Pre-curing	12/14°C	85-80%	37.5	138.5
	14/19°C	75-70%		
Curing and filling	15/22°C	70-80%	240	378.5

Percentage of microorganisms rendered inactive by treatment with the Bioxigen ionisation system

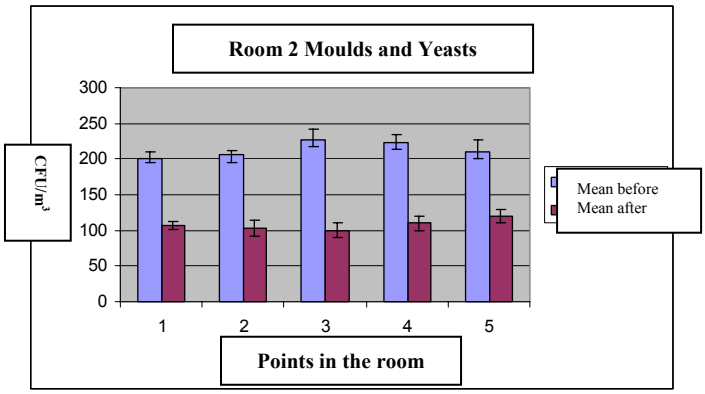
Room	Moulds/Yeasts	Bacteria
Salting	49.0	50.0
Pre-curing	49.5	48.0
Curing	47.5	53.2



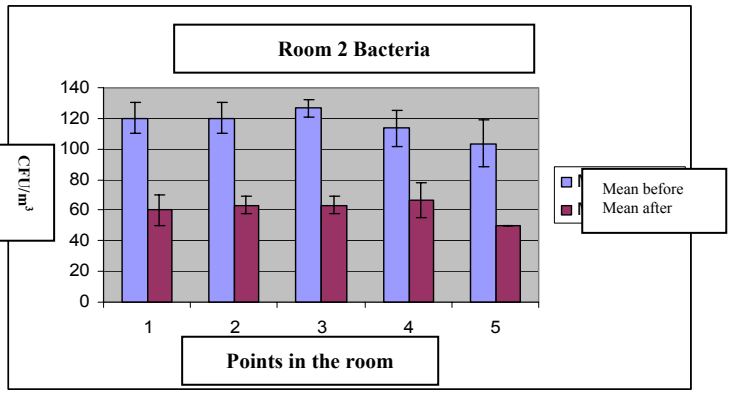
Graph 1: Mean variation in concentrations of yeasts and moulds before and after ionisation treatment.



Graph 2: Mean variation in concentrations of bacteria before and after ionisation treatment.



Graph 3: Mean variation in concentrations of yeasts and moulds before and after ionisation treatment.



Graph 4: Mean variation in concentrations of bacteria before and after ionisation treatment.

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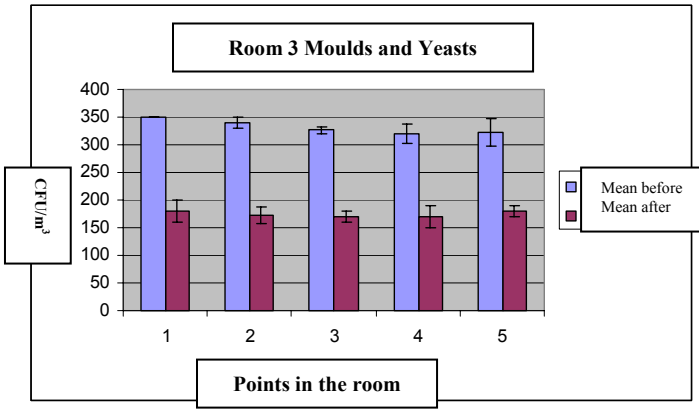
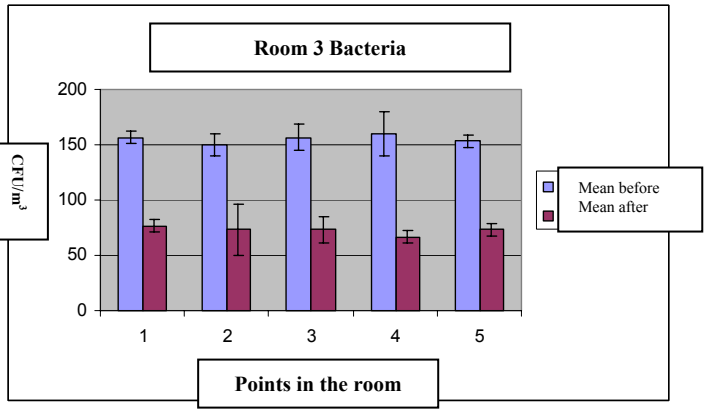
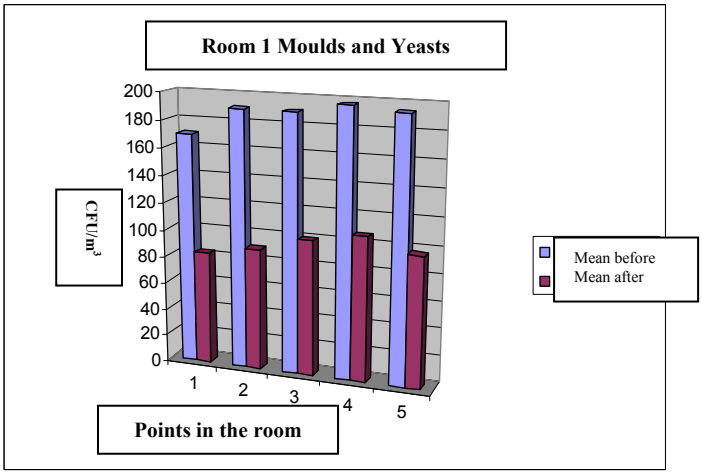


Graph 5: Mean variation in concentrations of yeasts and moulds before and after ionisation treatment.



Graph 6: Mean variation in concentrations of bacteria before and after ionisation treatment.



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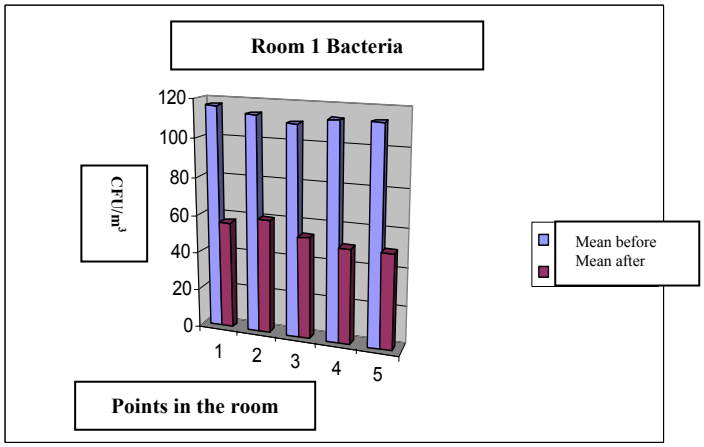
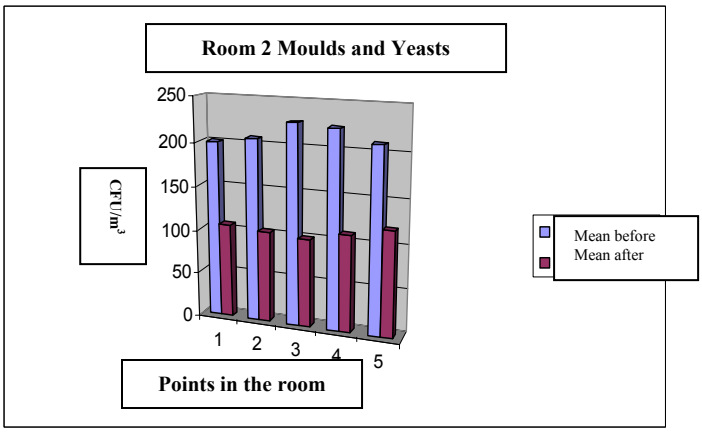
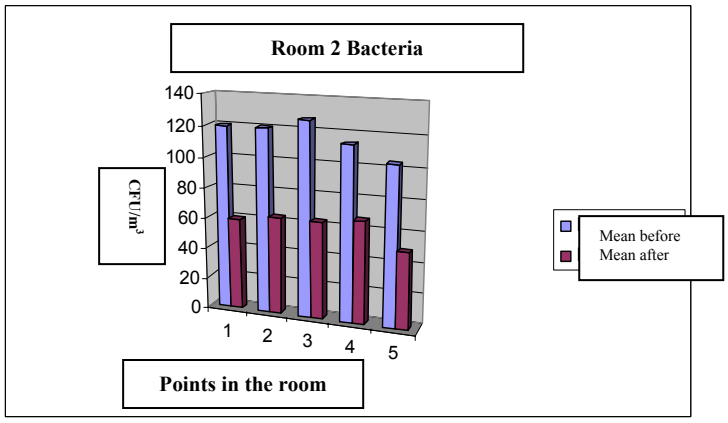


Graph 2: Mean variation in concentrations of bacteria before and after ionisation treatmente



Graph 3: Mean variation in concentrations of yeasts and moulds before and after ionisation treatment.



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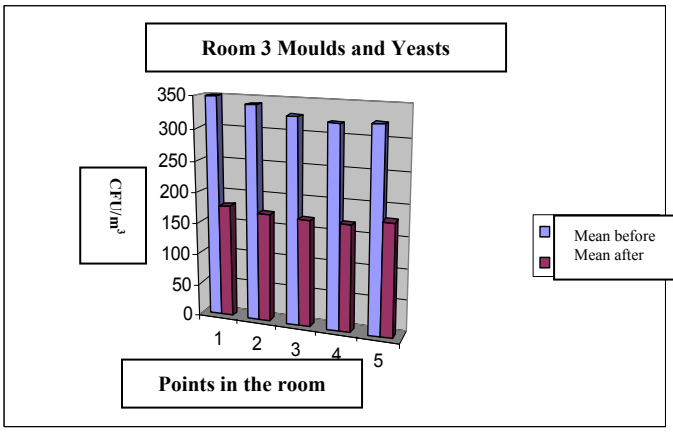
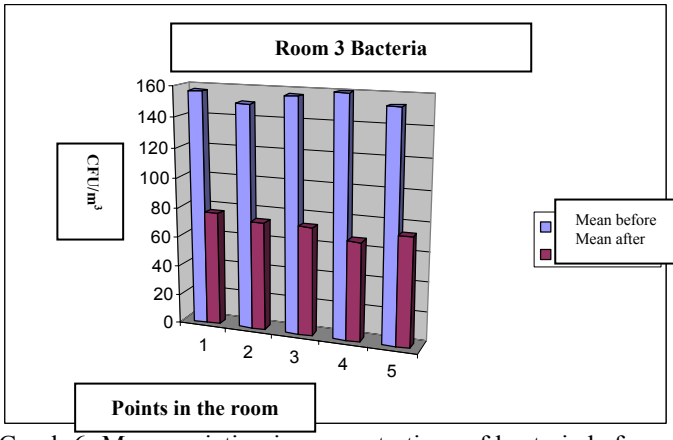


Graph 5: Mean variation in concentrations of yeasts and moulds before and after ionisation treatment.



Graph 6: Mean variation in concentrations of bacteria before and after ionisation treatment.

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