SANITIZERS ON MICROBIOLOGICAL QUALITY OF STRAWBERRIES 'OSO-GRANDE'

Polyanna Alves SILVA[1*]
Celeste Maria Patto de ABREU[2]
Estela de Rezende QUEIROZ[3]
Luís Roberto BATISTA[4]
Angelita Duarte CORRÊA[2]

1. Professora Doutora - FACICA, polyalves@gmail.com
2. Professora Doutora Associada II do Departamento de Química/UFLA, celeste@dqi.ufla.br; angelita@dqi.ufla.br
3. Doutoranda em Agroquímica – UFLA, estelaqueiroz@yahoo.com.br
4. Professor Doutor Adjunto do Departamento de Ciência dos Alimentos/UFLA, luisrb@dca.ufla.br

***AUTHOR FOR CORRESPONDENCE:** Dra. Polyanna Alves Silva
Endereço: Rua Simão Martins Ferreira, 64, centro. Campos Gerais, MG. Cep: 37160-000
Email: polyalves@gmail.com

Recebido em: 09/09/2013 - Aprovado em: 11/12/2013 - Disponibilizado em: 15/01/2014

ABSTRACT: The objective of this study was to evaluate the effect of two disinfectants on the microbiological quality of strawberries cv. 'Oso-grande' *in nature* sold in Lavras, MG, and stored for 6 days at room temperature. 5 treatments were used (control, sodium hypochlorite 100 and 200 mg L^{-1} and sodium dichloroisocyanurate (Hidrosan ®) 100 and 200 mg L^{-1} for 15 min) and 3 days of analysis, corresponding to days 0, 3 and 6, with 4 replicates of 10 fruits for each treatment. Microbiological tests performed were: coliform count at 35 ° C and heat tolerant, detection of *salmonella* spp., Aerobic psychrotrophs counts, isolation and identification of fungi and yeast counts. The dichloroisocyanurate and 200 mg L^{-1} inhibited the growth of filamentous fungi and psychrotrophs and the yeast count was lower when compared with other treatments.
Keywords: Sodium dichloroisocyanurate. Sodium hypochlorite. Coliforms. Aerobic psychrotrophs. Count of yeast and fungi.

SANITIZANTES NA QUALIDADE MICROBIOLÓGICA DE MORANGOS 'OSO-GRANDE'

RESUMO: Este trabalho foi realizado com o objetivo de avaliar o efeito de dois sanitizantes na qualidade microbiológica de morangos cv. Oso-grande ao natural comercializados em Lavras, MG, e armazenados por 6 dias, à temperatura ambiente. Foram utilizados 5 tratamentos (controle, hipoclorito de sódio 100 e 200 mg L^{-1} e dicloro isocianurato de sódio (Hidrosan®) 100 e 200 mg L^{-1} por 15 minutos) e 3 dias de análises, correspondente aos dias 0, 3 e 6, com 4 repetições de 10 frutos para cada tratamento. As análises microbiológicas realizadas foram: contagem de coliformes a 35ºC e termotolerantes, detecção de *salmonella* spp., contagem de aeróbios psicrotrófilos, isolamento e identificação de fungos e contagem de leveduras. O dicloro isocianurato a 200 mg L^{-1} inibiu o crescimento de fungos filamentosos e psicrotrófilos e a contagem de leveduras foi menor quando comparada com a de outros tratamentos.
Palavras-chave: Dicloroisocianurato de sódio. Hipoclorito de sódio. Coliformes. Aerobios psicrotrófilos. Contagem de leveduras e fungos.

INTRODUCTION

That sanitization is a relevant stage of fruit and vegetable processing and chlorine, in its diverse forms, is the most used sanitizer in foods. The chlorine based compounds are broad spectrum acting bactericidals that react with the proteins of microbial cell membranes, interfering in the transport of

nutrients and promoting cell components loss (Reis et al., 2008).

That antimicrobial activity of the chlorinated products thoroughly depends on the amount of available free chlorine, particularly in the form of hypochlorous acid that, in turn, depends on the pH and water temperature and on the amount of organic matter, besides the water temperature (Martinez-Téllez et al., 2005).

That sodium hypochlorite, marketed under the liquid form at levels of 1% to 10% of total residual chlorine, is the most used, among the inorganic chlorinated compounds. The chlorinated compounds, except for the chlorine dioxide, present similar forms of bactericidal action; when any inorganic or organic chlorinated products are in aqueous solution, they liberate hypochlorous acid, in its non-dissociated form, that presents germicidal action.

That sodium dichloroisocyanurate (NaDCC) is a chlorinated organic compound marketed in a powdered form or effervescent tablets (Hidrosan®). For complying with a specific production process for use in foods, it does not liberate heavy metals and trihalomethanes (carcinogenic compounds), when hydrolyzed. Therefore, the substitution of the chlorinated inorganic compounds, like sodium hypochlorite, by NaDCC, for use in foods has been the standard since the 1990's, in the USA and in Europe (Oliveira & Oliveira, 2004).

This study was conducted with the objective of evaluating the influence of sodium dichloroisocyanurate and sodium hypochlorite on the microbiological quality of strawberries cultivated in Itutinga, MG, stored at room temperature (21±1.4ºC and 55±5.7% RH), for a period of 6 days.

MATERIAL AND METHODS

Raw Material

The fruits of the Oso-grande cultivar were picked in the afternoon, in a commercial orchard in the area of Itutinga, MG located at 910 m of altitude, at 21°18'45" S. latitude and 44°41'15" W. longitude Gr, according to the Brazilian Institute of Geography and Statistics-IBGE (1959).

800 fruits were taken to the Biochemistry Laboratory in the Chemistry Department of UFLA, where they were selected as to size, maturation stage and absence of defects.

Treatments

5 treatments were used (control, sodium hypochlorite 100 and 200 mg L^{-1} and sodium dichloroisocyanurate (Hidrosan®) 100 and 200 mg L^{-1} for 15 min) and 3 analyses times, corresponding to days 0, 3 and 6, with 4 repetitions of 10 fruits per treatment.

Sample preparation and experiment setup

The fruits were separated at random into 5 lots of 160 fruits each and immersed in

the respective sanitizers: sodium hypochlorite 100 mg L^{-1}, for 15 minutes; sodium hypochlorite 200 mg L^{-1}, for 15 minutes; sodium dichloroisocyanurate (Hidrosan®) 100 mg L^{-1}, for 15 minutes; sodium dichloroisocyanurate (Hidrosan®) 200 mg L^{-1}, for 15 minutes and control.

Afterwards they were placed on paper towels on a bench, at room temperature for six days. The analyses were initiated soon after the immersion and, every 3 days, until the end of the storage period, the same was done for the control fruits.

Microbiological analysis

Coliforms at 35° C and thermotolerant coliform counts, by the most probable number method (MPN)

The procedure used was the method of the most probable number, of the American Public Health Association, described in the Compendium of Methods for the Microbiological Examination of Foods described by Silva et al. (2007) (Silva et al., 2012).

Coliforms at 35°C

25 g of strawberry pulp from each repetition of each treatment were weighed and 225 mL of peptone water 0.1% (H_2O p) was added. It was homogenized, under agitation, for 5 minutes, this solution the being dilution 10^{-1}. For each dilution, a tube with 9 mL of peptone water 0.1% (H_2O p) was made.

1mL of the dilution 10^{-1} was aurel into the first tube (10^{-2}) with 9 mL of peptone water 0.1%; 1 mL of this mixture was put in the second tube (10^{-3}) and so forth, until the dilution 10^{-5}.

For each dilution, three LST broth tubes were used, for inoculation. 3 mL from the tube of higher dilution was aurel and 1 mL was put in its corresponding lauryl broth. The procedure was repeated until the 10^{-5} dilution tubes. The the end of the process, the inoculated tubes were put in an incubation chamber, for 48 hours, at 37°C.

At the end of this time, readings were taken. Those tubes that darkened the medium (acid formation) and produced bubbles inside the Durham tube (CO_2) were considered positive for total coliforms. Any other option is interpreted as negative.

The quantification was conducted by the most probable number table (MPN) and the last dilution has to have at least one negative tube.

Thermotolerant coliforms

From the tubes positive for total coliforms, *Escherichia coli* (E.C.) was transferred to the tubes of broth with a platinum loop, always flaming. The tubes were incubated in bain-marie for 48 hours at 44.5°C.

The quantification was made by the most probable number table and the last dilution must have at least one negative tube.

Detection of *Salmonella* spp

The procedure described for *Salmonella* spp analysis was adapted from Uboldi Eiroa (1982), described by Silva et al. (2007) (Silva et al., 2012).

25g of the strawberries from each repetition of each treatment were weighed, homogenized in 225mL of sterile buffered peptone water and incubated for 18 hours at 35°C. Aliquots of 1mL of that pre-enriched culture were transferred to two tubes, each containing 10mL of selective enrichment broth, made up of tetrathionate broth and Rappaport broth, and incubated at 35°C for 24 hours. From those broths, an aliquot of each tube was sown in agar (*Hektoen Enteric Agar*) and incubated, at 35°C, for 24 hours. From the Hektoen agar, colorless or pink colored colonies, between translucent and slightly opaque, and whose base medium presented dark pink coloration were selected. Later, the colonies were sown in triple sugar iron agar (TSI) and lysine iron agar (LIA), with incubation at 35°C for 24 hours.

Aerobic psychrotroph counts

The counting of aerobic psychrotrophs was conducted according to Samson et al. (2000) described by Silva et al. (2007).

25 g of strawberry pulp from each repetition of each treatment were weighed and added with 225 mL of peptone water 0.1% (H_2O p). They were homogenized under agitation for 5 minutes, this solution being the dilution 10^{-1}. For each dilution a tube with 9 mL of peptone water 0.1% (H_2O p) was made.

1 mL of the 10^{-1} dilution was aurel , placing it in the first tube (10^{-2}), with 9 mL of peptone water 0.1%; 1 mL of this mixture was placed in the second tube (10^{-3}).

For each dilution, three tubes of LTS broth were used for inoculation. 3 mL from the tube with higher dilution was aurel and 1 mL was placed in its corresponding aurel broth. The procedure was repeated until the dilution tubes 10^{-5}.

100 µL were transferred to each dish containing PCA medium, incubating them for 10 days, at 7°C. After the incubation period, colony counts in the different dilutions were conducted and the results were expressed in CFU g^{-1}.

Isolation and identification of fungi and yeast counts

Dilution plating

25 g of the strawberries of each repetition of each treatment in which there was visual growth of fungi, were added to 225 mL of peptone water and agitated for 5 minutes, according to Samson et al., (2000), described by Silva et al. (2007) (Silva et al., 2012).

100 µL were transferred to each dish of medium containing dichloran rose-bengal chloramphenicol or DRBC and incubated for 7 days, at 25°C. After the incubation period,

counting of the colonies in the different dilutions was conducted and the results were expressed in UFC/g. After the counting, the fungi were transferred to the malt extract agar medium, until pure cultures were obtained. The identification of the fungi was carried out with the pure cultures, according to Samson et al. (2000), in standard culture media.

With the aid of a platinum loop, stained slides were prepared (methylene blue) and observed under a microscope for the identification of the genera of the filamentous fungi.

RESULTS AND DISCUSSION

In Resolution RDC Nº12 data are presented relative to the Microbiological Standards for Food Sanitation, in which it states that fresh and similar strawberries, *in natura*, whole, selected or not, have a tolerance of $2x10^3$ for coliforms at 45ºC/g and an absence of Salmonella sp./25g. according to the National Sanitary Surveillance Agency –ANVISA (2013). It was verified that the analyzed strawberries presented coliform absence (at 35ºC and thermotolerant) and *Salmonella* sp., for all of the treatments, plus the control, that can prove that the hygienic and sanitary care taken during the processing of the product is of fundamental importance and can contribute to their presenting low microbial counting.

During storage, it was verified that the strawberries without treatments presented high aerobic psychrotroph counts and those treated with 100 mg L^{-1} of sodium hypochlorite and 100 mg L^{-1} of sodium dichloroisocyanurate obtained lower counts than the control fruits, but superiors to those treated with 200 mg L^{-1} of sodium hypochlorite and 200 mg L^{-1} of sodium dichloroisocyanurate. Comparing the strawberries treated with 200 mg L^{-1} of sodium hypochlorite and those with 200 mg L^{-1} of sodium dichloroisocyanurate, it can be noticed that in those that received sodium dichloroisocyanurate at the 200 mg L^{-1} concentration there was no psychrotroph growth during the six days of storage at room temperature, which can be verified in Table 1.

Antoniolli et al. (2007) evaluated the quality of the fresh-cut 'Pérola' pineapple, stored under controlled atmosphere, and analyzed the following parameters: color, coliforms at 35 and 45 °C, mesophilic aerobic microorganisms, psychrotrophics, molds and yeasts. They concluded that total and fecal coliforms were not detected. The combination 5:15 (O_2:CO_2,%) slightly reduced the microbial growth, however the fresh-cut 'Pérola' pineapple appears to be a little insensitive to storage under controlled atmosphere, considering that at the end of the storage the slices were only slightly darkened and free from contamination that compromised the safety of the food.

TABLE 1 – Average values of aerobic psychrotroph counts in strawberries cv. Oso-grande, stored at room temperature, for a period of six days, under ambient conditions.

Sanitizer	Days	Psychrotrophs (UFC g^{-1})
Control	0	$0.7x10^3$
	3	$2.0x10^3$
	6	UNC
Sodium hypochlorite 100 mg L^{-1}	0	$0.4x10^3$
	3	$5.4x10^3$
	6	$4.0x10^3$
Sodium hypochlorite 200 mg L^{-1}	0	< 25
	3	$0.1x10^3$
	6	$0.3x10^3$
Hidrosan® 100 mg L^{-1}	0	$0.3x10^3$
	3	$0.3x10^3$
	6	$0.3x10^3$
Hidrosan® 200 mg L^{-1}	0	< 25
	3	< 25
	6	< 25

*UNC: Uncountable.

SOURCE – Biochemistry Laboratory in the Chemistry Department of UFLA, 2013.

Regarding the filamentous fungi and yeasts counts, it was verified that sanitizer use is of fundamental importance to inhibit their growth. All of the control samples presented high filamentous fungi and yeast counts, that can also be verified visually, mainly on the 6th day of storage. The samples treated with sodium hypochlorite presented higher filamentous fungi and yeast counts when compared with those treated with sodium dichloroisocyanurate . In the first days of storage, it can be verified that there was a difference among the sodium hypochlorite at 100 mgL^{-1} and 200 mg L^{-1} treatments, but, comparing the sodium dichloroisocyanurate at 100 mgL^{-1} and 200 mgL^{-1} treatments, it is verified that the 200 mgL^{-1} concentration was the most efficient treatment (Table 2). The hydrolysis of sodium dichloroisocyanurate offers advantages in relation to sodium hypochlorite, because it is acid, favoring the non-ionized form of the hypochlorous acid, besides its liberation being gradual, in function of the chemical balance of the reaction.

The presence of fungi in high quantities is undesirable as at the microbiological quality, because they are capable of producing a large variety of enzymes that provoke the deterioration of fruits (soft rot). Furthermore many filamentous fungi can produce metabolic toxins when they are growing in the foods.

Utto et al. (2008) studied the reduction of *Botrytis cinerea* in tomatoes, and related that the rates of filamentous fungi and yeasts are considered low when they remain between 10^3 and 10^4 $UFC.g^{-1}$.

The filamentous fungi identified were the *Rhizopus stolonifer*, that causes disease preferentially in postharvest during the commercialization process, and rarely in the field, also known as soft rot and *Cladosporium cladosporioides*, that provokes dark spots on damaged fruits. Based on the identified species, the results of this study demonstrated a natural fungal deterioration that happens in strawberry fruits, after their shelf life.

Silva et al. (2012) assessing the microbiological quality of strawberries sold in Lavras, MG, observed no growth of coliforms and *Salmonella* sp., in any of the cultivars in the early days of analyzes. At the end of storage was the presence of coliforms at 35 ° C, the cultivar Toyorrinho. It was also observed visual growth of filamentous fungi (*Rhizopus stolonifer* and *Cladosporium cladosporioides*) in strawberry cultivar Tudlla and Toyorrinho.

TABLE 2 – Average values of filamentous fungi and yeast counts in strawberries cv. Oso-grande, stored at room temperature for a period of six days, under ambient conditions.

Sanitizer	Days	Filamentous fungi (UFC g^{-1})	Yeasts (UFC g^{-1})	Contagem total
Control	0	$0.9x10^3$	$0.2x10^4$	$2.6x10^3$
	3	$8.2x10^3$	$2.0x10^4$	$28.2x10^3$
	6	UNC	$5.0x10^4$	UNC
Sodium hypochlorite 100 mg L^{-1}	0	$3.4x10^2$	$4.6x10^2$	$8.0x10^2$
	3	$6.0x10^2$	$4.5x10^2$	$1.1x10^3$
	6	UNC	INC	UNC
Sodium hypochlorite 200 mg L^{-1}	0	$2.0x10^2$	$2.5x10^2$	$4.5x10^2$
	3	$4.4x10^2$	$5.0x10^2$	$9.4x10^2$
	6	UNC	INC	UNC
Hidrosan® 100 mg L^{-1}	0	<30	$0.3x10^3$	$0.3x10^3$
	3	<30	$2.4x10^3$	$2.4x10^3$
	6	<30	$3.0x10^3$	$3.0x10^3$
Hidrosan® 200 mg L^{-1}	0	<30	$1.0x10^2$	$1.0x10^2$
	3	<30	$1.1x10^2$	$1.1x10^2$
	6	<30	$1.0x10^2$	$1.0x10^2$

SOURCE – Biochemistry Laboratory in the Chemistry Department of UFLA, 2013.

Dias et al. (2009) concluded that the strawberry is considered one of the fruits most sensitive to rot and the responsible agents for that fast deterioration are the fungi of the genera *Botrytis*, *Penicillium*, *Phomopsis* and *Rhizopus*.

CONCLUSION

The use of sanitizers was of fundamental importance, maintaining the filamentous fungi and yeasts count low. All of the studied sanitizers were efficient in maintaining the quality of strawberries cv. Oso-grande during six days of storage at room temperature.

However, the dichloroisocyanurate at 200 mg L^{-1} inhibited the growth of filamentous fungi and aerobic psychrotrophs and the yeasts counts were lower when compared with those of the other treatments.

BIBLIOGRAPHICAL REFERENCES

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. **Resolução RDC n. 12, de 02 de janeiro de 2001.** Disponível em: <www.file\\Avisa–Legislação–Resolução.htm>. Acesso em: 17 mar. 2013.

ANTONIOLLI, L. R.; BENEDETTI, B. C.; SIGRIST, J.; M. M.; SILVEIRA, N. F. de A. Quality evaluation of fresh-cut Pérola pineapple stored in controlled atmosphere . **Ciência e Tecnologia de Alimentos**, Campinas, v. 27, n. 3, p. 530-534, 2007.

DIAS, A. C. F.; COSTA, F. E. C.; ANDREOTE, F. D.; LACAVA, P. T.; TEIXEIRA, M. A.; ASSUMPÇÃO, L. C.; ARAÚJO, W. L.; AZEVEDO, J. L.; MELO, I. S. Isolation of micropropagated strawberry endophytic bacteria and assessment of their

potential for plant growth promotion. **World Journal of Microbiology and Biotechnology**, Oxford, v. 25, n. 2, p. 189-195, Feb. 2009.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍTICA. **Enciclopédia dos municípios brasileiros**. Rio de Janeiro, 1959. 670 p.

MARTÍNEZ-TÉLLEZ, M. A.; VARGAS-ARISPURO, L.; CUAMEA-NAVARRO, F.; MORÓN, C. Producción primária y manejo. In: GONZÁLEZ-AGUILAR, G. A.; GARDEA, A. A.; CUAMEA-NAVARRO, F. (Ed.). **Nuevas tecnologías de conservación de productos vegetales frescos cortados.** Hermosillo: CIAD, 2005. cap. 2, p. 17-34.

OLIVEIRA, L. M. de; OLIVEIRA, P. A. P. L. V. de. Revisão: principais agentes antimicrobianos utilizados em embalagens plásticas. **Brazilian Journal of Food Technology**, Campinas, v. 7, n. 2, p. 161-165, 2004.

REIS, K. C.; SIQUEIRA, H. H.; ALVES, A. P.; SILVA, J. D.; LIMA, L. C. O. Efeito de diferentes sanificantes sobre a qualidade de morango cv. Oso-grande. **Ciência e Agrotecnologia**, Lavras, v. 32, n. 1, p. 196-202, jan./fev. 2008.

SAMSON, R. A.; HOEKSTRA, E. S.; FRISVAD, J. C.; FILTENBORG, O. **Introdution to food-borne Fungi**. 4. ed. Baarn: Centraalbureau Voor Schimmelcultures, 2000.

SILVA, N. da; JUNQUEIRA, V. C. A.; SILVEIRA, N. F. A.; TANIWAKI, M. H.; SANTOS, R. F. S. dos; GOMES, R. A. R. **Manual de métodos de análise microbiológica de alimentos**. 3. ed. São Paulo: Varela, 2007. 552 p.

SILVA, P. A.; ABREU, C. M. P. de; BATISTA, L. R.; QUEIROZ, E. R.; CORRÊA, A. D. Avaliação da qualidade microbiológica de morangos comercializados na região de Lavras, MG. **Revista Brasileira de Armazenamento,** Viçosa, v. 37, n. 1, p. 14-17, 2012.

UBOLDI EIROA, M. N. **Curso de microbiologia de alimentos.** Campinas: ITAL, 1982. 83 p.

UTTO, W.; MAWSON, A. J.; BRONLUND, J. E. Hexanal reduces infection of tomatoes by *Botrytis cinerea* whilst maintaining quality. **Postharvest Biology and Technology**, Amsterdam, v. 47, n. 3, p. 434-437, Mar. 2008.