Combined natural antimicrobial treatments on a ready-to-eat poultry product stored at 4 and 8°C

A. G. Ntzimani, V. I. Giatrakou, and I. N. Savvaidis

Laboratory of Food Chemistry and Food Microbiology, Department of Chemistry, University of Ioannina, Ioannina 45110, Greece

ABSTRACT The aim of the present study was to evaluate the effect of natural antimicrobial agents EDTA, lysozyme, and 2 essential oils (rosemary and oregano) on the quality of a ready-to-eat poultry product (semicooked coated; SCC) stored under aerobic packaging conditions at 4°C (retail) and 8°C (abuse) for a period of 16 d. Treatments included the following: air-packaged chicken fillets (control, untreated); with EDTA (1.50% wt/wt); with lysozyme solution (1.50% wt/wt); with rosemary oil (0.20% vol/wt); with oregano oil (0.20% vol/wt); with a combination of EDTA and lysozyme solutions (1.50% wt/wt each); and with the combination of EDTA, lysozyme, and either rosemary or oregano essential oils (all added at concentrations previously mentioned). The shelf life of the SCC samples (untreated and treated) was determined using both microbiological and sensory analyses. Natural antimicrobial combinations consisting of EDTA, lysozyme, and rosemary or oregano essential oil affected the growth of Pseudomonas and yeasts and molds, whereas EDTA, lysozyme, and rosemary essential oil controlled Brochothrix thermosphacta population in the SCC chicken fillets stored at 4 and 8°C. The combination of EDTA, lysozyme, and either rosemary or oregano resulted in a shelf life extension of 5 d compared with the control samples at both 4 and 8°C, with the former combination producing a more sensorially acceptable product.

Key words: semicooked coated chicken fillet, lysozyme, ethylenediaminetetraacetate, essential oil, poultry

INTRODUCTION

Precooked and refrigerated ready-to-eat meat products are gaining interest because consumers purchase convenience foods that do not require extensive home preparation. Simultaneously, customers have become more knowledgeable about the content of the products they consume and expect their meal to be nutritious, palatable, and safe to eat. Consumers desire fresher and more natural meals that possess a “green” image and can conveniently be heated in a microwave oven. Therefore, food industries have been challenged to develop new methods for preserving ready-to-eat products possessing natural additives.

Several studies (Holzapfel et al., 1995; Skandamis et al., 2002) have recently demonstrated the beneficial effects of natural antimicrobials individually or in combination with preservative technologies (e.g., packaging) when applied on food systems. Lysozyme, a naturally occurring enzyme in foods, is being used frequently by the food industry (Gill and Holley, 2000). Nattress and Baker (2001, 2003) showed that lysozyme possesses antimicrobial action against spoilage bacteria of pork loins. The antimicrobial effect of lysozyme solution against lactic acid bacteria was investigated by Chun and Hancock (2000).

Chelators, often used as food additives, include the naturally occurring citric acid and the disodium and calcium salts of EDTA (Russell, 1991). The latter is known to potentiate the effect of weak acid preservatives against gram-negative bacteria by limiting the availability of cations, thus destabilizing the bacterial cell membranes (Shelef and Seiter, 1993). The combined effect of EDTA and lysozyme solutions was demonstrated in the inhibition of bacterial growth on ham and bologna (Gill and Holley, 2000).

Essential oils (EO), also called ethereal oils, are volatile liquids soluble in organic solvents and are known for their bactericidal, virucidal, and fungicidal properties (Burt, 2004). Because of their strong sensory characteristics, careful evaluation is needed before they are used as natural preservatives (Nychas, 1995) so that when added to food products they are sensorially acceptable to the consumer.

Although natural antimicrobial treatments have been used for the preservation of poultry meat (Tu and Mu-
stapha, 2002; Mecitoğlu et al., 2007), to our knowledge the combination EDTA, lysozyme, and EO for the preservation of fresh poultry meat and poultry products has not been reported to date. Thus, the objective of the present study was to investigate the effect of the aforementioned natural antimicrobial treatments in increasing the shelf life of a poultry product stored under aerobic packaging conditions at 4 and 8°C.

MATERIALS AND METHODS

Preparation of Semicooked Coated Fillets and Storage Conditions

Fresh semicooked coated (SCC) chicken fillets (schnitzel type, each approximately 150 ± 10 g) were purchased from a local poultry processing company (Pindos S.A., Ioannina, Greece) and prepared using a procedure described previously (Patsias et al., 2006). The SCC chicken fillets were transported to the laboratory in insulated polystyrene boxes on ice within 1 h of the baking process. Samples were packaged (1 fillet/pouch) in low-density polyethylene/polyamide/low-density polyethylene barrier pouches (75 μm thickness; Ver Pack, Thessaloniki, Greece) with an oxygen permeability of 52.2 cm³/m² per day per atmospheric mass unit at 75% RH, 23°C; a carbon dioxide permeability of 191 cm³/m² per day per atmospheric mass unit at 0% RH, 23°C; and a water vapor permeability of 2.4 g/day at 100% RH, 23°C.

In our study the following treatments were used: air-packaged SCC chicken fillets (control); air-packaged SCC chicken fillets with EDTA (1.50% wt/wt; AE); air-packaged SCC chicken fillets with lysozyme solution (1.50% wt/wt; AL); air-packaged SCC chicken fillets with rosemary oil (0.20% vol/wt; AR); air-packaged SCC chicken fillets with oregano oil (0.20% vol/wt; AO); air-packaged SCC chicken fillets with EDTA (1.50% wt/wt) and lysozyme solution (1.50% wt/wt; AEL); air-packaged SCC chicken fillets with EDTA (1.50% wt/wt), lysozyme solution (1.50% wt/wt), and rosemary oil (0.20% vol/wt; AELR); and air-packaged SCC chicken fillets with EDTA (1.50% wt/wt), lysozyme solution (1.50% wt/wt), oregano oil (0.20% vol/wt; AELO). Sampling was carried out at predetermined time intervals (0, 2, 4, 6, 9, 14, and 16 d).

Preparation of Solutions

The EDTA (C₁₀H₁₄N₂O₈∙Na₂∙2H₂O, 372.3 molecular weight, 99.5% purity, analytical grade; Serva, Athens, Greece), lysozyme (lyophilized) from chicken egg white (activity of approximately 50,000 IU/mg; Sigma, Athens, Greece), and EO (rosemary or oregano; obtained as pure, undiluted plant extracts; Kokkinakis, Athens, Greece) used in our study were prepared as follows: EDTA solution (50 mM) was prepared in sterile distilled water and the final pH was adjusted to 8.0 with the addition of the appropriate quantity of NaOH (1 N) solution. Lysozyme solution (250 μg/mL) was prepared in sterile distilled water, whereas the EO were used undiluted. The EDTA, lysozyme solutions, and EO were stored under refrigeration (4°C) before their application to the chicken samples.

Application of EDTA, Lysozyme Solutions, and Rosemary or Oregano Oils to the SCC Fillets

The antimicrobials used in our study were added to the SCC chicken fillets in the following order: EDTA was added first, followed by lysozyme and finally each of the EO. The EDTA and lysozyme solutions were added to the samples by spraying, followed by a mild, gloved massage of the samples, to achieve a final concentration of 1.5% (wt/wt) of each of these antimicrobials on the chicken product. Finally, rosemary or oregano oils were added on the surface (2 sides) of each SCC chicken fillet using a micropipette so as to achieve a 0.2% (vol/wt) final concentration of each oil on the sample.

Sensory Evaluation

The attributes (odor and taste) of microwave-cooked SCC chicken fillets were evaluated by a panel of 7 experienced judges on each day of sampling. Samples were cooked individually in a microwave oven at high power (800 W) for 3 to 4 min and immediately presented to the panelists. Panelists were not asked to evaluate any uncooked SCC chicken product. Freshly thawed samples (previously stored at −30°C, served as reference samples) were also heated, as described previously, and presented to the panelists. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature, and humidity. Panelists were asked to score odor and taste of microwave-cooked chicken samples using a 0 to 9 intensity scale, with 9 corresponding to the most-liked sample and 1 corresponding to the least-liked sample (del Río et al., 2007). The product was defined as unacceptable (score <6) after development of the first off odor. The evaluation scale was as follows: 9 = excellent, 8 = very good, 7 = good, 6 = acceptable, <6 = unacceptable.

Microbiological Analysis

Approximately 25 g of the SCC fillet was sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag containing 225 mL of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a Lab Blender 400 stomacher (Seward Medical, London, UK) at room temperature. Microbiological analyses were conducted using standard microbiological methods (APHA, 1984). For total viable counts (TVC) and Pseudomonas spp. enumerations, 0.1 mL of 1:10 prepared serial dilutions (0.1% peptone water) of chicken homogenates was spread onto the surface
of solid media. The TVC were determined using plate count agar (Merck, Darmstadt, Germany) after incubation for 72 h at 30°C. Pseudomonads were determined on cetrimide fucidin cephaloridine agar (code CM 559 supplemented with selective supplement SR 103; Oxoid, Basingstoke, UK) after incubation for 48 h at 30°C, and oxidase-positive colonies were enumerated. Brochothrix thermosphacta was determined on streptomycin sulfate–thallous acetate–cycloheximide (actidione) agar, prepared from basic ingredients in the laboratory after incubation at 48 h at 30°C. Finally, yeasts and molds were enumerated on rose bengal chloramphenicol selective agar (Merck) and incubated for 72 to 120 h at 30°C. All plates were examined for typical colony types and morphology characteristics associated with each growth medium.

Statistical Analysis

Experiments were replicated twice on different occasions with different SCC samples. Triplicate samples were analyzed per replicate. Microbiological counts were converted to log colony-forming units per gram and were subjected to ANOVA using Stat Graphics software (Statistical Graphics Corp., Rockville, MD). Means and SD were calculated and, when F-values were significant at the \( P < 0.05 \) level, mean differences were separated by the least significant difference procedure.

RESULTS

Microbiological changes of the SCC chicken fillets, both untreated (control) and treated (AR, AO, AEL, AELR, and AELO) and stored at 4 and 8°C, are shown in Figures 1, 2, 3, and 4. It must be noted that AE and AL individual treatments were also tested; however, results (not shown) revealed no statistically significant differences \( (P > 0.05) \) compared with the control samples.

At 4°C (retail temperature), the initial TVC of the SCC chicken fillets (Figure 1a) was approximately 3.59 log cfu/g (d 0). Analysis of the TVC data (Figure 1a) showed that the TVC population in SCC chicken fillets, stored at 4°C, reached a value of 7 log cfu/g (considered as the upper limit for microbiological acceptability in foods; ICMSF, 1978) after approximately 10 d for control samples, which is considered the shelf life of the product. The AO and AR treatments also reached a value of approximately 7 log cfu/g after 12 d of storage.

With regard to AELO and AELR samples (Figure 1a), a microbiological shelf life extension of 5 d was obtained for each sample. Our results indicate that a treatment combination consisting of lysozyme, EDTA, and EO (oregano or rosemary) may be effective in controlling TVC growth in the SCC chicken product.

The TVC data for the samples stored at 8°C (abuse temperature) were also recorded (Figure 1b). It was observed that at this temperature the TVC population in
SCC chicken fillets was higher \((P < 0.05)\) than at 4°C throughout the storage period, reaching a value of 7 log cfu/g after approximately 4 and 6 d for control samples and AO and AR samples, respectively.

As previously (4°C), the combination of the EDTA, lysozyme, and the EO was the most effective \((P < 0.05)\) in controlling growth of the TVC in the SCC chicken product. The basic difference was the fact that TVC values were statistically higher \((P < 0.05)\) for the samples stored at abuse temperature, meaning that the shelf life of the SCC chicken samples was longer at the lower storage temperature (4°C).

Irrespective of the antimicrobial treatments, analysis of the data revealed that *Pseudomonas* spp. counts increased during the entire storage period, reaching high populations, approximately 7 to 8 log cfu/g in all SCC chicken samples stored both at 4 and 8°C, with the exception of the AELR and AELO samples (Figure 2a and b, respectively). Of all the treatments examined in our study, the combination of EDTA, lysozyme, and EO proved to be the most efficient between d 9 and 16 of storage, producing significantly lower \((P < 0.05)\) counts compared with the control samples. Counts of *Pseudomonas* spp. were approximately 1 to 1.5 log cfu/g lower \((P < 0.05)\) compared with the control samples.

It is noteworthy that *B. thermosphacta* counts were significantly lower \((P < 0.05)\) with the AELR treatment compared with all other treatments between d 4 and 16 of storage at both 4 and 8°C (Figure 3a and b). Additionally, it was observed that rosemary oil was more effective against the growth of *B. thermosphacta* compared with oregano EO, added to both AR and AELR samples. Counts during storage at 4°C were significantly lower \((P < 0.05)\) than the respective counts during storage of the SCC chicken fillets at 8°C.

In our study, final yeasts and molds counts (Figure 4a and b), irrespective of treatment and temperature, were in the range of approximately 4 to 5 log cfu/g in all SCC chicken samples. Of all the antimicrobial treatments applied, the most effective against these species were the combinations including EDTA, lysozyme, and EO (AELR and AELO), producing lower counts \((P < 0.05)\) by approximately 1.0 to 2.0 log cfu/g compared with the control samples between d 4 and 16 of storage. Present results show that the combination of EDTA, lysozyme, and EO may more effectively control the growth of yeasts and molds in SCC chicken samples stored under aerobic conditions at 4°C. At abuse temperature (8°C), a difference of approximately 1.0 log cfu/g in yeasts and molds counts was recorded in SCC chicken samples upon treatment with the AELR and AELO combination samples compared with the control samples between d 4 and 14 of storage.

The results of the sensory evaluation (taste and odor) of SCC chicken fillets stored under aerobic conditions in the absence and presence of antimicrobials at both 4 and 8°C were determined. Generally, the taste attribute was a more sensitive parameter than odor; therefore, in our study the taste attribute was used for
the determination of the shelf life of SCC chicken untreated (control) and treated (AR, AO, AEL, AELR, and AELO) and stored at 4 and 8°C (Figure 5a and b, respectively). With regard to the odor attribute, no significant differences \( (P > 0.05) \) were recorded between control and treated samples stored at 4 and 8°C (results not shown).

At both storage temperatures, on d 0 of storage SCC chicken fillets had an extremely pleasant taste and odor (score 9). Data (based on taste evaluation) showed that the shelf life at both 4 and 8°C (Figure 5a and b) was longer for the AELR and AELO samples (15 and 9 d at 4 and 8°C, respectively), followed by AR and AO (12 and 6 d, respectively), AEL (11 and 5 d, respectively), and control (10 and 4 d, respectively). The results of the sensory evaluation (taste data; Figure 5a and b) correlated well with those of the microbiological analyses (TVC data; Figure 1a and b) at both storage temperatures used.

**DISCUSSION**

The increased demand for natural antimicrobials during the past few years stimulated investigations regarding their effects on real food systems (Tiwari et al., 2009). This study evaluated a combination including a chelator (EDTA), lysozyme, and EO (rosemary or oregano) and effects of these treatments on the shelf life, microbial flora, and sensory characteristics of chicken fillets, either in the absence or presence of these antimicrobials. Additionally, the effect of temperature abuse was investigated.

The initial microbiological analysis (TVC) of the chicken fillets was approximately 3.59 log cfu/g (d 0), most likely indicating postcooking cross-contamination as well as a high initial (pre-thermal treatment) microbial load \( (10^5–10^6 \text{ cfu/g}) \) of the chicken fillets given that during the pasteurization treatment, an approximately 2-log reduction in TVC is expected (Senter et al., 2000). In a related study, Patsias et al. (2006) also reported high initial TVC microbial load \( (TVC = 3.9 \text{ log cfu/g}) \) in a chilled, precooked chicken product.

When each of the 2 EO (oregano and rosemary) in the present study were added to the samples examined (AO and AR treatments) and stored at 4°C, a 2-d shelf life extension of the SCC chicken fillets was noted, which may be attributed to the antimicrobial effects of the EO phenolic components such as carvacrol, thymol, p-cymene, and γ-terpinene. Other studies (Skandamis and Nychas, 2001) reported a shelf life extension of 4 d after the application of oregano oil (1% vol/wt) on minced beef stored aerobically under refrigeration, whereas Tsigardia et al. (2000) showed that the addition of 0.80% \( (\text{vol/wt}) \) oregano oil resulted in a reduction of 2 to 3 log cycles in the total mesophilic bacteria of vacuum-packaged beef. Treatment of SCC chicken fillets by the EDTA–lysozyme combination led to a shelf life extension of 1 d for AEL samples (Figure 1a), which was attributed to the increased antimicrobial action of...
EDTA–lysozyme treatment combination (Branen and Davidson, 2004) in agreement with the results of Gill and Holley (2000). The shelf life of the SCC chicken fillets was longer and more extended in the samples in which the combinations of EDTA, lysozyme, and oregano or rosemary oil were added. These combinations have not been investigated in the past, and the 5-d shelf life extension may be attributed to the combined, synergistic effect of the natural antimicrobials used in the present study.

When the samples were stored at 8°C, the shelf life of the control and AO and AR samples was proven to be 4 and 6 d, respectively. This 2-d shelf life extension when oregano and rosemary oil were added, as previously noted, may be attributed to the antimicrobial effects of the EO phenolic components. Their mechanism of action is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents (Lambert et al., 2001; Burt, 2004; Holley and Patel, 2005). In other studies, Zhang et al. (2009) reported that treatment of fresh pork chops with extracts of rosemary and licorice reduced TVC by approximately 3.0 log cfu/g during refrigerated storage under modified atmosphere packaging conditions. Furthermore, Wan et al. (1998) and Singh et al. (2002) reported that the addition of EO (thyme and basil) to the washing water of iceberg lettuce and carrots managed to delay the growth of pathogens and spoilage bacteria.

As far as AEL samples (Figure 1b) are concerned, a 1-d extension of the microbiological shelf life was noted, which may be attributed to the increased antimicrobial action of the combination of the EDTA–lysozyme solutions (Branen and Davidson, 2004). Finally, the shelf life of AELO and AELR samples (Figure 1b) was extended by 5 d because of the combined effect of the antimicrobials used.

As expected, the microbiological shelf life of untreated and especially of treated SCC chicken samples was longer at 4°C (retail) than at 8°C (abuse), as also noted by Corbo et al. (2004) for minimally processed cactus pear fruit stored at 4°C and by Toivonen (1997) for storage of wrapped broccoli at 1°C compared with 5°C. Ravishankar et al. (1992) also reported that the shelf life (7 d) of the sausages kept at room temperature (18°C) increased to 65 d when a lower refrigerated storage temperature (2°C) was used.

Besides TVC counts, Pseudomonas spp. were examined in the present study. Results obtained showed that the combination of EDTA, lysozyme, and EO (AELR and AELO treatments) was effective in retarding the growth of Pseudomonas spp., partly attributed to the action of the chelating agent (EDTA) rendering them sensitive to antibacterial agents that they normally resist. The mode of EDTA action is to remove Mg2+ and Ca2+ ions from the gram-negative cell wall, affecting its permeability and thus allowing antimicrobial agents to

Figure 4. Changes (log cfu/g) in yeasts and molds of semicooked coated fillets stored at A) 4°C and B) 8°C in air (untreated; ■), with rosemary oil (□), with oregano oil (♦), with EDTA and lysozyme solutions (◊), with EDTA and lysozyme solutions and rosemary oil (▲), and with EDTA and lysozyme solutions and oregano oil (Δ). Each point is the mean of 3 samples taken from 2 replicate experiments (n = 6). Error bars show SD.
Brochothrix thermosphacta, a facultative anaerobic gram-positive bacterium, produces cheesy or dairy odors (Dainty and Mackey, 1992) upon chicken meat spoilage (Kakouri and Nychas, 1994). This bacterium was also examined in our study and it was observed that the AELR treatment was the only combination that was effective against B. thermosphacta growth ($P < 0.05$) between d 4 and 16 of storage of the SCC chicken fillets at both temperatures examined (Figure 3a and b). Counts during storage of the SCC chicken fillets at 4°C were significantly lower ($P < 0.05$) than counts during storage at 8°C. Gram-positive organisms tend to be more susceptible to the action of lysozyme than gram-negative organisms because of the absence of their protective outer membrane (Nattress and Baker, 2003). According to Ouattara et al. (1997), rosemary oil effectively inhibited the growth of B. thermosphacta in meat. In another study (Tu and Mustapha, 2002), EDTA alone exhibited an antibacterial effect upon B. thermosphacta, with a reduction of 2.6 log compared with the untreated control. Gill and Holley (2000) also showed that growth of B. thermosphacta was inhibited by the use of EDTA and lysozyme solutions. It must be stated that limited information exists on the effects of natural antimicrobials, including EDTA, lysozyme, and EO, on B. thermosphacta in fresh poultry meat and poultry products.

With regard to yeasts and molds, it must be noted that limited studies have been conducted in relation to the effects of natural antimicrobials (Ismail et al., 2000). It was observed that of all the antimicrobial treatments applied, the most effective against these species were AELR and AELO treatments, producing lower counts ($P < 0.05$) compared with the rest of the treatments examined. Gallo et al. (1988) observed that a yeast population of $10^2$ cfu/cm² on the skin of freshly slaughtered chicken increased about 2.5 log cfu/cm² during storage at 4°C after 8 d. According to Holley and Patel (2005), EO are known to possess anti-yeast and antifungal activity and generally are more active against yeasts and fungi than against bacteria. In other studies related to poultry products, Patsias et al. (2006) reported that growth of these species was suppressed in precooked chicken samples stored under vacuum packaging for 14 d. Finally, chitosan in combination with rosemary oil reduced yeasts population by approximately 2 log cycles in sausages during chilled storage (Georgantelis et al., 2007).

Of the microbial flora association examined in our study, gram-negative bacteria, such as the Pseudomonas spp., known to be one of the most resistant bacteria groups to antimicrobial agents, were suppressed in the SCC chicken fillets as a result of the combined action of EDTA, lysozyme, and EO used, whereas B. thermosphacta growth was delayed by the antimicrobial combination that included only rosemary EO. Finally, yeast and mold populations were affected most by the AELO treatment. As expected, growth of all microorganisms examined in the present study was affected by temperature, being slower at 4°C than at 8°C. To our knowledge, the use of natural antimicrobials has not yet

**Figure 5.** Changes in taste scores of semicooked coated fillets stored at A) 4°C and B) 8°C in air (untreated; ■), with rosemary oil (□), with oregano oil (♦), with EDTA and lysozyme solutions (◊), with EDTA and lysozyme solutions and rosemary oil (▲), and with EDTA and lysozyme solutions and oregano oil (△). The dotted line shows the acceptance limit. Each point is the mean of 3 samples taken from 2 replicate experiments ($n = 6$). Error bars show SD.
been reported in the literature with regard to chicken meat preservation at either refrigerated (4°C) or abuse (8°C) temperatures.

It is noteworthy that the presence of EDTA and lysozyme (AEL treatment) in SCC chicken samples stored at 4 and 8°C imparted a lemon taste that was well received by the panelists. Of the treatments AELR and AELO, the former (presence of rosemary oil 0.20% vol/wt) in chicken samples stored at 4°C produced a distinct and pleasantly acceptable taste, whereas the application of oregano oil (0.20% vol/wt) to the SCC chicken fillets was not as pleasant as that of the rosemary oil, resulting in a rather bitter taste, even though the concentration of the EO added to the SCC chicken fillets was the same. A lower oregano oil concentration (i.e., 0.10% vol/wt) would perhaps impart a more sensorially acceptable taste to the chicken product, although this hypothesis was not investigated. Similar sensory characteristics were obtained for AEL, AELR, and AELO treatments at 8°C as judged by the panelists. It is noteworthy that the potential use of EO such as rosemary or oregano as preservatives in foods needs to be carefully evaluated in terms of their sensorial acceptability.

Present results suggest that natural antimicrobials (EDTA, lysozyme, and rosemary oil to a larger extent than oregano oil) may be used as alternative chemical agents for poultry meat and poultry products storage, satisfying the consumer demands for preservative-free, ready-to-eat foods with improved sensory quality.

In conclusion, natural antimicrobial combinations consisting of EDTA, lysozyme, and rosemary or oregano EO (AELR and AELO treatments) affected the growth of *Pseudomonas* spp. and yeasts and molds, whereas the use of EDTA, lysozyme, and rosemary EO controlled *B. thermosphacta* population in the SCC chicken fillets stored at 4 and 8°C. The AELR and AELO treatments resulted in a shelf life extension of 5 d compared with the control (untreated) samples both at 4 and 8°C, with the former combination producing a more sensorially acceptable product.

ACKNOWLEDGMENTS

We thank the European Union for the financial support of the project “Double Fresh” (proposal/contract no. PL 023182). We acknowledge M. Zwietering and Z. Sosa Mejia (both of Wageningen University, Department of Agrotechnology and Food Sciences, Laboratory of Food Microbiology) for providing useful suggestions in relation to this project.

REFERENCES


