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Microbiological quality of tomatoes and peppers produced under the good agricultural practices protocol AGRO 2-1 & 2-2 in Crete, Greece

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Abstract

The efficiency of the good agricultural practices (GAP) protocol AGRO 2-1 & 2-2, in advancing microbiological-quality of tomatoes and peppers, was studied in greenhouses at Ierapetra, Crete, Greece. The 240 tested vegetables-samples, produced under AGRO 2-1 & 2-2, showed satisfactory quality: *Listeria monocytogenes* absent per 25 g; *Escherichia coli* < 20 Colony Forming Units per gram (CFU/g); total coliforms 4.37–4.68 log CFU/g; aerobic plate counts 5.78–5.92 log CFU/g. Based on actual results and practices evaluation, we conclude that AGRO 2-1 & 2-2 can reduce microbial hazards for consumers and furthermore can establish practices in compliance to basic Euro-Retailer-Produce GAP (EUREPGAP) requirements.

Keywords: Microbiological quality; Vegetables; Good agricultural practices; EUREPGAP

1. Introduction

Fresh vegetables supply consumers with vitamins, minerals, and fibers (FAO/WHO, 2004). Although they are colonized mainly by saprophytes and plant pathogens (Ligoxigakis, Fragkiadakis, Manganaris, Vakalounakis, & Thanassoulopoulos, 2002; Vakalounakis & Fragkiadakis, 1999; Vakalounakis, Wang, Fragkiadakis, Skaracis, & Li, 2004), they can also carry human pathogens (*Listeria monocytogenes, Escherichia coli* etc.) that occasionally cause outbreaks of foodborne illness (Beuchat, 1996; Garrett et al., 2003; Legnani & Leoni, 2004).

Quality systems, such as good agricultural practices (GAP), are recommended, in order to sustain best practices for farming, i.e. soil and water management, crop production, storage, waste disposal etc. (Codex Alimentarious Commission, 1997; FAO, 2003; FDA, 1998). GAP systems can also provide products of higher microbial safety that facilitate the implementation of Hazard Analysis Critical Control Points (HACCP) procedures in establishments offering minimally processed vegetables (Kokkinakis & Fragkiadakis, 2007). Consequently, monitoring of certain microbial-flora markers during vegetable production can contribute in advancing hygiene "from farm to table": can link GAP and HACCP procedures (Baines, Ryan, & Davies, 2004).

Since 1999, Greece has adopted the AGRO 2-1 & 2-2 GAP system in agricultural farms, to maintain consumer

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confidence in food safety; protect worker safety, and the environment (AGROCERT, 2005a). On the other hand, in 1997 a group of 26 retail organizations belonging to the Euro-Retailer Produce (EUREP) Working Group took the initiative to develop and harmonize widely accepted standards and procedures, for a potentially global certification of GAP. In 2001 EUREP, has produced the first version of EUREPGAP protocol that today as version 2.1 defines the minimum standard acceptable to many leading retail groups in Europe (EUREPGAP, 2004).

The basic targets of this study were: (I) To monitor certain microbial-flora markers, in order to check the efficiency of the Greek protocol AGRO 2-1 & 2-2 in advancing microbial food quality of tomatoes and peppers grown in greenhouses. (II) To evaluate whether overall greenhouses-management under AGRO 2-1 & 2-2, can establish actual farming and handling conditions that are in compliance to basic EUREPGAP requirements.

2. Methodology

2.1. Sampling

Four greenhouses were selected within a diameter of 30 km from Ierapetra, the major vegetables producing area in Crete and one of the 4–5 major ones in Greece. Two greenhouses (GRA and GRB) were growing vegetables under the GAP protocol AGRO 2-1 & 2-2, while the other two (GRC and GRD) applied no quality management system. GRA covered about $20 \times 10^3\,m^2$; GRB about $20 \times 10^3\,m^2$; GRC $6–7 \times 10^3\,m^2$; and GRC $5–6 \times 10^3\,m^2$. Sampling was carried out in 2005, in two sessions: during vegetables growing in spring, and during harvesting in summer. Samples were collected (ICMSF, 2002) as follows: (I) 15 tomatoes and 15 peppers were randomly collected per greenhouse in spring. Water was also tested (9 samples per greenhouse), in order to evaluate its possible effect on the vegetables microflora. (II) Similarly, during harvesting, vegetable samples were collected and also samples from the greenhouse's containers and the personnel's hands. Microbial analysis was concentrated on pathogenic microorganisms (*Listeria monocytogenes* or LMO), *Escherichia coli* (*E. coli*); as well as spoilage-microorganisms markers: total coliforms (TC), and aerobic plate count (APC). Totally we analyzed: 240 vegetable samples (120 tomatoes, 120 peppers) for LMO, TC, *E. coli*, and APC; 36 greenhouses water-samples for *E. coli*, *Streptococcus faecalis,* and TC; 60 samples of vegetable containers for *E. coli*, TC, and APC; 36 samples of greenhouses-personnel's hands, for TC, and APC.

2.2. Microbiological analysis

The *L. monocytogenes* detection and enumeration followed methods provided by the International Organization for Standards (ISO 11290-1:1997). Two-stage enrichment was used, with inoculation into "half Fraser" broth, followed by subculture into Fraser broth. This latter medium was then inoculated on to Oxford agar as well as Polymyxin-Acriflavin-Lithiumchloride-Ceftazidime-Aesculin-Manni-tol (PALCAM) agar, incubated for 24–48 h at 35 °C and read with a colony counter (Van Netten, Perales, van der Moosalijk, Curtis, & Mossel, 1989). The suspect colonies were used to inoculate tryptic soy agar with 0.6% yeast extract (TSA-YE); plates were incubated for 24 h at 35 °C and the colonies still suspect were confirmed with API Listeria kit (10300, API Listeria strip, bioMerieux SA, 69280 Marcy-l'Etoile/France). With the method used, enumeration of either large (more than 10 per g) or smaller bacteria numbers (1 per 10 g or 1 per 25 g) can be carried out (Van Netten et al., 1989). The 3 M Petrifilm *E. coli*/*Coliform* Count (EC), and aerobic count (AC) methods were used for enumeration of *E. coli*, total coliforms and APC (Gracias & McKillip, 2004). The 3 M Petrifilm Plates are sample-ready plates used for microbial enumeration on raw materials, in-process products, finished products, and the plant environment (Gracias & McKillip, 2004). For the microbial analysis of water the membrane filtration technique was used, as described in ISO 9308-1:2000 for *E. coli* and total coliforms, and ISO 7899-2:2000 for *S. faecalis*. By this technique small numbers of microbes can be detected; the water passes through the membrane restricted only by the amount of gross suspended matter present in it (ISO 9308-1:2000, ISO 7899-2:2000). For the evaluation of personnel's hands and vegetable's container hygiene, the Hy-Giene Monitor (Hy-Laboratories Ltd.) kit was used, that involves a visual interpretation guide for evaluation of microbial levels on personnel's hands (Raugel, 1999).

2.3. Evaluation of microbial safety

Codex Alimentarious Commission (1997) guidelines were followed for evaluating the microbial quality of fresh produce, i.e.: *L. monocytogenes* (not to be detected in 25 g), *E. coli* (satisfactory <20 CFU/g, acceptable 20–100 CFU/g, unsatisfactory >100 CFU/g). Concerning *S. faecalis*, it must not be present in potable water (Council Directive 98/83 EC). *L. monocytogenes* is naturally present in the soil and often found on produce, while *E. coli* and *S. faecalis* are more likely to contaminate fresh produce through vehicles such as raw or improperly composted manure, irrigation water containing untreated sewage, or contaminated wash water (Giese, 2003). Total coliforms (TC) and aerobic plate counts (APC) were also measured, even though fresh vegetables often carry high levels of these organisms as part of their normal flora (Gilbert et al., 2000). Total coliforms and aerobic plate counts are considered suitable to provide a general estimation of the total number of microorganisms on produce (Giese, 2003), and are very helpful in estimating/comparing the effect each step (growing, harvesting, storage etc.) has on vegetable microbial quality "from farm to table" (Kokkinakis & Fragkiadakis, 2007).