The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology 35(2) 75-85

ORIGINAL RESEARCH PAPER

STUDY OF THE COMBINED EFFECT OF SPICES AND MARINATION ON BEEF MEAT VACUUM PACKAGED

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Received 29 September 2011
Revised 10 October 2011

Fresh beef slices were marinated by immersion in marinades based on dry red wine, lime-tree honey, salt, spices and seasoning plants as thyme (*Thymus vulgaris),* marjoram (*Majorana hortensis*), garlic (*Allium sativum*) and horseradish (*Armoracia rusticana*). Control samples were represented by raw meat without marination treatment but stored in the same conditions as marinated samples. After marination, meat pieces were packed under vacuum and stored at refrigeration temperature of 4°C for 12 days. The influence of the combined effect of spices and marination on beef stability was evaluated by monitoring pH evolution, degree of lipid oxidation and by microbiological analysis. For control samples, a mean increase of 0.47 log CFU/g of total mesophilic aerobic bacteria was observed during the 48 h of storage, but for the samples marinated with the addition of spices was observed a decrease of 0.57 log CFU/g. The growth of LAB in control samples was generally limited and did not exceed 5 log CFU/g. During storage at 4°C, marination with the addition in the base marinade (wine, honey, garlic, pepper and salt) of thyme, marjoram and horseradish separately inhibited the growth of LAB while marination with the addition in the base marinade of thyme, marjoram and horseradish together resulted in significantly lower levels of LAB. All marination treatments resulted in significantly lower TBA and POV values at the end of storage compared to the control. Marination with dry red wine, lime-tree honey, thyme marjoram, garlic, and horseradish can evidently control total mesophilic aerobic bacteria, lactic acid bacteria and oxidation of beef meat.

Keywords: beef meat, spices, marination, lipid oxidation, lactic acid bacteria, mesophilic aerobic bacteria

Introduction

During storage, quality attributes of the product deteriorate due to lipid oxidation and microbial growth. Lipids oxidation is responsible for reduction in nutritional quality as well as changes in flavor (Aguirreźabal *et al.*, 2000), while microbial contamination can precipitate major public health hazards and economic loss in terms of food poisoning and meat spoilage. Thus, the application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss (Yin and Cheng, 2003). Much research has indicated that lipid oxidation and microbial growth in meat products can be controlled or minimized by using either synthetic or natural food additives (Gray *et al.*, 1996; Lee *et al.* 1997; Mielnik *et al*., 2003). Natural agents possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers, as they are considered natural (Sallama *et al*., 2004). Some spices and herbs used today are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities. The extracts of many plant species have become popular in recent years and the attempts to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications (Shan *et al.*, 2007). The antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies (Lis-Balchin and Deans, 1997). Many studies have reported that phenolic compounds in spices and herbs significantly contribute to their antioxidant and pharmaceutical properties (Cai *et al.*, 2004; Shan *et al*., 2005; Wu *et al*., 2006). Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo *et al*., 2004).

The polyphenolic compounds play a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and cardioprotective and vasodilatory effects (Benhammou *et al*., 2009). These functions have been attributed to their antioxidant activity by several mechanisms such as free radical scavengers, reducing agents, complexers of pro-oxidant metals, quenchers of the formation of singlet oxygen and stimulating the antioxidative defence enzymes activities (Bertoncelj *et al*., 2007).

Numerous types of food packaging in combination with different storage techniques can be used in order to extend the shelf-life of meat. One of the key technological measures needed during storage is the preservation of the meat from microbial spoilage and contamination/proliferation of pathogenic microorganisms (Jang and Lee, 2005; Brightwel *et al*., 2009; Pennacchia *et al*., 2011). The objective of the present study was to investigate the influence of marination on the development of total mesophilic aerobic bacteria and lactic acid bacteria during storage of raw beef and to evaluate the use of these marinades as means for the application of antimicrobial compounds from *Thymus vulgaris, Majorana hortensis*, *Allium sativum*, *Armoracia rusticana*, dry red wine and lime-tree honey. In addition to the microbiological analysis, the effects of the marinades on other parameters including lipid oxidation and pH of the samples were studied.

Materials and methods

Beef meat

The raw material, utilized in this research program, was represented by the beef thigh from adult animals. The meat was purchased in refrigerated state from a local slaughterhouse at maximum 24 hours post-slaughter. Salt was of food-suitable purity, being a largely used additive in meat industry. Marjoram (*Majorana hortensis)* and garlic (*Allium sativum)* have been purchased from Quatre épices Company (Bucharest, Romania), thyme (*Thymus vulgaris)* was acquired from Plantavorel Research Institute (Piatra Neamt, Romania), horseradish (*Armoracia rusticana*) was purchased from a local supermarket, lime-tree honey was purchased from S.C. Apisalecom S.R.L. (Bacau, Romania) and dry red wine, minimum 12% vol. alcohol content, from S.C. Viovin Prodserv S.R.L. (Odobesti, Romania).

Marinades

The marinades were: marinade 1 consists of dry red wine (300 ml/kg), honey (40 g/ kg), garlic (9 g/kg), pepper (2 g/kg) and salt (5%); marinade 2 consists of dry red wine (300 ml/kg), honey (40 g/ kg), garlic (9 g/kg), thyme (4 g/ kg), pepper (2 g/kg) and salt (5%); marinade 3 consists of dry red wine (300 ml/kg), honey (40 g/ kg), garlic (9 g/kg), marjoram (4 g/ kg), pepper (2 g/kg) and salt (5%); marinade 4 consists of dry red wine (300 ml/kg), honey (40 g/ kg), garlic (9 g/kg), horseradish (4 g/ kg), pepper (2 g/kg) and salt (5%) and marinade 5 consists of dry red wine (300 ml/kg), honey (40 g/ kg), garlic (9 g/kg), thyme (4 g/ kg), marjoram (4 g/ kg), horseradish (4 g/ kg), pepper (2 g/kg) and salt (5%). The marinades were left at room temperature with intermittent agitation for at least one hour, to allow the dry ingredients to hydrate. Control samples were represented by raw meat without marination treatment but stored under the same conditions as the marinated samples.

Marination and storage of samples

The adult beef meat was separated from the conjunctive tissue, and the fat was cut into pieces of the same size (10 X 6 cm) and thickness (1.5 – 2.0 cm) weighing approximately 200 g, cut along the muscular fibers. For each marination treatment, five meat slices were placed into polypropylene boxes. A 300 ml volume of the marinade per one kg of meat was then added to cover all the meat pieces, followed by agitation by hand to ensure an even distribution of the solid components of the marinades. All boxes were over-wrapped with a polyethylene cover and held at 4°C for 48 h. After approximately 24h, the meat pieces were turned over, to ensure uniform marination. Following marination, the meat samples were removed from the trays and the excess liquid was allowed to drain off for 5 minutes at 4°C and then they were vacuum packaged in polypropylene bags type Flexo-Vacuum PE 01, Flexopack SA Plastics Industry Greece (thickness: 80μm, yield: 13,3 m^2/Kg, O_2-TRAN 23^{0}C, 75% R.H.: 75 cc/m^2/24h/1atm, WVTR 38^{0}C, 90% R.H.: 5.0 g/m^2/24h, tensile strength: 30 N/mm^2, elongation at break: 340%) and were stored at 4°C for 12 days in a Siemens refrigerator type K643S123, Romania.

Chemical analysis

Initially it was determined the general composition of the beef including: water content according to the AOAC-1995 method, the total nitrogen content according to the SR ISO 9037:2007 method and the fat content according to the AOAC, 1984 method.

pH measurement

Before and after marination as well as during storage (every third day of storage in vacuum packages at 4°C) was measured the pH of the samples at 25°C using a Hanna digital pH-meter according to the AOAC method (1984).

Lipid oxidation

*Measurement of TBA value.*The 2-thiobarbituric acid (TBA) assay was carried out according to the procedure of Schmedes and Holmer (1989). Beef sample (10 g) was mixed with 25 ml of trichloroacetic acid solution (200 g/l of TCA in 135 ml/l phosphoric acid solution) and homogenized in a blender for 30 s. After filtration, 2ml of the filtrate were added to 2ml TBA solution (3 g/l) in a test tube. The test tubes were incubated at room temperature in the dark for 20 h; then the absorbance was measured at 532 nm by using UV–VIS spectrophotometer (model T80 Series UV/VIS Double Beam Spectrophotometer, PG Instruments Ltd, UK). TBA value was expressed as mg malonaldehyde per kg of beef meat.

Measurement of peroxide value. Peroxide value (POV) was determined according to the AOAC International (1999). The sample (3 g) was weighed in a 250 ml glass stoppered Erlenmeyer flask and heated in a water bath at 6°C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered using filter paper to remove meat particles. Saturated potassium iodide solution (0.5 ml) was added to the filtrate, which was transferred into the burette. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/l). POV was calculated and expressed as milliequivalent peroxide per kg of sample:

$$POV\ (meq/kg) = S \times N/W \times 1000,$$

where S is the volume of sodium thiosulfate solution used for titration (ml), N the normality of sodium thiosulfate solution (N = 0.01), and W the sample weight (kg).

Microbiological analysis

On each sampling day, 10 g of individual beef samples were aseptically removed from the bags and transferred to a stomacher bag with 90 ml of sterile distilled water. Serial decimal dilutions of the stomacher fluids were prepared.

For the enumeration of total mesophilic aerobic bacteria (TMAB), 1 ml of the appropriate dilution was spread on plate count agar (PCA), and the plates were incubated at 37°C, for 48 h. For enumeration of lactic acid bacteria (LAB), 1 ml of the appropriate dilution was pour-plated in de Man, Rogosa and Sharpe agar (MRS Agar), overlaid with 10 ml of the same medium and incubated at 30°C for 96 h.

After incubation, typical colonies for each group of microorganisms were enumerated. Colony counts were transformed to log CFU/g.

Results and discussion

The research used adult beef thigh post rigor at 24 hours after slaughter purchased from specialized stores in refrigerated state. The data resulted from chemical composition analyses of beef showed a relatively lean meat (5.94% ± 1.5% fat), with average protein content (17.5% ± 1.09%) and water content (76.1% ± 1.06), the average values being calculated using Microsoft Excel Programs for Windows, v. 2007, for three replications.

The influence of spices and beef meat marination on the evolution of pH value

The pH of the meat has a special importance in its processing, directly influencing shelf life, color and quality of the meat (Fernandes şi Tornberg, 1991; Simela, 2005). The marination of the adult beef in the marinade with the addition of different spices and seasoning plants influenced the pH values of beef meat (the pH of the marinades was 4.57). pH values were dependent on the type of treatment applied and time of storage at refrigeration temperature of 4°C (Fig. 1).

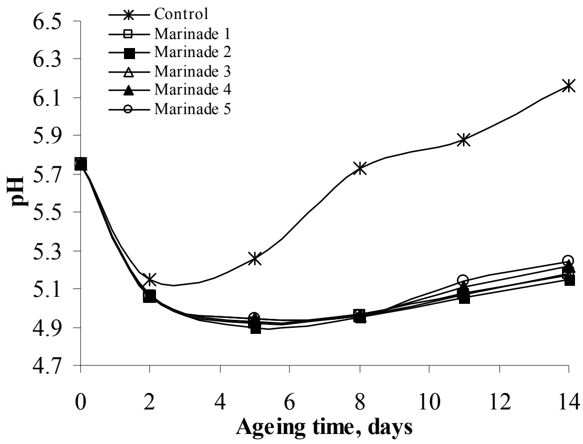

Figure 1. Changes in pH values of beef meat during marination (0-2 days) and subsequent storage at 4°C.

From the data presented in Figure 1, it can be seen the decreasing of pH values in the first days of storage in both control samples and experimental samples. The lowest value of pH was reached at the samples marinated in the base marinade consisting of wine, honey, garlic, pepper and salt with the addition of thyme (marinade 2) for 5 days of storage (two days of marination and three days of storage in vacuum packaging). The pH values of experimental samples recorded an increasing trend until the end of the storage time after 5 days of storage and at the control samples after 2 days of storage. The highest value of pH was reached at the control samples for the maximum ageing time, 14 days (pH 6.16). Vacuum packaging determines maintaining of pH values at low values, probably, one of the causes being dissociation of organic acids, lactic and acetic acid, accumulated in muscle tissue.

Microbiological analysis

The behavior of spoilage microflora during marination of beef and subsequent storage at 4°C are shown in Figures 2 and 3. For control samples, a mean increase of 0.47 log CFU/g total mesophilic aerobic bacteria was observed during the 48 h of storage (Figure 2). In contrast, during marination of the samples with wine, honey, garlic, pepper and salt (marinade 1) total mesophilic aerobic bacteria decreased by 0.14 log CFU/g. The addition of thyme, marjoram and horseradish in the marinade resulted in more pronounced decrease of total mesophilic aerobic bacteria compared to the marinade without spices and seasoning plants. The mean decrease in total mesophilic aerobic bacteria for samples marinated with addition of spices was 0.57 log CFU/g. Subsequent storage of beef resulted in a rapid increase of total mesophilic aerobic bacteria in control samples to > 2 log CFU/g. In contrast, no significant microbial growth occurred in marinated samples stored at 4°C. At the end of the storage period the mean total mesophilic aerobic bacteria was 4.2, 4.07, 4.03, 3.93 and 3. 24 log CFU/g for marinade 1, marinade 2, marinade 3, marinade 4 and marinade 5 while the mean total mesophilic aerobic bacteria in control samples was 7.05 log CFU/g.

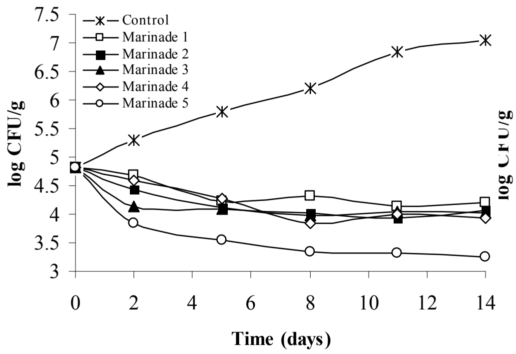

Figure 2. Changes in total mesophilic aerobic bacteria during marination (0-2 days) and subsequent storage of beef meat at 4°C.

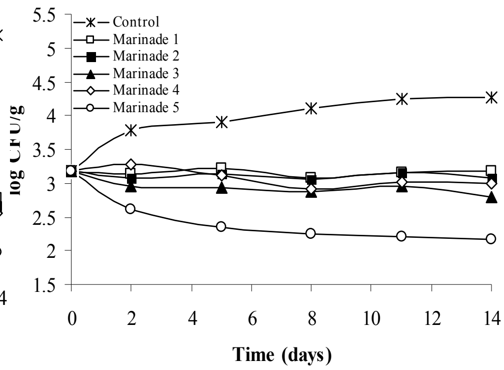

Figure 3. Changes in lactic acid bacteria levels during marination (0-2 days) and subsequent storage of beef meat at 4°C.

Lactic acid bacteria (LAB) dominate the meat microflora during storage of fresh meat in vacuum or modified atmosphere packaging or in processed meat environments of low pH and/or aw which do not allow for the growth of *Pseudomonas* spp. The growth of LAB in control samples was generally limited and did not exceed 5 log CFU/g at 4°C storage temperature. During storage at 4°C, marination with addition in the based marinade (wine, honey, garlic, pepper and salt) of thyme, marjoram and horseradish separately inhibited growth of LAB while marination with addition in the base marinade of thyme, marjoram and horseradish together resulted in significantly lower levels of LAB at the end of the storage period, mainly due to the reduction during the marination period (Figure 3). The inhibition of microbial growth on marinated beef samples during storage can be attributed to the changes in the meat environment caused by the marinades. All the marinades resulted in a significant decrease of meat pH compared to the control (Figure1). The pH changes in meat during marination may explain the observed inhibition of microbial growth in the marinated samples during storage. This inhibition however, can also be attributed to the fact that the marinades contain ethanol and phenolic compounds which are reported to have inhibitory effects on microbial growth (Kataoka, 2005; Rodríguez Vaquero *et al*., 2007). Rodríguez Vaquero *et al*. (2007) reported the antimicrobial activity of non-flavonoid compounds like gallic acid, vanillic acid, protocatechuic acid, and caffeic acid and flavonoid compounds including rutin, quercetin and catechin, present in wine, against various bacterial strains. Honey can also inhibit the growth of foodborne pathogens and food spoilage organisms (Mundo *et al.*, 2004; Taormina, Niemira, & Beuchat, 2001). Moreover, the use of garlic and pepper in marinade formulations may also contribute to their overall inhibitory effect due to the antimicrobial properties of allicin, found in garlic and piperine of black pepper (Ankri and Mirelman, 1999; Dorman and Deans, 2000; Benkeblia, 2004). Oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) are amongst the most active EOs, while lemon balm (*Melissa officinalis*) and marjoram (*Origanum majorana*) display a good antimicrobial activity against Gram-positive and Gram-negative bacteria, respectively. Recently, some researchers have reported the efficacy of plant EOs as antimicrobial agents against food-borne pathogens and spoilage microflora in meat (Busatta *et al*., 2008; Carraminana *et al*., 2008; Gutierrez *et al*., 2009).

Lipid oxidation

Marination had a significant impact on the development of rancidity in the beef pieces. To determine the effect of different marinade on the rancidization process, we measured lipid peroxides and lipid aldehydes during meat storage. The POV (peroxide value) measures primary products of lipid oxidation and is used to determine the oxidative state of lipid containing foods, while TBA analysis measures the formation of secondary products of lipid oxidation such as malondialdehyde which contribute to the acrid flavor of oxidized oil (Juntachote *et al.*, 2007; Jung *et al*., 2009).

All marination treatments resulted in significantly lower TBA values at the end of storage compared to the control (Figure 4). The initial TBA value was 0.632 mg MDA/kg, and after 14 days of storage, it ranged from 0.369 to 0.322 mg MDA/kg in the samples marinated in the based marinade consisting of wine, honey, garlic, pepper and salt (marinade 1), 0.369 to 0.308 mg MDA/kg in samples marinated in the based marinade with the addition of thyme (marinade 2), 0.352 to 0.259 mg MDA/kg in samples marinated in the based marinade with the addition of marjoram (marinade 3), 0.374 to 0.292 mg MDA/kg in samples marinated in the based marinade with the addition of horseradish (marinade 4) and 0.267 to 0.170 mg MDA/kg in samples marinated in the based marinade with the addition of thyme, marjoram and horseradish (marinade 5). These values were significantly lower than those of control samples (0.438 to 0.640 mg MDA/kg). A significant difference was also noted between TBA values of the samples marinated in the based marinade (marinade 1) and the samples marinated in the based marinade with the addition of thyme, marjoram and horseradish (marinade 5). The based marinade being rich in phenolic compounds maintained the TBA values nearly constant throughout storage but the addition of thyme, marjoram and horseradish, which possess high antioxidant activity, resulted in a significant decrease in TBA values throughout the storage period (Figure 4). The decrease in TBA values during the 14 days of storage was attributed, also, to vacuum packaging, which creates less oxygen available for oxidation. TBA value is routinely used as an index of lipid oxidation in meat products in store (Raharjo and Sofos, 1993), and the rancid flavor is initially detected in meat products between TBA values of 0.5 and 2.0 mg MDA/kg (Gray and Pearson, 1987).

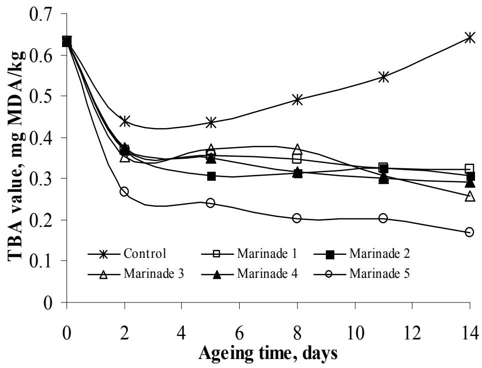

Figure 4. Changes in 2-thiobarbituric acid value (TBA) during marination (0-2 days) and subsequent storage of beef meat at 4°C.

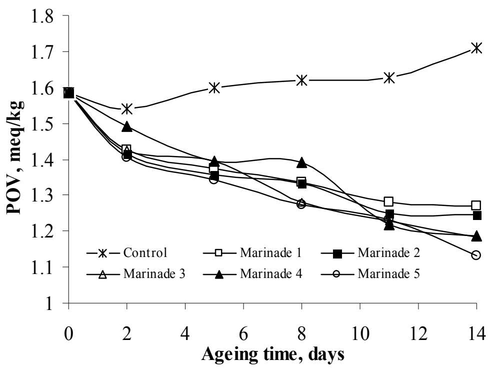

Figure 5. Changes in the peroxide value (POV) during marination (0-2 days) and subsequent storage of beef meat at 4°C.

Figure 5 shows changes in POV value in raw beef meat marinated and stored at 4°C. The initial POV value was 1.587 meq/kg and after 14 days of storage, it ranged from 1.425 to 1.268 meq/kg in the samples marinated in the based marinade (marinade 1), 1.415 to 1.246 meq/kg in samples marinated in marinade 2, 1.425 to 1.185 meq/kg in samples marinated marinade 3, 1.493 to 1.186 meq/kg in samples marinated in marinade 4 and 1.404 to 1.130 meq/kg in samples marinated in marinade 5. These values were significantly lower than those of the control (1.710 meq/kg). Results showed that the addition of natural agents possessing antioxidant activity in the marinades, destined to improve quality of beef meat, reduced POV throughout the storage period in comparison with control samples (Figure 5). Beneficial effects of thyme, marjoram and wine are justified by some authors through the high content in phenolic compounds. Jang *et al*., (1997) reported that the phenol compounds have an important role in the stabilization of lipid oxidation, antihypertensive and antithrombic effects, and reduce carcinostatic properties. Honey, garlic and horseradish can, also, prevent deteriorative oxidation reactions in foods such lipid oxidation in meat. The components in honey responsible for its antioxidative effect are flavonoids (chrysin, pinocembrin, pinobanksin, quercetin, kaempferol, luteolin, galangin, apigenin, hesperetin, myricetin), phenolic acids (caffeic, coumaric, ferrulic, ellagic, chlorogenic), ascorbic acid, catalase, peroxidase, carotenoids and products of the Maillard reaction (Bertoncelj, 2007). Garlic is rich in organosulfur compounds and their precursors (allicin, diallyl sulfide and diallyl trisulfide) are believed to play a key role in this antioxidant activity (Ankri and Mirelman, 1999; Kumar and Berwal, 1998). Previous studies (Manesh and Kuttan, 2003; Kinae *et al*., 2000) showed that isothiocyanate and allyl isothiocyanate from horseradish inhibit lipid oxidation and the food-poisoning bacterial activities of *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*.

Conclusions

The data presented in this study showed that the tested marinades were effective on the proliferation of total mesophilic aerobic bacteria, lactic acid bacteria and also resulted in reduced lipid oxidation. Considering that the growth of mesophilic aerobic bacteria, lactic acid bacteria and oxidation are the two main types of failure for meat quality, the results of the present study indicate that marination with marinades consists of dry red wine, lime-tree honey, salt, spices and seasoning plants as thyme (*Thymus vulgaris),* marjoram (*Majorana hortensis*), garlic (*Allium sativum*) and horseradish (*Armoracia rusticana*) can be used as an effective and natural preservation method.

Acknowledgements

This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project, ORGANIZING THE NATIONAL INTEREST POSTDOCTORAL SCHOOL OF APPLIED BIOTECHNOLOGIES WITH IMPACT ON ROMANIAN BIOECONOMY, project co-financed by the European Social Fund through the Sectoral Operational Programme Human Resources Development 2007-2013.

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