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OPINION PAPER

RECENT INNOVATIONS IN CONSUMER SCIENCE TECHNIQUES FOR FOOD PRODUCT DEVELOPMENT

INTRODUCTION

During the past two decades we have seen a gradual increase in corporate efforts to develop new products. Whereas in the 1960s it was sufficient to have an idea for a product, do some testing and move forward with the winning idea and product formulation, since the 1980s it has been necessary to have new ways of thinking and working at the so-called "early stage of development".

Thinking principally of product ideation and concept development, and looking back now at twenty-five years of efforts, we can see two distinct areas of effort that have matured tremendously. The first is the development of ideas. The world of "ideation" has grown significantly, perhaps moved forward by the business mantra "innovate or die". The second is the optimization of these ideas. The world of concepts as guides for products is beginning to emerge from a simple practice of company-based testing to a science of the consumer mind.

Two approaches grounded in solid science, based upon research that has appeared in the refereed scientific literature are presented here. In turn, we move forward, presenting applications of those academically accepted, proven ideas. Perforce much of the application will come from business cases, simply because a great deal of the academic literature deals with research among convenient populations, often students or others available to the researcher. Where we present data, we do so with actual business cases, making every attempt to link the business application to archival sensory and consumer research literature.

IDEATION TO CREATE NEW IDEAS FOR FOODS AND BEVERAGES

Successful invention comes from identifying and addressing unfulfilled, possibly unexpressed needs. Those responsible for product concept development often turn to consumers in an effort to unearth the next big idea. In-person focus groups have long been the gold standard for involving consumers in the invention process. Focus groups account for a great deal of today's research, and indeed it is not unusual in corporations to hear of some professionals who spend a great deal of their time "on the road", meeting with these groups.

Qualitative approaches

Extended "online" focus groups

As Internet penetration goes "mainstream", researchers and marketers can effectively reach and interact with consumers in an online environment. Thus, there is the option

of having extended online focus groups using online message boards where respondents log in and participate in a group discussion that can last for several days.

Some marketers believe consumers don't know what they want and aren't reliable sources of information when it comes to identifying new concept opportunities. Conversely, others believe by "listening to the voice of the customer" they will be able to determine where the so-called development "sweet spots" lie. In a sense, both are correct. Consumers know what they want, but they do not naturally express their desires in terms of product features, functionality and concepts, which unfortunately is what the marketer and product developer would like them to do. Instead, consumers are comfortable relaying anecdotes, experiences and stories, each of which provides insight into need-states, that fertile ground for new product development. Therefore, it is critical to engage consumers in conversations, interactions and activities that permit them to offer clues as to what new concepts they would find appealing. It then falls to the marketer to follow those clues to "the" answers.

"Online" collaborative filtering

Rather than relying only on solicited consumer or expert comments, usually gathered by market research (traditional questioning in focus groups), researchers have recognized that the online environment can be used as a factory of ideas with a type of self-correcting process (FLORES et al., 2003). The organizing principle differs from focus groups and draws its inspiration from the practice of competitive intelligence. The online process mines the market environment in order to detect "weak signals". These weak signals herald product opportunities.

Basically, the approach presents consumers with "pieces of ideas" on the Internet through a program that first asks the respondents to choose which ideas are "relevant", then to rate some of these ideas and finally to add their own ideas. The approach thus automates the process of idea generation.

Zoom out

Zoom out is a projective technique which blends visualization and projection, where the moderator begins by instructing respondents to imagine themselves in a specific setting, such as a grocery store. Using their five senses and their imaginations, respondents explore the store, paying particular attention to the shoppers and the food and beverage items they are buying. Respondents then identify an individual who is shopping for a certain kind of product. Next, respondents are directed to focus on the selected individual's hand and notice everything about it – skin texture, jewelry, nails, etc. Then participants "zoom out" until they can see the individual's entire arm, and once again, note all its detail. This process continues until participants can view the entire individual in his or her immediate surroundings. After briefly discussing who and what they saw, respondents answer questions about their particular individual's behavior in the grocery store, why the person selected the food and beverages he/she did, and what things they might have wanted and why. In many cases, respondents' answers provide insight into their own beliefs, attitudes and need-states, providing the foundation to identify "sweet spots" for subsequent concept development (PRIDDY, 1991).

Word and metaphor

Metaphor techniques such as the Zaltman Metaphor Elicitation Technique, or ZMET (ZALTMAN and COULTER, 1995) allow respondents to express one thing in terms of another. ZMET requires respondents to find images that represent their

emotions, feelings and fears regarding a specific topic or situation. Respondents present their images along with an in-depth, insightful explanation of why the images were selected and what these images represent. Through their explanations, the respondents divulge insights into their need-states and desires.

New ideas, bits of thought and ideational productivity

Often there is an expectation that ideation, whether conducted with consumers or within an organization, will result in fully developed concepts. The objective of an idea generation session is to produce a large number of starter ideas and idea fragments rather than concepts. People find the prospect of composing finished ideas daunting, but seem to have little problem ideating pieces of the ideas. These pieces can then be combined, developed and refined into complete, innovative concepts. Once the new concept development opportunity has been established, the company can use it as the starting point(s) for producing ideas, and eventually, fully-formed new concepts.

Direct thinking and improve ideation

Providing tools which allow the participants to expand and diversify their thinking in a directed way is a powerful key to successful idea generation.

1. BrainWriting. The session leader begins by giving each participant one sheet of paper, each of which has been primed with a different issue to solve (such as, "What is the next big idea in breakfast products?"), or a possible solution to a problem ("Portable breakfast cereals in a pouch"). Each participant silently reads what is written on the paper, and then writes his or her additional ideas, remaining silent. When a participant runs out of suggestions he or she exchanges papers with a fellow participant. The participant then reviews the ideas on the new sheet of paper and adds new ideas to the page. This process is repeated in silence until the group has no more new ideas to suggest.

2. Windtunneling. Use of a technique called Windtunneling (WENGER, 2008) is an attempt to have the more unique ideas surface earlier rather than later in the process by forcing the participants to dig for original suggestions. Ideation partners are divided into pairs, and one partner is assigned the role of the "Windtunneler" and the other the "Listener". The pair is given an opportunity around which to generate as many ideas as possible. For the next six minutes, the Windtunneler says everything that comes into his or her mind that might address the problem in a non-stop flow and the Listener captures the most interesting ideas he or she hears during the Windtunneler's tirade. The participants then reverse roles and repeat the six-minute process.

3. SCAMPER. This is an effective tool to generate new ideas by deliberately changing an existing product or service. Participants are asked to think about a specific product or service, and then follow the disciplined steps prescribed by SCAMPER (see Table 1). For example, participants in an ideation session for sportswear might be told to think of a water bottle and then be instructed to come up with a new idea using R – reverse. One resulting idea might be clothing that releases moisture onto the wearer's skin to cool the body.

4. Clean Slate. The participants acknowledge the rules under which they cur-

Table 1 - The steps for SCAMPER, an ideation tool.

S = Substitute	Substitute a different feature for one that exists now, such as artificial sweetener instead of sugar.
C = Combine	Combine two things that are not usually found together, such as chocolate and wine.
A = Adapt	Change one aspect of an item, such as adding thermal packaging to ice cream for on-the-go consumption.
M = Maximize/ Minimize	Make something smaller, bigger, more powerful, and/or less powerful, such as intense flavors in bite-sized servings.
P = Put new uses	Find a new use for something, such as using bread as a bowl for soups and salads.
E = Eliminate	Remove a feature, such as food packaging that does not require an external heat source.
R = Reverse	Change something completely, such as beverages that have extra sugar, caffeine and other stimulants, rather than less.

rently operate, and then consciously break each rule to develop a new idea. Such rules might include "ketchup must be red". The rules are shared with the group, and then participants generate ideas that do not conform to the rules they wrote. The acts of acknowledging the current state of being, and then deciding as a group to ignore that state, give participants explicit permission to think of new ideas that otherwise would not have been acceptable.

5. Cherry Split. This is an attribute-listing technique in which ideation participants break an opportunity or challenge into small parts, and then reassemble the parts to form new ideas (MICHALKO, 1991). The process begins by stating the opportunity in two words, such as "nutritious snack". Next, the opportunity is split into two separate attributes: nutritious could be "healthy and organic" and snack might split into "light and satisfying". The resulting four attributes are then split. Healthy becomes "balanced and nourishing", organic becomes "tasty and fresh", light is split into "fluffy and size", and satisfying becomes "filling and flavorful". Participants continue to split each attribute until there are 20 or more. Next, participants review the list of attributes one at a time, looking for ideas to create a new nutritious snack item. Finally, the attributes are re-assembled to generate even more ideas.

6. Excursions

a. Street Excursion: Participants literally take a walk and use whatever they see to trigger new associations and ideas for food products. Certain environments can be particularly rich sources of ideas: highways, parks, zoos, industrial areas, and so on.

b. Example Excursion: A technique which involves finding examples or parallels from other contexts, such as geology, electricity or the weather, and then using those parallels to generate ideas for new food products.

c. Imaging Excursion: Participants select a random word and then let their chosen word trigger a picture in their minds. They allow the picture to take its own course, much like watching a movie. After a few minutes, they "replay the movie" and let it suggest ideas for the opportunity.

7. "Other People's Shoes" or "Someone Else's Perspective". Ideation participants simply ask, "How would _____ take advantage of this opportunity?" and fill in the blank with the name or description of a person. The person can be someone famous, or someone who fits a target market profile, or someone completely unrelated to the participants. By asking this question, participants are forced to adopt a new frame of reference, which provides them with new attitudes, capabilities and resources with which to ideate.

OPTIMIZATION OF THE IDEAS

Quantitative approaches

Ideation is only the first part of concept development for a company. The next stages comprise different ways of testing the ideas developed through ideation.

We will deal with three types of testing of ideas. The first two, experimental design of concepts and concept screening, typically deal with customer problems that must be solved by the company. The third, databanks of ideas is a new approach to concept research, in which the aim is to create a database of ideas that "work", and a means to recombine these ideas into new, appropriate product concepts (MOSKOWITZ et al., 2005).

Product concept

This concept describes the characteristics of the product. The concept may be a bare-bones affair with a limited amount of information and almost no "persuasive" elements. In contrast, the concept may be an extended paragraph or two, with a picture, a description of the product in complete sentences, and perhaps even a reason or two for buying it. The second type of concept is the positioning concept. This concept talks about what is special about the product and tries to present the product in such a way that the customer wants to buy the product or service. We might expect to see more flowery language in the positioning concept, as well as more circuitous language, that is appropriate for persuasion as well as for description.

Concept Screening

The goal of concept screening is to assess the appeal of the concept and, where possible, use that measure to predict performance of the product in the marketplace. At the same time, concept screening is also used to create a "signature" of the concept (i.e., product idea) on attributes, ranging from judgmental (buy/not buy) to emotional (how the product would be described in emotional terms), to functional (for which situations is the product designed).

The most popular method is to analyze the proportion of the responding population who rate a specific concept at the top of the scale points. This is known as the top-box or top-two box score, when associated with a 5-point scale such as the purchase intent scale:

1 = definitely not buy;
2 = probably not buy;
3 = might/might not buy;
4 = probably buy;
5 = definitely buy.

Consumer researchers are accustomed to dividing the five-point (or other multipoint) scale into two regions, to denote accept versus reject. Such division into parts and the use of percents or incidence (proportion of people accepting vs. rejecting) derives from the intellectual history of market researchers.

Concept screening is typically used to discover a so-called "winner" or concept that performs well and can be used to guide product development. Concept screening is often done in conjunction with a normative database: typically the norms come from a company's bank of previously-tested concepts.

Experimental design of concepts

Systematic variation of concepts teaches the researcher and product developer far more than concept screening does. One learns far more when the researcher systematically varies the components of a concept, presents these combinations to respondents, collects the ratings and analyzes the data. Some of the insights that can be obtained from experimental design include "what works", the "existence of segments" and "interactions among concept elements". These types of learning go beyond simply providing the researcher and developer with a concept that wins.

Systematic, statistical design of concepts has a much longer history, primarily in statistics (BOX et al., 1978), mathematical psychology (LUCE and TUKEY, 1964), and marketing, rather than in product development (GREEN and SRINIVASAN, 1980).

In retrospect, the early methods using experimental design now seem to be a bit archaic and not particularly productive. Perhaps one of the reasons is that experimental design was not particularly popular in the food industry, especially for concepts, due to the labor and cost involved. The early studies were reserved for high-profile projects that focused on products that could return millions of dollars in profits. Foods and beverages do not typically return that much profit, so conjoint analysis was rarely used. If used, the approach was typically garbed in hard-to-understand statistics.

The model showed how each test element in the concept "drove" interest in or rejection of the concept. A bit later the conjoint approach was improved by creating a system that could develop individual-level models, that showed which concept elements drove the responses for each individual person. This advance led to many other developments, such as segmentation. Finally, experimental design became even easier with the advent of the Internet, which could be used to set up studies, acquire data and analyze results.

How experimental design of ideas or conjoint analysis is currently done

Today, experimental design follows a series of fairly routine procedures that generate utility values or contribute specific elements to the overall reaction. Since the approach is sequential and systematic, we list the steps in the procedure and discuss each one briefly. Note that when we talk about elements, we mean the pieces of the concept, the small phrases, which when conjoined, generate a concept.

Step 1 - Assemble raw materials. These raw materials are small, single-thought elements. The elements may come from ideation, or they may be deconstructed, i.e., they may be part of existing product ideas currently in the marketplace. When consumers participate in the conjoint studies they do not generally know where the elements come from, nor does it make a difference. When the raw materials focus on small changes in an existing product, the analysis will identify what works within a slight modification of an existing product. In contrast, when the elements come from different products that are mixed/matched to create new-to-the-world products, the analysis will generate new-to-the-world combinations.

Step 2 - Classification. Sort the elements into silos (buckets, categories) and elements which researchers in marketing call variables and elements, respectively. No matter what the terminology, the objective here is to put the elements into "silos" or "groups", such that the elements in a single silo are similar to each other, and would not appear together in a concept. Classification here is mainly a bookkeeping exercise and should not affect the results.

Step 3 - Create an experimental design. The design lays out the different combinations. At the design stage, the researcher has to be very careful. The elements must appear independent of each other (called orthogonality) and there must be a minimal number of test concepts in order not to burden the research (called minimality). The concept may contain, at most, one element from each silo. Some practitioners, including the authors, believe that it is important that the concepts are not necessarily complete – i.e., in some concepts, one or more silos are absent. In this way, the analysis by regression (see below) can estimate the absolute impact of each element. When some silos are absent from concepts, the independent variables are no longer collinear. Other practitioners, with just as much vehemence, are willing to settle for collinear predictors, and do not mind relative impact values (rather than absolute impact values) and insist that for the concept to "make sense", one element from each silo must be present. Which approach is correct depends on whether the data will be used later, to be compared with the results from other studies. If the information will be used after the study for the purpose of "learning" and "knowledge-building", then the concepts must have silos absent, so that some are incomplete. If the data is not be used in the future, i.e., it is critical for the concepts to be complete, then the researcher will abandon the absoluteness of the results in favor of more complete concepts.

Step 4 - Create test concepts based on the design. The experimental design prescribes what particular elements will appear in each concept. The respondent does not know what design lies behind a concept, but merely sees the combination. The computer automatically creates the combination in "real time" by reading a file and assembling the elements. We can see an example of a test concept in Fig. 1. If we were to follow the design as shown schematically, we could manually assemble the components of each concept, place them in the template which shows the rating question and then present the combination to the respondent.

Step 5 - Run the study. Today, research on the Internet makes the field work quite easy to do. The researcher drafts a letter that invites the respondent to participate. The letter need not provide too much detail. At the time of this writing, the response rates to e-mail invitations vary widely. With most panels the response rate is about 5%, whereas if the respondent is paid for actual participation, the response rate can be considerably higher. During the course of the interview, the computer program presents the test concepts according to the experimental design, acquires

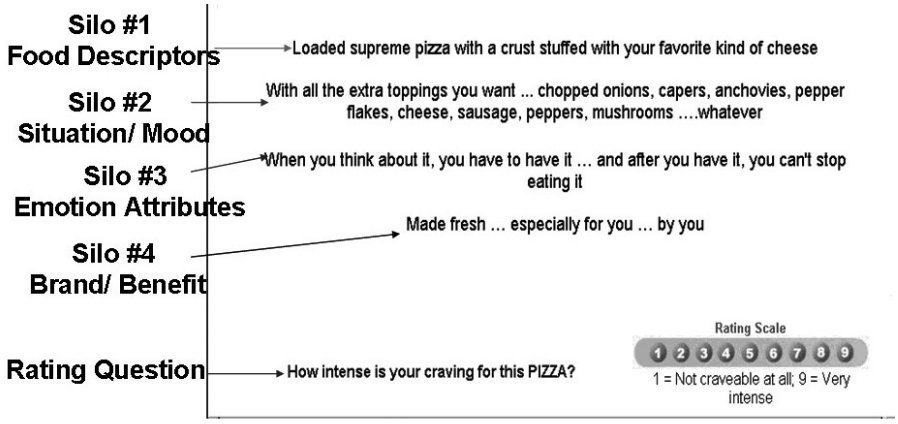

Fig. 1 - Example of a test concept.

the data and then creates a model relating the presence/absence of the elements to the rating. The rating may either be a category rating scale (e.g., anchored 1-9 scale), or a binary transformation so that the high ratings (e.g., 7-9) are coded as 100 to denote acceptance of the concept, and low ratings (e.g., 1-6) are coded as 0 to denote rejection of the concept. Keep in mind that this type of binary analysis, that looks at accepters versus rejecters, characterizes market research.

Step 6 - Develop individual-level models and aggregate the individual models. Modern research has advanced to the point where individual-level data can be computed by the non-statistician. Canned statistical programs can relate the presence/absence of the concept elements to the rating or the binary transformation of the rating. The programs come back with simple models of the form: Rating = k_0 +k_1(Element #1)… k_n(Element #n).

Step 7 - Create average models for specified subgroups of interest. Typically, the researcher looks at well-defined subgroups, such as product users versus non-users, as well as the more usual demographics such as gender, age, income, education, etc. For the most part, the conventional ways of dividing people into groups does not generate radically different patterns of the elements that drive a respondent. That is, a male shows the same patterns of responses as a female, all other factors being equal. Occasionally, some subgroups react differently to the elements, as shown by the average model. Thus in Fig. 2 we see similar patterns of utilities for males and females, and opposite user patterns for frequent versus infrequent users.

Step 8 - Segment the respondents based on the pattern of utilities. Marketers have known for decades that one of the keys to a successful product is to find groups of individuals who really like the product. Although the conventional hope is that the typical ways to divide the respondents (age, income, etc.), typically these individuals show similar utility patterns. What attracts one group attracts another. A better way divides the entire group of respondents into relatively cohesive subgroups, such that the individuals in the subgroups show similar patterns of what they like and dislike. This is called concept-response segmentation. We can get a sense of the similarity of conventional subgroups (gender), and the dissimilarity among segments from Fig. 2, for two concept-response segments of persons who responded to concepts about potato chips.

Step 9 - Look at the utility values of the elements to identify ideas that do well, and those that do not. In Table 2 we can see all the utilities of 36 concept elements (omissis). We again focus on potato chips, whose utilities are plotted in Fig. 2. On the left side of Table 2 we see the elements, on the right side we see the utility or impact scores. The additive constant is the conditional probability that an item would be rated as interesting (7-9 on a 9-point scale), if no elements were pres-

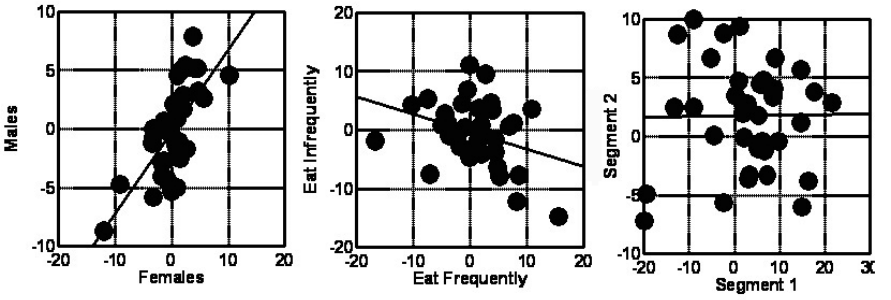

Fig. 2 - Scattergram plot of different utilities for comparable subgroups. Each circle corresponds to an element in a study on responses to concepts about potato chips.

Table 2 - Performance of 36 concept elements for total panel for potato chips. The elements are sorted from high to low within each concept.

	Additive Constant	39
	The product features	
A1	Thin sliced, golden and salty potato chips	8
A3	Classic potato chips, with a light taste and crispy crunch	7
A4	Potato chips with ridges, perfect for holding dip or spices	7
A8	Potato chips marinated before cooking for a unique flavor, golden color, and extra crunch	6
A7	Potato chips ... salty, golden and crunchy	5
A5	Crunchy kettle style potato chips	3
A2	Potato chips baked for a healthier snack	-1
A6	Chips made from sweet potatoes, a contrast in flavor and texture	-11
A9	Potato chips with the unique texture that only comes from special potatoes … brushed with olive oil and topped with parmesan cheese, garlic and basil	6
	Emotions, situation, slogan	
B7	With all the flavors you want … roasted garlic & herbs, sharp cheddar and spicy jalapenos, sweet and sour smoky BBQ, sour cream, onion & chive … whatever	11
B9	So good … you practically have to lick your fingers & lips twice after each bite	8
B4	Premium quality … that great classic taste, like it used to be	4
B6	100% natural … and new choices every month to keep you tantalized	4
B1	Potato chips are a party pleaser	2
B8	You can imagine the taste as you walk in the door	1
B3	Cooked in pure vegetable oil	-1
B5	You can just savor it when you think about it during work and school	-2
B2	With a chilled glass of water or carbonated beverage	-3
	Usage and response	
C2	When you think about it, you have to have it … and after you have it, you can't stop eating it	5
C3	Fills that empty spot in you ... just when you want it	2
C9	It feeds THE HUNGER	1
C5	Now you can escape the routine … a way to celebrate special occasions	0
C6	A joy for your senses ... seeing, smelling, tasting	0
C7	An outrageous experience … shared with family and friends	-1
C8	Pure ecstasy	-2
C1	Quick and fun … eating alone doesn't have to be ordinary	-3
C4	When you're sad, it makes you glad	-5
	Brand, quality promise	
D7	Made fresh ... especially for you ... or packaged to maintain that fresh flavor	4
D4	From Lay's/ Ruffles	3
D3	From Pringles	1
D8	Simply the best potato chips in the whole wide world	1
D6	From Bistro Gourmet	-5
D5	From Cape Cod	-6
D2	From Utz	-7
D9	With the safety, care and cleanliness that makes you trust it & love it all the more	-7
D1	From Herr's	-8

ent. Obviously all concepts have elements, so the additive constant is a computed value, which can be used as a baseline. The additive constant is 39, meaning that without elements, we expect 39% of the respondents to rate potato chips 7-9 on the interest scale. The individual utilities are the additive conditional probabilities of a concept being interesting if the element is added to the concept. Some elements add to the percent of interested respondents, other elements detract.

Step 10 - Create better performing concepts by combining winning elements. If the first objective of experimentally designed concepts is to reveal what works and what does not, then the second objective is to combine elements into new concepts that will presumably work better. By putting together winning elements that seem to go together by judgment, the developer can create better ideas.

By recombining the elements into new combinations, and choosing the best performing elements, it becomes possible to see approximately "how high is up", i.e., how well one can perform. Such information "jump-starts" the concept development process.

CONCLUSION

A review of business-practice methods for developing new ideas in the food industry starting with qualitative methods of the current business environment, move onto practitioner-based methods of ideation, and move forward to the integration of ideas into concepts by experimental design is here reported.

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REVIEW

ANALYSIS OF THE ITALIAN PUBLIC FOOD CONTROL SYSTEM AND ITS RELATIONSHIP TO EUROPEAN HYGIENE AND SAFETY LEGISLATION

ANALISI DEL SISTEMA ITALIANO DEL CONTROLLO UFFICIALE DEGLI ALIMENTI E SUOI RAPPORTI CON LA LEGISLAZIONE EUROPEA SULL'IGIENE E LA SICUREZZA

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ABSTRACT

This paper provides an overview of the Italian Public Food Control system, describing the skills and roles of each part of the Public Authority that is well defined by national legislation. There is a lack of coordination among the official structures involved in control, with frequent overlapping of activities. The paper, also, focuses on the problem of implementing the EU safety and hygiene legislation in Italy. The complex administrative structure in the area of food control could be a barrier

RIASSUNTO

Il lavoro riporta una descrizione sistematica dell'organizzazione del sistema pubblico italiano per il controllo dei prodotti alimentari, analizzando i compiti ed i ruoli di ogni singola Autorità, ben definiti dalla legislazione nazionale. In realtà manca ancora un adeguato coordinamento fra le strutture impegnate nel controllo ufficiale con conseguenti frequenti sovrapposizioni. Viene anche affrontato il problema dell'attuazione in Italia della legislazione europea sulla sicurezza ed igiene degli alimenti. La

- Key words: consumers, EU legislation, food safety, legislation -

to the development of an effective national food safety system that adequately enforces current EU policy. The regions are becoming the main channel through which EU directives and regulations are implemented, but structural differences between regions and the political choices of the local governments create some difficulties for receiving and implementing EU legislation. The impact varies throughout the country. The establishment of a National Authority for Food Safety, which will be scientifically and hierarchically independent can only be justified if it can guarantee concrete coordination of all the control structures as well as act as an advisory body.

complicata struttura amministrativa, nel settore del controllo ufficiale sugli alimenti, può rappresentare una barriera allo sviluppo di un efficace sistema nazionale per la sicurezza alimentare, in grado di mettere in atto adeguatamente la linea di condotta dell'Unione Europea. Le Regioni sono diventate il principale canale attraverso il quale i regolamenti e le direttive dell'Unione Europea sono applicati a livello nazionale, ma le differenze strutturali e le scelte politiche delle singole autorità regionali, possono determinare un differente impatto nella reale applicazione della legislazione europea. Infine la creazione dell'Agenzia Nazionale per la Sicurezza Alimentare, che dovrà essere scientificamente e gerarchicamente indipendente, sarà giustificata solo se, oltre il suo ruolo di organo consultivo, garantirà anche il coordinamento fra le strutture di controllo.

1. INTRODUCTION

In recent years the feed and food sectors have been involved in various crises from BSE to the more recent cases of early childhood product contamination. These events highlight the need to strengthen monitoring and controls throughout the production chain, but, above all, to gather information and data through standardised data collection which concurs with correct risk assessment (1)*.

At the European level, a new approach to developing food laws (VAN DER MEULEN and VAN DER VELDE, 2004) as well as new regulations and directives has been created based on the principle that feed and food operators, at all stages of production, processing and distribution, are responsible for satisfying the requirements of the feed and food laws (Regulation 2002/178/EC, art. 17).

The basic feed and food laws are found in Regulation 2002/178/EC of the European Parliament and Council of 28 January 2002. One of its objectives is to establish common definitions and lay down guiding principles and legitimate objectives for food law in order to ensure a high level of health protection and efficiency within the internal market.

This Regulation established the European Food Safety Authority (EFSA) which is an independent body dedicated to risk assessment which should ensure the smooth functioning of the internal market (VARZAKAS and JUKES, 1997).

The Regulation also seeks to harmonise the existing food laws and requirements at the Community level of all the Member States by providing a basic

* See end of paper for websites and abbreviations.

framework of definitions, principles and requirements for future European food laws. Regulation 2002/178/EC was derived from "White Paper" (2000), which originated, in part, from the agreement on the application of Sanitary and Phytosanitary Measures (The "SPS Agreement") that came into force on 1 January 1995 (2). This agreement was adopted in order to avoid any trade restrictions due to unjustified sanitary barriers, which are often adopted by national administrations in order to promote their own products and prevent foreign ones from being imported. Analogously Regulation 2002/178/EC is based on the principle that the free movement of safe and wholesome food and feed is essential for the Community market and this can only be achieved if food and feed safety requirements do not differ significantly between Member States. Through the SPS Agreement the members are committed to establishing scientifically-based measures to protect public health through international guidelines and international standards (JUKES, 2000).

In order to allow the Member States to apply the community feed and food laws, apply animal health and welfare rules and controls and monitor and verify compliance by business operators, it was important to define a coordinated framework of general rules in order to organise the official control at the European Union level. So, the European Parliament and Council issued Regulation 2004/882/EC on 29 April 2004, which describes the official controls for complying with feed and food laws and animal health and welfare rules.

The European Commission recently adopted the Commission Decision of 29 September 2006 which sets out the guidelines for audits under Regulation 2004/882/EC of the European Parliament and Council for official controls to verify compliance with feed and food laws, animal health and animal welfare rules. The guidelines explain the objectives of the audit system for verifying the effectiveness and suitability of official controls regarding feed, food, animal health and animal welfare, including compliance with national control plans while these guidelines are not legally binding, they give member states useful indications about applying the regulations (FORTE, 2006).

The proliferation of EU legislation on foodstuffs confirms that the supranational standards have taken a leading role in the Member States (FERRETTI and MAGAUDDA, 2006). Many papers have analysed the changes in feed and food control systems introduced in the Member States by new EU legislation. For instance, in Spain, the Spanish Agency of Food Safety has been established (GARCIA and JUKES, 2004), with a similar development in Greece (VARZAKAS *et al.*, 2006a, b); the Danish food safety system has been reorganised and centralised (NIELSEN, 2006) over the last decade; the food system in Germany has become very complex and has lacked uniformity since the EU began influencing national legislation (LENZ, 2006); in the Portuguese food safety system the responsibilities of the various agents for food control often overlap (DOMINGUES, 2006); the establishment of the Food Standards Agency is a major governmental innovation in the UK food safety system (WALES *et al.*, 2006). The project "Consumer Trust in Food: A European Study of the Social and Institutional Conditions for the Production of Trust (TRUSTINFOOD 2002-04)" (5), has underlined that, although European legislation has been readily integrated into Italian regulations, the complex Italian administrative structure in the area of food control is a barrier to developing an effective national food safety system which adequately enforces current EU policy (FERRETTI and MAGAUDDA, 2006).

The aim of this paper is to provide a systematic and detailed description of the Italian feed and food control system,

and focus on the problem of implementing EU legislation regarding food in Italy. The institutions and organisations involved in food control are identified according to some analytical categories such as: regulation, control and repression. At the central level the responsibilities of the institutions are limited to legislation and co-ordination, while the functions at local level are mainly control and repression.

2. FEED AND FOOD SAFETY CONTROL IN ITALY

Matters pertaining to food safety and quality involve several Ministries and the current system is the product of a recent reorganisation. The reform of the 2001 Constitution, in particular article 117 (Constitutional Law No 3, 18 October 2001), introduced concurrent legislative powers for both the central and regional governments. So, human and animal health in general and food and feed safety, in particular, have become matters of shared responsibility at the national and regional levels, while remaining the competence of the State in international relations and international maritime, aerial and border prophylaxis, including veterinary matters, as well as controlling the importation of products destined for nutrition. While the Regions have become increasingly more autonomous, the central authority retains responsibility for national co-ordination, guidance and inspection (6). The regions are gradually becoming the main channel through which EU directives and regulations are implemented in the country. However, structural differences between regions and the political choices of the local governments cause some difficulties when receiving and implementing EU legislation, resulting in a varied impact throughout the country (FERRETTI and MAGAUDDA, 2006). Even if EU Regulations are directly applicable in all Member States, they are not always immediate. While awaiting new national European-based regulations, national laws still remain in force if they do not contradict new European laws (FORTE, 2006). For example, in Italy, Legislative Decree No 123, 3 March 1993, regarding official food product controls, which applies Directive 89/397/EEC, was in force, despite the fact that this directive was repealed by Regulation 2004/882/EC, until the end of 2007, when it was repealed by Legislative Decree No 193, 6 November 2007. The Presidential Decree No 327, 26 March 1980 (Regulation of execution of Law No 283, 30 April 1962 and successive modifications), dealing with hygienic discipline and sale of foodstuffs and drinks (FORTE, 2006), is still in force.

3. THE MINISTRIES

At least four Ministries in Italy are related to feed and food safety controls (Fig. 1):

- Ministry of Health;
- Ministry of Agriculture Foodstuffs and Forestry Policies;
- Ministry of the Economy and Finances;
- Ministry for the Environment and Protection of the Territory and the Sea.

3.1 Ministry of Health

The Ministry of Health was instituted by Law No 317, 3 August 2001; its organization was defined by Presidential Decree No 129, 28 March 2003 and modified by Presidential Decree No 189, 14 March 2006. It is the main organ of the Italian National Health Service (SSN), the public system that guarantees health assistance to all citizens. The responsibilities of this Ministry are limited to preparing legislation, co-ordinating the activities of the Regions and Autonomous Provinces through the Standing Conference for Relations be-

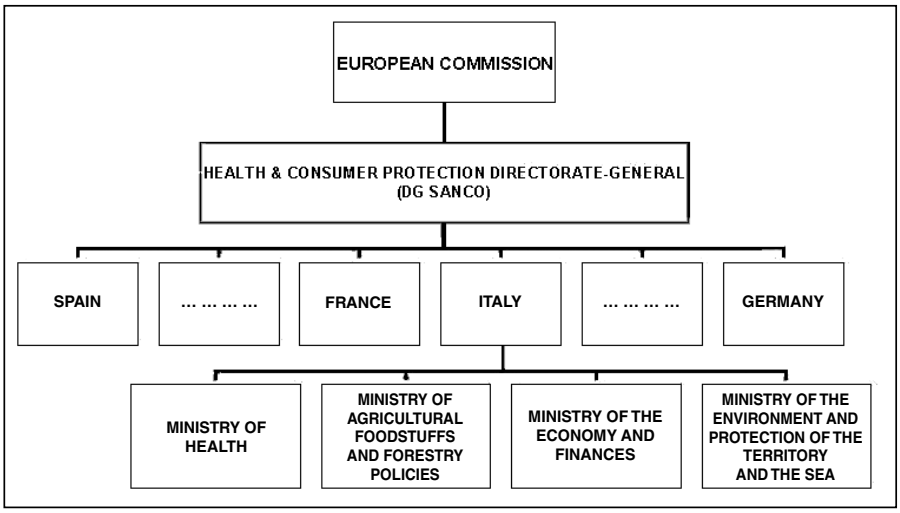

Fig. 1 - Food and feed safety control in Italy.

tween the State, the Regions and Autonomous Provinces of Trento and Bolzano, and representing Italy at the EU and international level.

The Ministry of Health consists of four Departments, each subdivided into general directorates: quality; innovation; prevention and communication; veterinary public health, nutrition and food safety (Fig. 2).The central level carries out regulation and coordination activities, while the local level carries out control and inspection activities (Fig. 2).

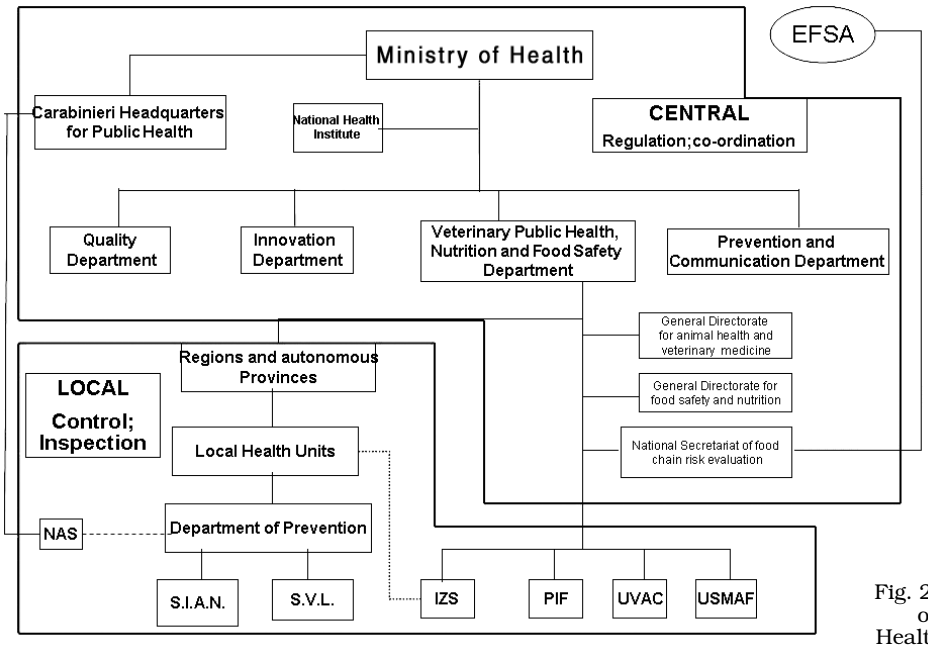

Fig. 2 - Structure of Ministry of Health and Associated Bodies.

Note: see list at end of paper for abbreviations.

3.1.1 Department for Veterinary Public Health, Nutrition and Food Safety

The Department for Veterinary Public Health, Nutrition and Food Safety was instituted by Legal Decree No 202, 1 October 2005 and modified by Law No 244, 30 November 2005. Most of the competencies for animal health, food and feed safety and animal welfare are assigned to this Department at the national level.

It is organised into two General Directorates and one Secretariat (Fig. 2):

- General Directorate for Animal Health and Veterinary Medicine;
- General Directorate for Food Safety and Nutrition;
- The National Secretariat of Food Chain Risk Evaluation.

At the beginning of 2006, a task force was established to prepare the Multi-annual National Control Plan, according to Regulation 2004/882/EC, art. 41 (6). The 'Control plan' describes the competent authority with general information about the structure and organisation of its official control systems (Regulation 2004/882/EC, art. 2). The control plans should allow the Commission inspection services to determine if the official controls in the Member States are organised in accordance with the criteria laid down in Regulation 2004/882/EC.

The General Directorate for Animal Health and Veterinary Medicine has jurisdiction in animal health, animal registries, zoonosis control, welfare and animal reproduction, technical zoo hygiene, the hygiene and safety of animal feeding, drug and veterinary procedures, veterinary pharmacy surveillance, manufacturing of veterinary medicines, raw materials and veterinary devises. The Directorate includes the "Fight and Emergency Against Animal Disease National Centre" and the "Central Crisis Unit".

The Directorate drafts the annual National Animal Nutrition Plan (PNAA), according to Legislative Decree No 223, 17 June 2003; this document establishes the principles underlying the official controls in animal feeding. Through vigilance and feed control, the PNAA should ensure the health and safety of the products of animal origin destined for human consumption. The Plan is drafted and revised annually through risk analysis based on the evaluation of the inspection data gathered in previous years and compliance with any new Community norms. The PNAA is carried out through investigations by sample analyses throughout the production chain. In case of non-conformity, prescribed interventions are enacted to protect public health. The PNAA is applied through the collaboration of several institutions that have different roles and skills. The Ministry of Health coordinates the activity of surveillance and control at the national level, issues the PNAA to the Regions and Autonomous Provinces which, through Local Health Committees, instructs Local Health Units to guarantee appropriateness. The surveillance and inspection of animal nutrition products from EU or extra-EU countries is carried out by Border Inspection Posts (PIF) and Veterinary Offices which ascertain compliance with community requirements (UVAC). Samples are analysed by Experimental Zoo Prophylaxis Institutes (IZS), that are coordinated by the National Health Institute (ISS), which performs second level analyses. For specific requirements, the Regions and Autonomous Provinces, Local Health Units, call on control organisations of the state (Military police for Public health, State Forestry Corps, Central Inspectorate for quality control of agricultural and food products, Finance Police, Police). The PNAA data should be updated regularly by the Local Health Units and reported to the Health Committee, which in turn, should present it to the Ministry of Health. The Ministry collects the national data annually, and transmits it to the EU by 1 April of the following year (7).

The General Directorate for Food Safety and Nutrition is responsible for hygiene and the safety of food products

of animal and plant origin, dietary nutrition, herbal products, food supplements, nutrition labels, dietary education, transgenic foods, additives, flavourings, contaminants, packaging material, phytosanitary products, food chain inspection plans, food alert management and food export. It is also responsible for coordinating all surveillance and monitoring of chemical residues (The National Residues Plan – PNR) on food of animal origin that might be harmful to the public health. The PNR, following Legislative Decree No 158/2006, and applying EU Directive 2003/74/EC, is involved in prohibiting the use of hormonal, thyrostatic action and β-agonist substances in livestock. Samples from all phases of animal production: bovine, pig, ovine-goat, equine, aviculture, fish hatcheries, game, dairy, eggs and honey are analysed under the PNR. If restricted substances are detected at levels above the established limits, a series of interventions are activated, according to the PNR, to protect public health. With respect to the PNR, the General Directorate for Food Safety and Nutrition is the administrative authority corresponding with the European Community. The National Health Institute (Fig. 2) coordinates the PNR, in the skill of the National Reference Laboratory for Residues. In practice, the Ministry of Health organises the PNR, according to EU norms, and establishes it in the Regions and the Autonomous Provinces. In the Regions and Autonomous Provinces, the Local Veterinary Services (Fig. 2), depending on the Local Health Committee (except in the Autonomous Province of Bolzano, where the Veterinary Services depends on the Local Agriculture Committee), distribute the samples among the Local Health Units; the Veterinary Services carry out the sampling.

The samples are analysed in the laboratories of the Experimental Zoo Prophylaxis Institutes and ARPA laboratories. The Local Committee sends all the data relative to the samplings and their analyses to the Ministry of Health; the information is collected and presented to the European Commission together with a proposal for the next year (8).

In the same way, this General Directorate coordinates and defines the National Monitoring Plan for Pesticide Residues on food (P.N.R.A.), according to Ministerial Decree 23 December 1992. This annual plan is part of the EU coordinated program for monitoring the maximum residue levels in food. This Ministerial Decree dictates to the Regions and Autonomous Provinces the minimum number of samplings required for each type of product. The samples are analysed in the laboratories of the Experimental Zoo Prophylaxis Institutes and ARPA laboratories. All the data relative to the samplings carried out and to the analytical results are sent to the Ministry of Health by 31 March of the following year; the Ministry of Health - General Directorate for food safety and nutrition will send them to the EU Commission by 31 August.

The National Secretariat of Food Chain Risk Evaluation (Fig. 2) is in charge of evaluating physical, chemical and biological risks. The Secretariat is the point of reference in Italy for the European Food Safety Authority (EFSA).

The offices of the Department for Public Veterinary Health, Nutrition and Food Safety may carry out inspections directly or through a branch of the military police force (Carabinieri). Since its reorganisation in 2003, the Department has been carrying out audits on regional controls system, in parallel to these inspections. The purpose is to create a totally uniform approach to applying the law at the national level. The audit is carried out in two parts. The first involves a "Systemic Audit" of the regional Administration, to verify the efficacy and regional management efficiency in the veterinary and food fields. The second is a "Sectorial Audit" which evaluates the regional admin-

istration and Local Health Units with respect to their planning and execution of inspections, which can mean inspecting some businesses (6).

3.1.2 Ministry of Health: Import controls

Through the Peripheral Veterinary Offices and the Airport and Border Health Offices, the Ministry of Health carries out health checks on imported products.

The 36 border inspection posts (PIF) are responsible for checking imported animals, food of animal origin and feed (Fig. 2). These are Peripheral Veterinary Offices of the Ministry of Health are recognised and qualified, according to community procedures, to carry out veterinary checks on live animals and products of animal origin that come into EU from extra-EU countries or are in transit to other EU countries. These checks are in accord with Directives 97/78/EC and 91/496/EEC, applied in Italy with Legislative Decrees No 80, 25 February 2000 and No 93, 3 March 1993. They are located in all airports, ports and borders as part of a system of checks which is completely integrated at the European level. Goods that have been certified by PIF can circulate freely throughout the European Union.

Each PIF is qualified to check one kind of product depending on its setup and according to the EU categories that are annually reviewed. Since 1991, PIFs are subject to periodic inspections by Community inspectors in order to verify structural and functional requirements, as well as the work that is specified by the norms.

The Veterinary Offices for Compliance with Community Requirements (UVAC), (Fig. 2), are responsible for intra-community trade and are a specific Italian control office. These are the Peripheral Veterinary Offices of the Ministry of Health and that were instituted by Legislative Decree No 27, 30 January 1993, applying Directive 89/608/EEC on mutual assistance between administrative authorities, in order to ensure the correct application of veterinary and zoo-technical legislation. They were the results of the abolition of border controls between EU Member States and a consequence of the Single Market (1993). The functions and tasks of UVAC were determined by the Ministry of Health Decree, 18 February 1993. Each of the 17 operating UVAC has a territorial competence that generally covers the territory within one region, but in some cases it covers two regions. The priorities of UVAC are to: determine the level of control depending on the type of goods and origin; apply Ministry of Health norms, in concomitance with regional Veterinary Services and Local Health Units; coordinate and verify uniformity of the control activities of the Local Health Veterinary Services, in collaboration with the regions; manage the flow of information related to goods exchanged in the Community and to give technical and legislative advise in case of community legal disputes. In order to carry out the above works, the flow of goods from other EU countries should be known. There are two instruments: the first, introduced by Legislative Decree No 28, 30 January 1993, obliges addressees of live animals or animal products from Member States to notify the UVAC and the Local Health Veterinary Service about the entrance of such goods to at least one day in advance. To notify them effectively, business operators must be registered at their nearest UVAC and, in some cases, have an appropriate agreement with them. The second instrument is a computerised network linking veterinary authorities. Originally it only dealt with live animals (ANIMO System - ANImal MOvement) but now it has been extended to some kinds of animal products from the EU. On the day of consignment, the competent authority of the Member State of origin which issued the health certificate, must communicate the consignment of animals being dispatched to the central competent authority of

destination and the competent authority of the place of destination. This obligation is laid down in Article 4 of Directive 90/425/EEC, concerning veterinary and zoo-technical checks applicable in intra-Community trade for certain live animals and products in an effort to harmonise the internal market. The health authority in Italy is the Ministry of Health with the Veterinary Service and Local Health Units.

The PIF and UVAC are coordinated by the Department for Public Veterinary Health, Nutrition and Food Safety - General Directorate for Animal Health and Veterinary Medicines (Presidential Decree No 189, 14 March 2006, art. 2) (Fig. 2).

The Port, Airport and Border Health Offices (USMAF) are responsible for checks on imported food of non-animal origin (Fig. 2). These offices are located at the largest ports and airports and form a protective filter against the risk of importing disease. This is the first structure to carry out health checks on transport, goods and persons entering the EU territory. They carry out international prophylaxis and public health checks established by Ministerial Decree 2 May 1985 and successive modifications. They supervise the importation of goods, mostly of plant origin, for human consumption and phytosanitary products. The Port, Airport and Border Health Offices are coordinated by the Department for Public Veterinary Health, Nutrition and Food Safety - General Directorate for Food Safety and Nutrition (Ministerial Decree 14/12/2006).

3.1.3 The Military Police (Carabinieri) Headquarters for Public Health

The Carabinieri Headquarters for Public Health designated in Legislative Decree No 202, 1 October 2005, operate through the Anti-Adulteration and Health Units (NAS). Although hierarchically linked to the Carabinieri (Ministry of Defence), they operate under the supervision of the Ministry of Health (Fig. 2). NAS personnel have the title of "health inspector", thus giving them the authority to inspect any areas where medicines and food are produced, distributed and stored (9). Each NAS unit investigates illegal adulteration of foodstuffs, often in collaboration with inspectors of the Central Inspectorate for Quality Control of Agricultural and Food Products (ICQ) of the Ministry of Agriculture Foodstuffs and Forestry Policies (Fig. 3) and plans national checks and surveillance (coordinated with Local Health Units).

Health inspectors check hygiene, product sampling and analysis, document authorisation and the control systems installed by businesses and their effectiveness. If crimes arise, the inspectors, as police authority, notify the competent legal authorities for further investigation (9).

The Carabinieri Headquarters for Public Health are made up of a central headquarters, three field offices, in Milan, Rome and Naples, and 35 local inspection units, located throughout Italy. Over the years, NAS has extended its expertise to include international prophylaxis of infectious diseases, maritime, aerial and border health, the production and sale of speciality medications for human and veterinary use (including homeopathic medicines), vaccines, viruses, serums, cosmetic and herbal products, medical and diagnostic devices, hygiene, public health and veterinary police and the production and legal commerce of narcotics for the preparation of pharmaceutical specialities (9).

3.1.4 National Health Institute

Since 1978 (Law No 833, 23 December 1978), the National Health Institute (ISS), under the Ministry of Health, is the public authority that carries out work and technical-scientific tasks and technical coordination. Its activities include research, control, training and consultation in the interest of protecting public health. One of its most important activities, mandated by the Minister of

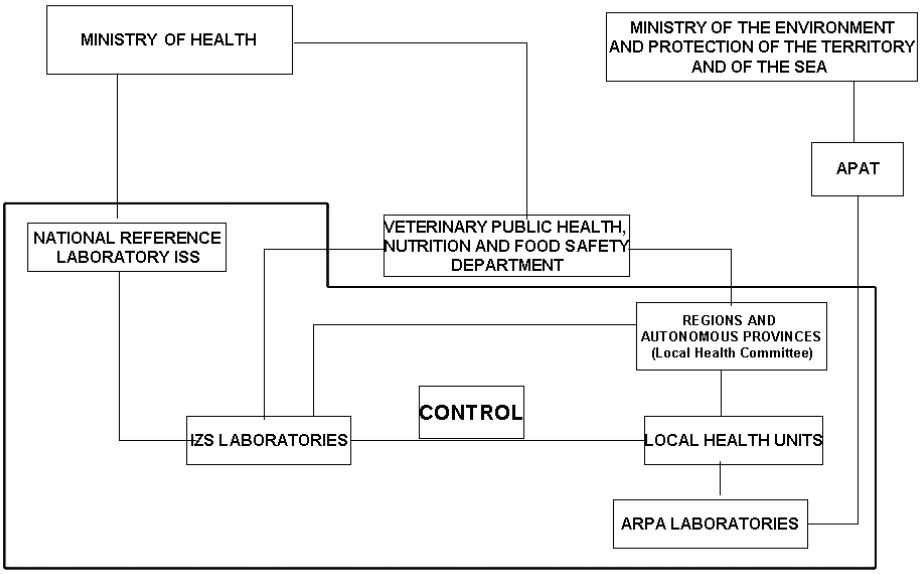

Fig. 3 - Network of Public Laboratories of National Health Service.

Health or the Regions, is the inspection and quality control of food products and packaging. It also supervises the laboratories of the Italian National Health Service (SSN) involved in food control. It is made up of 7 Departments and 4 National Centres (10).

The mission of the Department for Food Safety and Public Veterinary Health is to protect human health and welfare through the development of knowledge, tools and strategies for preventing and controlling animal diseases and improving food safety. It collects, analyses and publishes scientific data, acting as a liaison and working with Italian food safety and animal health organisations, with special reference to coordinating the research activities of the Experimental Zoo Prophylaxis Institutes (Fig. 3). It serves as a European Reference Laboratory for studying residues and food contaminants and as a National Reference Laboratory for veterinary medicine residues and food contaminants. Furthermore, the Department works as a Laboratory Accreditation Body (ORL) for food analysis; it also collaborates with WHO-FAO for Public Veterinary Health (10).

The four National Centres are technical scientific structures that carry out research, control, advice and training activities, which sometimes involve different departments within the ISS. They also play a coordinating role with outside institutions. They are organized into different Units.

The National Centre for Food Quality and Risk Assessment ensures food safety and nutritional value. It evaluates the risk of adverse health effects due to contaminated food and provides information to the public on food, food safety and nutrition (10).

3.1.5 Role of Regions and Autonomous Provinces

Concerning food and drink safety, the administrative organisation of the Regions and the Autonomous Provinces, as laid out by regional and provincial law, creates offices within more complex structures (e.g. management, departments, areas, services, etc.) which locally apply regional skills to public and

veterinary health and hygiene, planning, addressing, coordinating and controlling the local health units that are operative in the food hygiene sector (1). These structures are part of the Health Committees in almost all the Regions and Autonomous Provinces.

3.1.6 Local Health Units (AUSL)

The Local Health Units are organisationally, administratively, technically and legally autonomous (Fig. 2). They provide health assistance in both public and accredited private structures. Law No 833, 23 December 1978, establishes (art. 14) the skills concerning hygiene in the production, manufacture, distribution and commerce of food and drink, prophylaxis and veterinary police, the inspection and veterinary surveillance of animals for human consumption, the installations for slaughtering and transformation, feed, animal nutrition, transmittable diseases from animals to humans, reproduction, breeding and animal health and veterinary medicines.

The above-mentioned functions, in light of art. 7 of Legislative Decree No 502, 30 December 1992, modified by art. 8 of Legislative Decree No 517, 7 December 1993, are carried out by the Food Hygiene and Nutrition Service (S.I.A.N.) and Local Veterinary Services (S.V.L.) of the Departments for Prevention (Fig. 2).

SIAN is in charge of food of non-animal origin, while SVL is in charge of food of animal origin, animal health and welfare, and feed.

SVL is organised into three different areas responsible for animal health (area A), food of animal origin (area B), animal breeding and animal production (area C) (6). These distinct areas of activity are not always present in all the Local Health Units and the allocation of resources may differ according to their priorities.

3.1.7 Regional Agencies for Environmental Protection

The Agency for Environmental Protection and Technical Services (APAT), established by art. 38 of Legislative Decree No 300, 30 July 1999, carries out scientific and technical activities in the national interest to protect the environment, water resources and soil. It was created by merging the National Environmental Protection Agency (ANPA) with the Department for Government Technical Services, according to the regulatory provisions of Presidential Decree No 207, 8 August 2002. APAT is technically, scientifically and financially autonomous and is subject to the guidelines and overseeing of the Ministry for the Environment and Protection of the Territory and Sea. It is monitored by the National Audit Office (Fig. 3), (11).

The Regional Agencies for Environmental Protection (ARPA) were established by the regulations of Legislative Decree No 496, 4 December 1993, amended by Law No 61, 21 January 1994.

APAT is a network of 21 Regional (ARPA) and Provincial (APPA) agencies established by special regional laws. The creation of APAT shows the cohesion of the network, while respecting local realities and promoting uniform development in terms of cooperation and collaboration (12).

3.1.8 Experimental Zooprophylaxis Institutes

These are authorised public health authorities with administrative autonomy that are the technical and operative arm of the Italian National Health Service (SSN) regarding animal health, the health and quality of food of animal origin and cattle hygiene (13).

These institutes were established by Law No 503, 23 June 1970, modified by Law No 745, 23 December 1975. The Experimental Zooprophylaxis Institutes (IZS), with ten central seats and about ninety peripheral sections, are an important operative arm of the Italian National Health Service that ensures epidemiological surveillance, experimental

research, personnel training, laboratory support and official food control diagnosis (14). They work closely with Local Health Units, particularly with Local Veterinary Services (Fig. 2).

They are financed and monitored by the Department for Public Veterinary Health, Nutrition and Food Safety (Presidential Decree No 189, 14 March 2006, art. 2). Such financing, together with possible additional resources from the Regions, is used for many purposes such as institutional tasks (Ministerial Decree No 190, 16 February 1994, art. 3), research (financed by the European Union or corporations or national and local Institutions) and planning of activities of regional and/or provincial interest. In addition, the Experimental Zooprophylaxis Institutes have laboratories of excellence called National Reference Centres (Ministerial Decree 4 October 1999), which have the following functions: confirming diagnoses from other laboratories; standardising and promulgating methods of analyses; initiating "ring tests" between IZSs; collaborating with other EU or extra-EU reference centres and providing assistance and specialised information to the Ministry of Health. The National Reference Centres are also a reference for international organisations (WHO, OIE, FAO).

3.1.9 Public Laboratories of the National Health Service Network

Laboratory services for animal health, food and feed are provided by a network of public laboratories at the regional level.

The 105 laboratories of the Regional Agencies for Environmental Protection (ARPA) are wholly responsible for the analysis of contaminants, pesticides and foodstuffs of plant origin. ARPA is made up of laboratories responsible for environmental monitoring and those responsible for food control, previously known as Multi-Territorial Prevention Offices (PMP). ARPA reports to the Local Health Units (Fig. 3), although they may perform analysis for more than one AUSL on a regional basis (6).

Ten Experimental Zoo Prophylaxis Institutes (IZS) with about ninety field diagnostic units at the provincial level are responsible for the analysis of food of animal origin, feed and animal health. The IZSs are under the control and supervision of the regions, while being coordinated by the Department for Public Veterinary Health, Nutrition and Food Safety (Fig. 3).

The laboratories are independently accredited by the National System for Laboratory Accreditation (SINAL), according to norm UNI ISO/IEC 17025, or by the Laboratory Accreditation Body (ORL), which is part of the National Health Institute. All ten IZS laboratories are accredited; while to date, 68 of the 105 ARPA laboratories are accredited (6). Through a computerised system, the public laboratories of the National Health Service Network have access to a data collection centre for the results of analysis carried out by the public laboratories. The centre belongs to the Informative Health Service (S.I.S.) of the Ministry of Health. The S.I.S. was instituted according to Law No 462, 7 August 1986, bringing urgent measures in matters of prevention and repression of food adulteration.

3.1.10 National Agency for Food Safety

Italy has been waiting for about five years for a National Authority for Food Safety to be set up. The purpose would be to give Italy, like other Member States of the EU, a reference structure connected to the European Food Safety Authority (EFSA), as required by art. 22 of Regulation 2002/178/EC.

While awaiting the foundation of a National Authority for Food Safety, a National Committee for Food Safety was temporarily established, on June 17th 2004. This National Committee, put under the auspices of the Department for Public Veterinary Health, Nutrition and Food Safety (Law No 244, 30 Novem-

ber 2005, art. 1), must guarantee that there is no political bias given the scientific nature of risk evaluation (S.I. Ve. M.P., 2006).

The function and organisation of this Committee and that of the Strategic Committee were defined in the Ministerial Decree 26 July 2007. According to article 22 of Regulation 2002/178/EC, the National Food Safety Committee cooperates with EFSA to ensure that the mission is accomplished and that it takes part in the Advisory Forum, according to article 27 of Regulation 2002/178/EC. This Committee provides scientific and technical support to Public Administrations involved in risk management in matters of food safety, and provides scientific opinions, upon the request of Strategic Committee, Central Public Administrations and the Regions and Autonomous Provinces of Trento and Bolzano. It approves the annual and multi-annual plans of scientific and technical activity, planned by the Strategic Committee and the National Secretariat of Food Chain Risk Evaluation. The Committee is composed of 18 members with expertise in the following areas: food additives, spices, technological coadjutants and food packaging, additives, animal feed products, plant safety, health and residues, genetically-modified organisms, organic products, dietary products, food, allergies and human nutrition, biological threats, residues and food chain contaminants, health and animal welfare.

Law No 31, 28 February 2008 established that the National Food Safety Committee became the National Authority for Food Safety; as of 15 January 2008, this Authority became the National Agency for Food Safety, with its seat in Foggia; some legislative measure will establish its organisation, function and administration.

The task of the Agency is controversial. It is an advisory organ and therefore provides support, protection and information to citizens in the area of food safety; it gives advice on the design and bills in parliament in matters of food safety and conducts studies in specific fields connected to food and potential risks for human health. It also has a role in coordinating several competent bodies on the national level. It should be the only reference body in a position to operate easily and timely in response to emergencies. The scientific committee has to have a central role in dialoguing with the EFSA and in expressing opinions on urgent issues in matters of health risk assessment. From a careful assessment of the results of control bodies, from their coordination and by strengthening of weak structures, it should optimize the controls.

The organisation within the Ministry of Health and the associated bodies, described above, has some weak points:

- Without better coordination between the Central, Regional and Local Authorities an effective control of food will be difficult and expensive to apply;

- On the local level, the Bodies directly dependent on the Department for Veterinary Public Health, Nutrition and Food Safety (IZS, PIF, UVAC and USMAF) must interface with the Regional and Local Bodies (e.g. AUSL); especially when implementing control plans like the PNAA, PNR and P.N.R.A.

3.2 Ministry of Agriculture Foodstuffs and Forestry Policies

The Ministry of Agriculture Foodstuffs and Forestry Policies, (MiPAAF), reformed by Presidential Decree No 79, 23 March 2005 and recently again reordered by Presidential Decree No 18, 9 January 2008, draws up and coordinates agricultural, forestry, foodstuffs and fishing policies, at the national and international levels. In addition, it guarantees agricultural and food product quality.

MiPAAF includes (Fig. 4) the Department of European and International Policies, the Department of Economic and Ru-

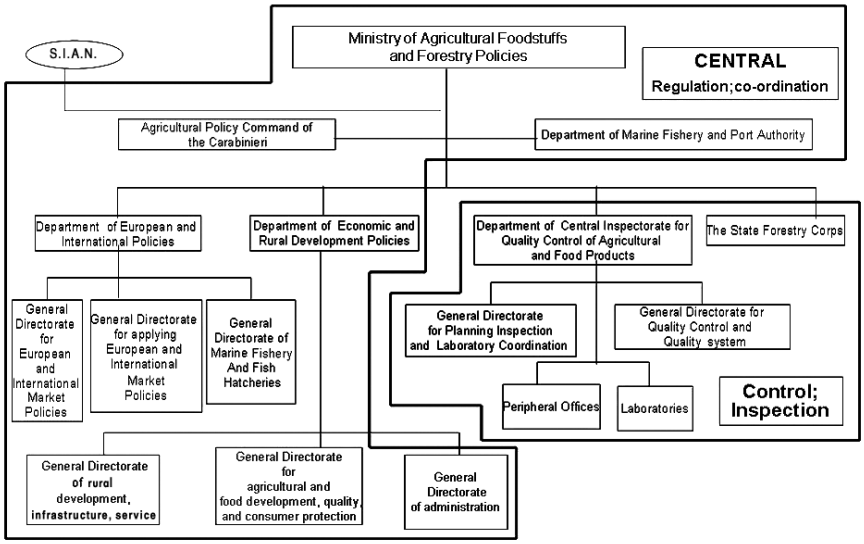

Fig. 4 - Structure of Ministry of Agricultural Foodstuffs and Forestry Policies and Associated Bodies.

ral Development Policies, the State Forestry Corps, the Central Inspectorate for Quality Control of Agricultural and Food Products, the Agricultural Policy Command of the Carabinieri, the Department of Marine Fishery and Port Authority.

All of the tools needed to exercise the functions of a central and regional administration in agriculture, foodstuffs and forestry matters converge in a centralised information system called SIAN, which has been managed by an outside agency, since 2001. The Agency for Agriculture Subsidies (AGEA) and the Mountain Information System (SIM) also serve in this system. The web site "www.sian.it" supplies SIAN services to agriculture. In particular, SIAN ensures the development of services for the management of Common Agricultural Policy subsidies to agricultural producers. In the food safety field, SIAN supplies data banks that are vital for conducting official controls: the plant health products data bank, plant health products sale declarations, registry of plant varieties and the list of egg packaging centres.

The Department of European and International Policies (Fig. 4) deals with market policies of agriculture, food, fishing and fish hatcheries and deals with the European Union during the drafting and applying of EU laws. It is organised into three general Directorates:

- the General Directorate for European and International Market Policies of market;
- the General Directorate for applying European and International market Policies;
- the General Directorate of Marine Fishery and Fish Hatcheries.

The Department of Economic and Rural Development Policies (Fig. 4) is responsible for national and EU policies of structural and rural development, agricultural and food product quality, consumer protection and the communication and promotion of foodstuffs at national and EU levels. It is organised into three General Directorates: Rural Development, Infrastructure and Services; Agricultural and Food Development, Quality and Consumer Protection; Administration.

The General Directorate for Agricultural and Food Development, Quality and Consumer Protection is involved in activ-

ities concerning products with a protected geographical indication (IGP), protected designation of origin (DOP) (Regulation 2006/510/EC) and with the recognition of guaranteed traditional specialities (STG) (Regulation 2006/509/EC). In general it is responsible for applying Law No 164, 10 February 1992, which concerns the designation of wine origin and geographic indications; organic agriculture and the certification of environmentally friendly agriculture; the application of the Codex Alimentarius/Food Standards.

As regards specific competencies, the MiPAAF delegates some activities to other agencies including the Agency for Agriculture Subsidies (AGEA), the National Institute for the Agricultural Economy (INEA), the National Union for the Equine Protection (UNIRE), the Institute of Services for Agricultural and Food Market (ISMEA) and the National Institute for Foods and Nutrition (INRAN).

3.2.1 The State Forestry Corps

According to Law No 36, 6 February 2004, the State Forestry Corps (Fig. 4) is a police force, skilled in the defence of Italian agriculture and forestry and the protection of the environment, landscape and ecosystems. It assists in works of public order and safety, as well as in territorial control, with special reference to rural and mountain areas. It also checks food safety and prevents and represses related crime. It also works in the interest of national civil protection. The Corps is involved in the surveillance of parks, protected areas of natural beauty and state nature reserves; it controls freshwater fishing and illicit refuse dumping. Beyond these traditional competencies, the Corps is also involved in controlling international commerce of fauna and flora species that are threatened or in danger of extinction, as established by the Washington Convention (CITES) (15). In some special areas, central operating units were established like the Agricultural, Food and Forest Unit (NAF), which is involved in consumer safety and the correct application of Community regulations for agriculture and forests. In particular, Division II of Service I (The Central Environmental, Forestry, Agricultural, Food and Civil Police), the "Agriculture and Food Police":

- coordinate the activation of community regulations regarding agricultural food and forestry;
- coordinate norms on food safety, consumer and biological safety, including BSE, OGM and organic crops;
- manage the Agriculture, Food and Forest Unit (NAF);
- act as liaison with the Central Inspectorate for Quality Control of Agricultural and Food Products, the Agricultural Policy Command of the Carabinieri and other organisations involved in Community and/or national fraud to guarantee food safety to citizens (16).

3.2.2 The Central Inspectorate for Quality Control of Agricultural and Food Products

Its statutes follow those of the Central Inspectorate for Fraud Suppression, with Law No 462, 7 August 1986 (Fig. 4). It is the technical arm of the state for preventing and suppressing fraud in the preparation and commerce of agricultural and food products, and in agricultural and forestry consumables. It reports directly to the Minister (Legal Decree No 1, 11 January 2001, converted into Law No 49, 9 March 2001) and operates with complete autonomy. Its operating structure was reorganised for the first time by Ministerial Decree No 44, 13 February 2003, subsequently by Law No 231, 11 November 2005 when it assumed a departmental structure with two general directorates (both located in Rome) and recently by Presidential Decree No 18, 9 January 2008 (Fig 4):

- The Directorate General for Planning, Inspection and Laboratory Coordination;

- The Directorate General for Quality Control and Quality System.

There are twelve managing inspection offices throughout Italy, with fifteen local units. There is also a network of five chemical laboratories for analysing the samples collected by local inspection offices. A central laboratory for second level analyses is located in Rome and its mission is to assess the quality of chemical analysis, and improve methods.

As a consequence of the 2007 Italian Finance Law (Law No 296, 27 December 2006 *subs* 1047), the Central Inspectorate for Fraud Suppression changed its name to the Central Inspectorate for the Quality Control of Agricultural and Food Products (ICQ). It is in charge of the accreditation and surveillance of public and private bodies involved in certification of DOP, IGT and STG products.

The ICQ oversees the labelling and market presence, DOP, IGP and STG products, winemaking, olive oil, dairy products, organic food, cereal derivatives (pasta, flour, durum wheat flour, pastry products), honey, vegetable preserves, eggs, seeds, animal feed, fertilisers and bio-stimulants, phytosanitary products.

New norms have extended their competencies (Law No 231/2005) to include checking for irregular trading of agricultural and food products from EU extra-EU countries. Moreover, it carries out exceptional controls to help agriculture that has been hit by market crises and to combat fraud which gives rise to unfair competition between operators (Law No 71, 29 April 2005).

In the winemaking sector, Ministerial Decree 4 August 2006 assigned the Central Inspectorate the role of surveilling consortia for the protection of wines by denomination of origin, to guarantee that they respect the approved control procedures without discrimination. It operates in tandem with other organisations, such as the Carabinieri Headquarters for Public Health, the Finance Police, the State Forestry Corps, the Carabinieri, the Police, the Agricultural Policy Command of the Carabinieri, and the Customs Agency.

3.2.3 The Agricultural Policy Command of the Carabinieri

The Agricultural Policy Command of the Carabinieri (Fig. 4), instituted on 5 December 1994 with the name of "Carabinieri for the Protection of Community and Agricultural Food Norms" works throughout Italy and, if necessary, on foreign soil, to enforce the law and directives of the Ministry of Agricultural Foodstuffs and Forestry Policies. According to Presidential Decree No 79, 23 March 2005, the Command is directly accountable to the Minister of Agricultural Foodstuffs and Forestry Policies (Fig. 4) and oversees Community aid for agricultural food, fishery and hatcheries, the sale and/or withdrawal of agricultural food products, and aid to developing countries.

Moreover, it specifically controls the application of regulations and contributions, in tandem with the ICQ, to prevent and suppress fraud in the agricultural food sector. To better achieve more widespread action, the Command consists of an Operational Coordination Unit and three Carabinieri Anti-Fraud Units (N.A.C.), located in Rome, Parma and Salerno. To this end, checks are planned and inquiries initiated in various skilled areas of the Ministry of Agricultural Foodstuffs and Forestry Policies (17).

Special assessments are made at every phase within the agricultural food sector, from seeds to products; recently there has been an increased surveillance of organic crops. Equal attention has been placed on zootechnical areas like fish, vegetables, fruit, milk and its derivatives, olive oil and wine. The Command has close ties with OLAF (European Office of the Fight Against Fraud) in Brussels, with which it coordinates anti-fraud measures throughout the EU (17).

3.3 The Ministry of Economy and Finance

The Ministry of Economy and Finance, instituted in 1999 (Legislative Decree No 300, 30 July 1999), was formed from the union of the Ministry of the Treasury, Budget and Economic Planning with the Ministry of Finance. This latter became the fourth Department of the Ministry of Economy and Finance, called the Department of Fiscal Policies (DPF), whose tasks are to define and coordinate fiscal policy. State financial administration is attributed to four Fiscal Agencies: State Resources, Customs, Revenue and Territory (Legislative Decree No 300, 30 July 1999) (Ministerial Decree 28 December 2000). The DPF draws up fiscal policy, deals with regulations and coordinates the Agencies that guarantee taxpayer revenue, manage fiscal observance and provide collection services. Currently, the Department of Fiscal Policies (DPF) is governed by Presidential Decree No 107, 26 March 2001 and Ministerial Decree 21 November 2001. (Ministry of Economy and Finance - March 2006).

3.3.1 The Customs Agency

The Customs Agency manages administrative services (collection, legal disputes in Agency rights and the internal taxation within international exchange, the inland duties on production and consumption, excluding those on industry tobacco) working closely with EU organisations for harmonisation and unification (Legislative Decree No 300, 30 July 1999, art. 63, modified by Legislative Decree No 173, 3 July 2003). As a consequence of Council Regulation (EC) No 1383/2003 of 22 July 2003, concerning the Customs response to goods suspected of infringing upon intellectual property rights and the measures taken against goods which have infringed on such rights, enacted as of 1 July 2004, the Agency, in expectation of issuing the regulation (Commission Regulation 2004/1891/EC), sent out Circular No 32/D of 23 June 2004 (18), so that its offices might assimilate the above regulation. This circular contains EU-approved operating instructions for suppressing the commerce of counterfeit goods, and protecting goods with protected geographical indication (IGP) and designation of origin (DOP).

The Customs Agency has laboratories throughout Italy: 15 specialised laboratories subdivided into three macro-areas connected across the LIMS (Laboratory Information Management System) net with logistics to transfer samples rapidly (19). The Customs' laboratories are accredited (rule UNI EN ISO/IEC 17025) at SINAL. They analyse features that are particular to products in order to create a customs and fiscal classification, which not only regulates the tax but also helps prevent and suppress tax fraud. They collaborate with the Ministry of Health by analysing imported foodstuffs (20).

The Customs laboratories prepare analyses for the public that are used for study, consultancy, research and experimentation.

4. CONCLUSIONS

The organisation of the Italian Public Food Control system described in this paper shows that, the National legislation clearly defines the skills and roles of each Authority involved. However, there is a lack of coordination among the various authorities which results in frequent overlapping, even among Departments within the same Ministry. This causes problems among food operators, in that some operators may be inspected repeatedly, while others may never be inspected. Other weaknesses are related to institutional complexity and continuous Ministerial revisions, in which new duties are added, without clearly removing the existing ones. In order to ensure an efficient, effective coordination and con-

sistent control, as required by art. 4 of Regulation 2004/882/EC, the Ministries and Regions need to define the system of official control coordination and the flow of information among the control bodies. An effective coordination can only be achieved through communication and meetings between the Central Authorities and Regional and Local Authorities. The structural differences between Regions and the political choices of the local governments create difficulties with respect to receiving and implementing EU legislation, which can have a varied impact throughout the Nation.

To date, there has been little effort at the national level to adapt the official control system to the new EU hygiene legislation. In fact, a report of the European Commission (DG SANCO) 8145/2006 of a mission carried out in Italy from 20 November to 1 December 2006, to evaluate official controls related to safety of food of animal origin, shows that none of the competent authorities in the six regions visited had developed a control system in the framework of Regulation 2004/882/EC and the new Hygiene Regulations (21).

Italy has only implemented measures in the form of guidelines ("Guidelines for the Correct Application of Regulation 2004/852/EC (European Parliament and Council) on foodstuff hygiene" and "Guidelines for the Correct Application of Regulation 2004/853/EC (European Parliament and Council) on animal product hygiene"), but these are at a very early stage, because they were only published in February 2007. Guidelines regarding Regulations 2004/882/EC and 2004/854/EC are being drafted by the Department for Public Veterinary Health, Nutrition and Food Safety and it is not known when they will be ready for approval by the Regions.

It is important to ensure that the staff performing the official controls receives adequate training as required by art. 6 of Regulation 2004/882/EC.

A good starting point for a complete implementation of the EU legislation on food in Italy would be to complete multi-year national control plans in compliance with Regulation (EC) No 882/04, with the aim of applying a totally uniform approach. Each multi-year national control plan should contain general information on the structure and organisation of the systems of feed and food control, animal health and animal welfare control in the Member State including: the general organisation and management of official controls at the national, regional and local levels, the control systems applied to different sectors and the coordination between the different services of competent authorities responsible for official controls in these sectors.

It is important to underline that the recent establishment of the National Agency for Food Safety will be justified only if it guarantees coordination between the control structures in addition to its tasks as an advisory body.

ABBREVIATIONS

AGEA	Agenzia per le Erogazioni in Agricoltura
ANPA	Agenzia Nazionale Protezione ambiente
APAT	Agenzia per la Protezione dell'Ambiente e per i Servizi Tecnici
ARPA	Agenzia Regionale per la Protezione dell'Ambiente
AUSL	Azienda Unità sanitaria locale
DPF	Dipartimento per le Politiche Fiscali
ICQ	Ispettorato Centrale per il controllo della qualità dei prodotti agroalimentari
INRAN	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione
ISMEA	Istituto di Servizi per il Mercato Agricolo Alimentare
ISS	Istituto Superiore di sanità
IVA	Imposta sul valore aggiunto
IZS	Istituto Zooprofilattico Sperimentale
MiPAAF	Ministero delle Politiche Agricole Alimentari e Forestali
NAC	Nucleo Antifrodi Carabinieri
NAF	Nucleo Agroalimentare e Forestale
NAS	Nucleo Antisofisticazioni e Sanità

ORL	Organismo di Riconoscimento Laboratori
PIF	Posto di Ispezione Frontaliera
PMP	Presidi Multizonali di Prevenzione
PNAA	Piano Nazionale Alimentazione Animale
PNR	Piano Nazionale Residui
PNRA	Piano Nazionale Residui Antiparassitari
SIAN	Servizio Igiene Alimenti e Nutrizione (Fig. 2)
SIAN	Sistema Informativo Agricolo Nazionale (Fig. 3)
SIM	Sistema Informativo della Montagna
SINAL	Sistema Nazionale Accreditamento Laboratori
SIS	Servizio Informativo Sanitario
SSN	Servizio Sanitario Nazionale
SVL	Servizio Veterinario Locale
UNIRE	Unione Nazionale Incremento Razze Equine
USMAF	Ufficio di Sanità Marittima Aerea e di Frontiera
UVAC	Ufficio Veterinario per gli Adempimenti degli obblighi Comunitari

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Law No 317, 3 August 2001, "Conversione in legge, con modificazioni, del D.L. 12 giugno 2001, n. 217, recante modificazioni al D.Lgs. 30 luglio 1999, n. 300, nonché alla L. 23 agosto 1988, n. 400, in materia di organizzazione del Governo" (Conversion in law, with modifications, of Legal Decree No 217, 12 June 2001, bringing modifications of Legislative Decree No 300, 30 July 1999, as well as of Law No 400, 23 August 1988, in matters of Government organisation) (Italian Republic Official Gazette No 181, 06/08/2001).

Law No 36, 6 February 2004, "Nuovo ordinamento del Corpo forestale dello Stato" (New ordering of the State Forestry Corps) (Italian Republic Official Gazette No 37, 14/02/2004).

Law No 71, 29 April 2005, "Conversione in legge, con modificazioni, del D.L. 28 febbraio 2005, n. 22, recante interventi urgenti nel settore agroalimentare" (Conversion in law, with modifications, of Legal Decree No 22, 28 February 2005, bringing urgent interventions in agricultural and food sector) (Italian Republic Official Gazette No 99, 30/04/2005).

Law No 231, 11 November 2005, "Conversione in legge, con modificazioni, del D.L. 9 settembre 2005, n. 182, recante interventi urgenti in agricoltura e per gli organismi pubblici del settore, nonché per contrastare andamenti anomali dei prezzi nelle filiere agroalimentari" (Conversion in law, with modifications, of Legal Decree No 182, 9 September 2005, bringing urgent interventions in agriculture and for the public organisms of the area, as well as for contrast anomalous courses of the prices in the agricultural and food chains) (Italian Republic Official Gazette, No 263, 11/11/2005).

Law No 244, 30 November 2005, "Conversione in legge, con modificazioni, del D.L. 1° ottobre 2005, n.202, recante misure urgenti per la prevenzione dell'influenza aviaria" (Conversion in law, with modifications, of Legal Decree No 202, 1 October 2005, bringing urgent measures for the avian influenza prevention) (Italian Republic Official Gazette No 279, 30/11/2005).

Law No 296, 27 December 2006, "Disposizioni per la formazione del bilancio annuale e pluriennale dello Stato (Legge finanziaria 2007)" (Dispositions for formation of the national annual and pluriannual budget) (Italian Republic Official Gazette, No 299, O.S., 27/12/2006).

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Legal Decree No 496, 4 December 1993, "Disposizioni urgenti sulla riorganizzazione dei controlli ambientali e istituzione della Agenzia nazionale per la protezione dell'ambiente" (Urgent dispositions for reorganisation of environmental controls and institution of National Environmental Protection Agency) (Italian Republic Official Gazette No 285, 4/12/1993).

Legal Decree No 1, 11 January 2001, "Disposizioni urgenti per la distruzione del materiale specifico a rischio per encefalopatie spongiformi bovine e delle proteine animali ad alto rischio, nonché per l'ammasso pubblico temporaneo delle proteine animali a basso rischio. Ulteriori interventi urgenti per fronteggiare l'emergenza derivante dall'encefalopatia spongiforme bovina" (Urgent dispositions for the destruction of the specific material at risk for Bovine Spongiform encephalopathies and of proteins animals to high risk, as well as for the temporary public heap of proteins animals at low risk. Further urgent interventions to face the emergency deriving from the Bovine Spongiform encephalopathy) (Italian Republic Official Gazette No 8, 11/11/2001).

Legal Decree No 202, 1 October 2005, "Misure urgenti per la prevenzione dell'influenza aviaria" (Urgent measures for the avian influenza prevention) (Italian Republic Official Gazette No 229, 01/10/2005).

Legislative Decree No 502, 30 December 1992, "Riordino della disciplina in materia sanitaria, a norma dell'articolo 1 della L. 23 ottobre 1992, n. 421" (Reordering of the discipline in sanitary matters, to rule of article 1 of Law No 421, 23 October 1992) (Italian Republic Official Gazette No 305 O.S., 30/12/1992).

Legislative Decree No 27, 30 January 1993, "Attuazione della direttiva 89/608/CEE relativa alla mutua assistenza tra autorità amministrative per assicurare la corretta applicazione della legislazione veterinaria e zootecnica" (Application of Directive 89/608/EEC on the mutual assistance between administrative authorities, in order to assure the correct application of the veterinary and zootechnical legislation) (Italian Republic Official Gazette No 28 O.S., 04/02/1993).

Legislative Decree No 28, 30 January 1993, "Attuazione delle direttive 89/662/CEE e 90/425/CEE relative ai controlli veterinari e zootecnici di taluni animali vivi e su prodotti di origine animale applicabili negli scambi intracomunitari" (Application of Directives 89/662/EEC e 90/425/EEC concerning veterinary and zootechnical checks in certain live animals and animal origin products applicable in intra-Community trade) (Italian Republic Official Gazette No 28 O.S., 04/02/1993).

Legislative Decree No 93, 3 March 1993, "Attuazione della direttiva 90/675/CEE e della direttiva 91/496/CEE relative all'organizzazione dei controlli veterinari su prodotti e animali in provenienza da Paesi terzi e introdotti nella Comunità europea" (Application of Directives 90/675/EEC e 91/496/EEC concerning the organisation of veterinary checks on produts and animals entering the Community from third countries) (Italian Republic Official Gazette No 78 O.S., 03/04/1993).

Legislative Decree No 123, 3 March 1993, "Attuazione della direttiva 89/397/CEE relativa al controllo ufficiale dei prodotti alimentari" (Accomplishment of directive 89/397/EEC relevant to the official control of the food products) (Italian Republic Official Gazette No 97, 27/04/1993).

Legislative Decree No 270, 30 June 1993, "Riordinamento degli Istituti Zooprofilattici Sperimentali, a norma dell'art. 1, comma 1, lettera h), della legge 23 ottobre 1992, n. 421" (Reordering of Experimental Zoo Prophylaxis Institutes to rule of art. 1, *comma* 1, lecter h) of Law No 421, 23 october 1992) (Italian Republic Official Gazette No 180 O.S., 03/08/1993).

Legislative Decree No 517, 7 December 1993, "Modificazioni al D.Lgs. n. 502, 30 dicembre 1992, recante riordino della disciplina in materia sanitaria, a norma dell'art. 1 della legge 23 ottobre 1992, n. 421" (Modifications to the Legislative Decree No 502, 30 December 1992, bringing reorganisation of discipline in sanitary matters, to rule of the art. 1 of the Law No 421, 23 October 1992) (Italian Republic Official Gazette No 293, 15/12/1993).

Legislative Decree No 300, 30 July 1999, "Riforma dell'organizzazione del Governo, a norma dell'articolo 11 della L. 15 marzo 1997, n. 59" (Reform of the organisation of the Government, to rule of article 11 of the Law No 59, 15 March 1997) (Italian Republic Official Gazette No 203 S.O., 30/08/1999).

Legislative Decree No 80, 25 February 2000, "Attuazione della direttiva 97/78/CE e 97/79/CE in materia di organizzazione dei controlli veterinari sui prodotti provenienti da Paesi terzi" (Application of directives 97/78/EEC and 97/79/EEC concerning the organisation of veterinary checks on products entering the Community from third countries) (Italian Republic Official Gazette No 82, 07/04/2000).

Legislative Decree No 68, 19 March 2001, "Adeguamento dei compiti del Corpo della Guardia di finanza, a norma dell'articolo 4 della L. 31 marzo 2000, n. 78" (Adaptation of the tasks of the Finance Police, to rule of article 4 of the Law No 78, 31 March 2000) (Italian Republic Official Gazette No 71, 26/03/2001).

Legislative Decree No 223, 17 June 2003, "Attuazione delle direttive 2000/77/CE e 2001/46/CE relative all'organizzazione dei controlli ufficiali nel settore dell'alimentazione animale" (Accomplishment of directives 2000/77/EC e 2001/46/EC, concerning the organisation of official controls on animal nutrition field).

(Italian Republic Official Gazette No 194, O.S., 22/08/2003).

Legislative Decree No 173, 3 July 2003, "Riorganizzazione del Ministero dell'economia e delle finanze e delle agenzie fiscali, a norma dell'articolo 1 della Legge 6 luglio 2002, n. 137" (Reorganisation of Ministry of Economy and Finances and of the fiscal agencies, to rule of art. 1 of Law No 137, 6 July 2002) (Italian Republic Official Gazette No 161, 14 /07/2003).

Legislative Decree No 100, 27 May 2005, "Ulteriori disposizioni per la modernizzazione dei settori della pesca e dell'acquacoltura e per il potenziamento della vigilanza e del controllo della pesca marittima, a norma dell'articolo 1, comma 2, della legge 7 marzo 2003, n. 38" (Further dispositions for modernization of fishing and fish hatcheries field and for expansion of surveillance and control of maritime fishing, to rule of article1, comma 2, of Law No 38, 7 March 2003) (Italian Republic Official Gazette No 136, 14/06/2005).

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Legislative Decree No 193, 6 November 2007, "Attuazione della direttiva 2004/41/CE relativa ai controlli in materia di sicurezza alimentare e applicazione dei regolamenti comunitari nel medesimo settore" (Application of directive 2004/41/CE concerning controls in matters of food safety and application of EU regulations in the same field) (Italian Republic Official Gazette No 261, O.S. No 228, 9/11/2007).

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Ministerial Decree 4 October 1999 "Centri di referenza nazionali nel settore veterinario" (National Reference Centres in veterinary area) (Italian Republic Official Gazette No 300, 23/12/1999).

Ministerial Decree 28 December 2000 "Disposizioni recanti le modalità di avvio delle agenzie fiscali e l'istituzione del ruolo speciale provvisorio del personale dell'amministrazione finanziaria a norma degli articoli 73 e 74 del decreto legislativo 30 luglio 1999, n. 300" (Dispositions bringing the rules of starting of the fiscal agencies and the institution of the special temporary role of the personnel of the financial administration to rule of the articles 73 and 74 of the legislative decree No 300, 30 July 1999) (Italian Republic Official Gazette No 9, 12/01/2001).

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Ministerial Decree 4 August 2006 "Vigilanza sul controllo della produzione dei vini di qualità prodotti in regioni determinate (V.Q.P.R.D.)" (Surveillance on control of production of wines with denomination of origin) (Italian Republic Official Gazette No 190, 17/08/2006).

Ministerial Decree 14 December 2006 "Individuazione degli uffici dirigenziali di livello non generale" (Identification of managerial office of not general level) (Italian Republic Official Gazette No 36, 13/02/2007).

Ministerial Decree 26 July 2007 "Organizzazione delle funzioni di cui al Regolamento (CE) del 28 Gennaio 2002, n. 178, del Parlamento Europeo e del Consiglio in materia di valutazione del rischio della catena alimentare" (Function organisation to the sense of Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 on matters of risk assessment of food chain) (Italian Republic Official Gazette No 231, 04/10/2007).

Presidential Decree No 327, 26 March 1980, "Regolamento di esecuzione della L. 30 aprile 1962, n. 283, e successive modificazioni, in materia di disciplina igienica della produzione e della vendita delle sostanze alimentari e delle bevande" (Regulation of execution of Law No 283, 30 April 1962 and successive modifications, in matters of hygienic discipline and sale of foodstuffs and drinks) (Italian Republic Official Gazette No 193, 16/07/1980).

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Presidential Decree No 207, 8 August 2002, "Regolamento recante approvazione dello statuto dell'Agenzia per la protezione dell'ambiente

e per i servizi tecnici, a norma dell'articolo 8, comma 4, del D.Lgs. 30 luglio 1999, n. 300" (Regulation bringing approval of Statute of Environmental Protection and technical services Agency, complies with article 8, comma 4, of Legislative Decree No 300, 30 July 1999) (Italian Republic Official Gazette No 222 O.S, 21/09/2002).

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Provision of 9 February 2006 "Accordo, ai sensi dell'articolo 4 del decreto legislativo 28 agosto 1997, tra il Ministero della salute, le regioni e le province autonome relative a Linee Guida applicative del Regolamento n. 853/2004/CE del Parlamento Europeo e del Consiglio sull'igiene dei prodotti di origine animale" (Agreement, to the senses of article 4 of the legislative decree 28 August 1997, between the Ministry of the health, the regions and the autonomous provinces relative to "Applicative guidelines of Regulation n. 853/2004/EC of the European Parliament and the Council on the hygiene of products of animal origin") (Italian Republic Official Gazette No 259, 7/11/2006).

Provision of 9 February 2006 "Accordo, ai sensi dell'articolo 4 del decreto legislativo 28 agosto 1997, tra il Ministero della salute, le regioni e le province autonome relative a Linee Guida applicative del Regolamento n. 852/2004/CE del Parlamento Europeo e del Consiglio sull'igiene dei prodotti alimentari" (Agreement, to the senses of article 4 of the legislative decree 28 August 1997, between the Ministry of the health, the regions and the autonomous provinces relative to "Applicative guidelines of Regulation n. 852/2004/EC of the European Parliament and the Council on the hygiene of foodstuffs") (Italian Republic Official Gazette No 259, 7/11/2006).

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PAPER

REDUCTION OF MICROBIAL LOAD AND SENSORY EVALUATION OF MINIMALLY PROCESSED VEGETABLES TREATED WITH CHLORINE DIOXIDE AND ELECTROLYSED WATER

RIDUZIONE DELLA CARICA BATTERICA E VALUTAZIONE SENSORIALE DI VEGETALI MINIMAMENTE PROCESSATI, TRATTATI CON DIOSSIDO DI CLORO E ACQUA ELETTROLIZZATA

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ABSTRACT

The efficacy of gaseous and liquid ClO_2 or neutral electrolysed water (NEW) to decontaminate minimally processed vegetables, without affecting sensory quality, was evaluated using aerobic plate counts and trian-

RIASSUNTO

È stata valutata, mediante conta aerobica su piastra e tests triangolari, l'efficacia del diossido di cloro sia allo stato gassoso sia liquido e dell'acqua elettrolizzata neutra (AEN), nella decontaminazione dei vegetali minimamente pro-

- Key words: chlorine dioxide, decontamination, electrolysed oxidising water, microbial quality, minimally processed, sensory quality -

gle tests. Gaseous ClO_2 yielded >1 log cfu/g reduction in lettuce and cabbage, but caused browning. Aqueous ClO_2 did not reduce aerobic plate count of lettuce and cabbage, but yielded >1 log reduction in carrots. Lettuce was sensorially affected by ClO_2 washings, but cabbage and carrots were not. NEW yielded >1 log reduction in lettuce, cabbage and carrots; a 5 min treatment did not damage their sensory quality. Aqueous ClO_2 was most appropriate to decontaminate carrots and NEW for lettuce, cabbage, and carrots. Gaseous ClO_2 was effective in decreasing lettuce and cabbage microbial load but significantly affected their sensory quality.

cessati (IV gamma), senza influenzarne le proprietà organolettiche. Il ClO_2 gassoso consentiva una riduzione della carica microbica a >1 log cfu/g nella lattuga e nel cavolo provocando tuttavia il loro imbrunimento. Il ClO_2 liquido non riduceva la carica microbica nella lattuga e nel cavolo ma consentiva una riduzione della carica microbica nelle carote (<1 log). Il lavaggio con ClO_2 influenzava le proprietà organolettiche della lattuga ma non quelle del cavolo e della carota. L'AEN riduceva la carica microbica a >1 log nella lattuga, nel cavolo e nella carota; dopo un trattamento di 5 minuti non sono state rilevate variazioni delle proprietà organolettiche. Il ClO_2 acquoso risultava essere più idoneo per la decontaminazione delle carote, mentre l'AEN risultava migliore per il trattamento della lattuga, del cavolo e della carota. Il ClO_2 gassoso risultava efficace per diminuire la carica batterica nella lattuga e nel cavolo ma ne influenzava le caratteristiche organolettiche in maniera significativa.

INTRODUCTION

Minimally processed vegetables (MPV) are a heterogeneous group of commodities prepared and handled by single methods to maintain their living fresh state and nutritional and sensory quality, while providing convenience to consumers and ensuring food safety (ARTÉS and ALLENDE, 2005). The combination of good manufacturing practices, chilling storage and modified atmosphere packaging is applied to maintain quality. Because microorganisms can spoil MPV, a decontamination method that could slow down microbial spoilage could be useful for extending their shelf-life as long as it does not impair their sensory quality. Fumigation or washing with ClO_2 and washing with electrolysed oxidising water are among the novel methods potentially useful to decontaminate MPV.

ClO_2 is a powerful oxidiser useful for decontaminating MPV when used in solution as well as in its gaseous stage. There have been several reports on the efficacy of gaseous ClO_2 against pathogenic bacteria inoculated onto vegetable peel (HAN *et al.*, 2000; 2001a). ClO_2 gas is more effective than the aqueous form, and uninjured surfaces are easier to decontaminate than injured ones (HAN *et al.*, 2001b). On lettuce, LEE *et al.* (2004) reported more than 5 log reductions of *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* cells with no effect on the appearance of the leaves. SY *et al.* (2005b) studied the inactiva-

tion of yeasts and moulds of the natural microbiota of several fresh and fresh-cut produce, together with the effect of ClO_2 on the sensory quality. They reported log reductions in yeasts and moulds as high as 4.16 on the skin of strawberries (SY *et al.*, 2005a), and damage on the sensory properties of MP cabbage, MP carrot and MP lettuce (SY *et al.*, 2005b). It has been recently reported that ClO_2 gas can extend the shelf-life of whole strawberries from 8 to 16 days (MAHMOUD *et al.*, 2007), and delay the physiological transformation and retain the postharvest quality of whole green bell peppers (JIN-HUA *et al.*, 2007).

The efficacy of liquid ClO_2 to decontaminate vegetables has been tested by several authors. COSTILLOW *et al.* (1984) reported that holding cucumbers in water with up to 105 ppm ClO_2 for 15 min had very little effect on the numbers of yeasts, moulds and lactic acid bacteria. On the other hand, REINA *et al.* (1995) reported a 3.5 log reduction in total aerobes and 1.3 log in total moulds using 5.1 ppm ClO_2. ZHANG and FARBER (1996) reported that treating MP lettuce and MP cabbage with 5 ppm ClO_2 for 10 min reduced *L. monocytogenes* populations by 0.8 log CFU/g in both fresh cut vegetables. Populations of the same microorganism inoculated onto bell peppers were reduced by 3.7 log CFU/5g after treatment with 3 mg/L ClO_2 (HAN *et al.*, 2001b). PAO *et al.* (2007) achieved 5 log reductions in counts of *Salmonella enterica* and *Erwinia carotovora* inoculated onto tomato surface by using 20 ppm ClO_2.

Electrolysed oxidising water is generated in an electrolytic cell from tap water or a sodium chloride solution pumped into it (NAKAJIMA *et al.*, 2004). There are three types of electrolysed water: acidic, alkaline and neutral. The first two are produced simultaneously on both sides of a membrane dividing the electrolytic cell. The latter, namely neutral electrolysed water (NEW), can be produced by different methods: by using no separating membrane to divide the products of the electrolytic cell (VENCZEL *et al.*, 1997), by mixing acidic and alkaline electrolysed waters (DEZA *et al.*, 2003; RICO *et al.*, 2008), or directly from the anodic side of the cell (GUENTZEL *et al.*, 2008). Most of the literature on decontamination with electrolysed water deals with the use of the acidic water, while the present work deals with NEW.

The efficacy of NEW to disinfect fresh-cut vegetables was reported by IZUMI (1999), who reported log reductions as high as 1.8 on trimmed spinach leaves compared to a water washed control, and no effect of NEW containing up to 50 ppm free chlorine on the colour of carrot slices, trimmed spinach leaves and cucumber slices. Other authors have reported inactivation levels up to 5 log (DEZA *et al.*, 2003; ONGENG *et al.*, 2006; GUENTZEL *et al.*, 2008).

Most of the research on decontamination of vegetables has been performed on pathogenic microorganisms, and on a limited amount of vegetable matrixes with little emphasis on sensory quality. Therefore, the goal of this research was to assess the decontaminant efficacy of gaseous and liquid ClO_2 and of NEW on the total plate count of some MPV, as well as their potential deleterious effect from the sensory point of view.

MATERIALS AND METHODS

Processing of the vegetables

Three types of vegetables were bought in a local wholesale company where produce is delivered fresh daily (Van Landschoot, Gent, Belgium). The following vegetables were stored at 7°C and processed within one day: carrot (*Daucus carota* L.), Iceberg lettuce (*Lactuca sativa* var. *capitata* L.) and white cabbage (*Brassica oleracea* var. *capitata* L.). Lettuce heads were shredded in 1-2 cm

strips with a sharp knife; carrots were peeled, then grated into 0.3x0.3x4 cm sticks, and cabbage was shredded into 1 mm thick pieces using a Compacto Kitchen Cutter (Philips, Eindhoven, the Netherlands). Vegetables destined for gas treatment were immersed in tap water for 1 min and dried for 1 min by means of a manual kitchen centrifuge (Zyliss, Bern, Switzerland). The washing protocol for vegetables destined for dipping treatments is indicated in the corresponding section.

ClO_2 gas treatment

A closed cabinet of 49 litre capacity was used (Fig. 1). One hundred grams of MPV were placed on perforated shelves in the cabinet, forming monolayers, and processed at 0.4-0.6 mg ClO_2/L air, 25±3°C and 91% relative humidity. A thermohygrometer (Digitron 2020R, Devon, UK) was used to measure relative humidity and temperature. A 1.000 mg/L solution of ClO_2 was prepared by diluting a stock solution (Vernagene, Bolton, UK). ClO_2 was separated from the solution by a stripping column at a flow rate of 100 mL solution/min. Pumped ClO_2 solution accessed the top of the stripping column, where ClO_2 going downwards was separated by a counterflowing air stream driven by fans. The column was operated up to 10 min, and then the samples were left in the cabinet for 5 min more. Experiments were run in triplicate. Washed, but non fumigated samples, were used as controls.

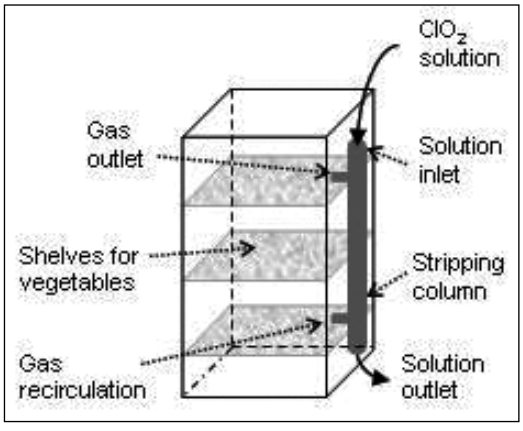

Fig. 1 - Schematic representation of the device for treating vegetables with chlorine dioxide.

Determination of ClO_2 in solution

The concentration of ClO_2 in solution was determined by an iodometric method. Five mL of ClO_2 solution was mixed with 5 mL of buffered 7% (w/v) KI (Sigma-Aldrich, Steinheim, Germany) solution pH 7, in an Erlenmeyer flask. The mixture was titrated with a 0.01N sodium thiosulfate solution (Aldrich, St. Louis, MO, USA) to a clear colourless endpoint, using soluble starch (Difco, Becton Dickinson, Meylan, FR) as indicator.

Determination of ClO_2 in air

To determine the concentration of gaseous ClO_2, samples were taken out of the cabinet by means of an air sampling pump (Gylair 3, Sensydine, UK) for up to 15 seconds. Two impingers were serially placed between the cabinet and the pump, so that the air sample was scrubbed through a buffered 7% (w/v) KI solution pH 7. The contents of the impingers were quantitatively transferred to an Erlenmeyer flask. Then, 3 mL of 6N HCl (Merck, Darmstadt, Germany) was added. The mixture was titrated with a 0.01N sodium thiosulfate solution to a clear colourless endpoint, using soluble starch as indicator.

Dipping treatments

Washing treatments of MPV were performed by immersing 10 g of sample in 200 mL of each disinfectant solution (1:20 ratio) in a sterile 400 mL Stomacher bag with gentle, continuous agitation using a shaker (OS10, Ika-Werke,

Staufen, Germany) at 120 rpm, for 1, 5, 10, and 20 min, at 26°±2°C. At the end of the contact time, the liquid was drained off, and the treated samples were immediately diluted with Peptone Saline Solution (PSS) (see total aerobic count section) and analysed for microorganisms. A control with tap water was run up for respective times. Moreover, samples of non-washed MPV were taken for comparison. Three runs for each MPV were performed.

Production of aqueous disinfectants

Aqueous ClO_2 solutions at 5, 10, or 20 mg/L were prepared by diluting a commercial solution (Vernagene, UK) with tap water. NEW was produced with a bench-top electrolyser (Ecodis 0,20.2-4A/2, Schoten, Belgium) consisting of an electrolytic cell with two anodes and one cathode, without a separating membrane. NEW with different free chlorine concentrations can be produced in this device by regulating the flow rate of incoming water or very diluted NaCl solution, the current and voltage supplied to the electrodes, and the salt concentration of the feeding solution. For this research, two kinds of NEWs were used, one with a free chlorine concentration of 4.9-5.1 mg/L (pH 7.7) produced by feeding the cell with tap water at 15 L/h and setting the voltage at 15 V and the current at 0.65 A; the other with a free chlorine concentration of 38-43 mg/L (pH 8.3) produced by feeding the cell with a 0.05% sodium chloride (VWR, Fontenay sous Bois, FR) solution at 15 L/h and setting the voltage at 15 V and the current at 1.70 A. Tap water used as control contained 0.05-0.09 mg/L free chlorine and had pH 7.3.

Free chlorine measurement

Free chlorine concentration in NEW was determined according to the DPD method by using a HI9133 Kit and corresponding photometer for free and total chlorine measurements (HANNA Instruments Inc., Woonsocket, RI, USA).

Total aerobic plate count

Microbiological counts were determined by taking 30 g of sample (the complete 10 g in the case of dipping treatments) and diluting tenfold with sterile PSS (8.5 g/L NaCl (VWR, Fontenay sous Bois, FR) and 1 g/L peptone (Oxoid, L34)) in a sterile Stomacher bag, and homogenising for 60 seconds with a Colworth Stomacher (Steward Laboratory, London, UK). Tenfold dilution series were made in PSS for plating. Plate Count Agar (Oxoid, CM325, Hampshire, UK) was used for total aerobic plate count (TPC), pour plated and incubated at 30°C for 3 days (ISO 4833:2003).

Sensory analysis

Triangle tests were conducted in order to detect differences caused by treatments, using 14 experienced panellists (BOTTA, 1995). Potential deleterious effects caused by gaseous ClO_2 were evaluated using the treatment conditions described earlier. In order to assess the effect of liquid ClO_2, MPV were treated for 5 min with 20.0 mg/L of ClO_2; for NEW, the conditions 5 min of exposure and 40 mg/L free chlorine were used. These conditions were selected because they allowed decontamination within a relatively short treatment time. Washing treatments were performed by immersing 80 g of MPV in 1.6L liquid (1:20 ratio) in a bowl shaken by an orbital shaker operated at 120 rpm, then spun dried for up to 15 seconds and stored at 7°C for 3-4 h before evaluation.

Statistical analyses

Data were analysed for differences of the effect of time and ClO_2 or free chlorine concentration by using ANOVA. Mean differences among different treatment

conditions for each MPV were analysed by using ANOVA as well; when data supported parametric examinations, Duncan's test was applied, when not, Brown-Forsythe's test for non homecedastic groups was used. Statistical analyses were carried out using SPSS 12.0 (SPSS Inc., Chicago, IL, USA), with P=0.05. Significant differences in triangle tests were established according to BOTTA (1995) with P=0.05.

RESULTS AND DISCUSSION

ClO_2 gas treatment

The ClO_2 gas treatment yielded more than 1 log reduction in the TPC of MP lettuce and MP cabbage compared to water washing (Table 1). There is no information available on the minimum logarithmic reduction that can result in shelf-life prolongation; however, a treatment capable of reducing the native microflora of a MPV by 1 log seems to be promising according to our experience.

Table 1 - Effect of gaseous ClO_2 treatment for 15 min on the total plate count (log CFU/g ± SD) of MP lettuce and MP cabbage. n=3.

MPV	Iceberg lettuce	White cabbage
[ClO_2] (mg/L)	0.62±0.08	0.41±0.03
% RH	90.6±0.2	90.8±0.7
T (°C)	27.8±0.6	22.3±0.1
Initial count	5.86±0.21	3.49±0.12
Log reduction	1.02±0.70	1.37±0.32

The method showed an important drawback, however. Subjective examination of MP cabbage and MP lettuce revealed that ClO_2 caused browning during treatment and furthermore the MP lettuce lost green colour. Results of triangle tests demonstrated that the differences between untreated and treated samples could be objectively perceived (Table 2). Similar results regarding MP cabbage and MP lettuce browning were also reported by SY *et al.* (2005b). Additionally, MP lettuce degreening was reported by SINGH *et al.* (2002), who suggested that this could be due to oxidation of chlorophyll. Since phenol oxidation by ClO_2 has been reported (NAPOLITANO *et al.*, 2005), MP cabbage and MP lettuce browning may be caused by oxidation of phenols. It is therefore advisable to test the suitability of antibrowning agents, which could minimise this deleterious effect of ClO_2.

Liquid ClO_2 treatment

Table 3 shows the results of the decontamination of three MPV by liquid ClO_2 at different concentrations and contact times with respect to the raw material. No significant differences (P>0.05) were observed for MP lettuce and MP cabbage. For MP carrots, both time and ClO_2 con-

Table 2 - Results of triangle tests on the effect of different decontamination treatments on the sensory quality of several MPV. n=14.

MPV	ClO_2 gas (0.4-0.6 mg/L air)	ClO_2 liquid (20 mg/L solution)	NEW (40 mg free Cl/L solution)
Iceberg lettuce	+[a]	+	-
White cabbage	+	-	-
Carrots	[b]	-	-

[a] Panel detected (+) or did not detect (-) statistically significant differences (P=0.05) between untreated and treated samples.
[b] Not tested.

Table 3 - Logarithmic reduction (log CFU/g ± SD) after washing several MPV with aqueous chlorine dioxide with respect to raw material*. n=3.

MPV	$[ClO_2]$ (mg/L)	Contact time (min) 1	5	10	20
Iceberg lettuce[i]	0	0.35±0.39	0.18±0.13	0.08±0.32	0.32±0.13
	5	0.13±0.04	0.58±0.13	0.23±0.24	0.57±0.19
	10	0.28±0.12	0.11±0.42	0.49±0.25	0.67±0.32
	20	0.17±0.22	0.50±0.44	0.72±0.28	0.78±0.66
White cabbage[i]	0	0.46±0.39	0.48±0.13	0.77±0.26	0.83±0.12
	5	0.83±0.14	1.08±0.18	1.14±0.28	1.01±0.09
	10	0.58±0.20	0.95±0.44	1.11±0.45	1.12±0.23
	20	0.77±0.58	1.39±0.65	1.29±0.70	1.28±0.44
Carrots	0	0.49±0.08[a]	0.53±0.23[ab]	0.72±0.28[abc]	0.77±0.06[abc]
	5	0.92±0.20[cd]	1.11±0.14[de]	1.17±0.09[de]	1.12±0.19[de]
	10	0.86±0.18[bcd]	1.13±0.19[de]	1.39±0.18[efg]	1.37±0.16[ef]
	20	1.34±0.09[e]	1.69±0.24[fgh]	1.71±0.21[gh]	1.89±0.24[h]

[a-h] Values sharing a common letter are not significantly different (P=0.05) according to Duncan's test.
[i] For each MPV, means are not significantly different (P=0.05) according to Brown-Forsythe's test.
* Raw material counts: 5.67±0.09 log CFU/g for lettuce, 4.40±0.32 log CFU/g for cabbage and 4.95±0.52 log CFU/g for carrots.

centration effects were statistically significant (P<0.05), the longer the treatment time and the higher the ClO_2 concentration, the greater the decontamination effect. The interaction of these two factors was not significant. There were no significant differences between decontamination levels achieved using ClO_2 concentrations of 5 and 10 mg/L for the respective times. Significant differences were found, however, between results obtained at those intermediate ClO_2 concentrations (5 and 10 mg/L) and those found by dipping at 0 or 20 mg/L for the respective times. When results were analysed with respect to decontamination achieved by using water washing for the respective times, at least 1 logarithmic reduction could be due to the biocide effect of the ClO_2 at concentrations of 20 mg/L and at times equal to or greater than 5 min.

A triangle test (Table 2) showed that samples of MP carrots treated with 20 mg/L of aqueous ClO_2 for up to 5 min could not be detected by the panel from samples washed with tap water for the same time (P>0.05). The same result was found for MP cabbage but not for MP lettuce.

These results show that this treatment can decrease the microbial load without damaging sensory attributes when applied to MP carrots. However, washing solutions became orange during treatment, and transfer of solids from carrot tissue to water has been reported (EL-BELGHITI and VOROBIEV, 2005). Washing shredded carrots could consequently result in important losses of nutrients due to leaching. Therefore, an evaluation of the impact of this kind of treatment on the nutritional quality of shredded carrots is strongly advised. It could also be possible to test other kind of carrot cuts, which, although not as common as grated carrots on the market, could be less prone to leaching.

As mentioned earlier, HAN *et al.* (2001b) demonstrated that gaseous ClO_2 is more efficient than the liquid form to decontaminate vegetable surfaces. The superiority of the gaseous form might indicate that it is not necessary to study the performance of the liquid form. From the practical point of view, however, implementation of a fumigation system to apply ClO_2 gas in an already working industrial facility is more complicated and expensive than merely substituting the currently-in-use disinfectant added to the wash water. In the first case, completely new devices would need to be installed to apply a gaseous disinfectant. In the second case, already installed dumping tanks could be used.

NEW treatment

The results of the treatment of three MPV with NEW with different free chlorine concentrations for different washing times are shown in Table 4. Statistically significant differences ($P<0.05$) were found for the three MPV. The effect of the free chlorine concentration was significant but that of time was not. In general, the higher the free chlorine concentration, the greater the decontamination. However, in the specific case of MP lettuce, similar reductions were reached using 4.9 and 39 ppm free chlorine. It is possible that the highest feasible decontamination of MP lettuce with this method was already reached using NEW with just 4.9 ppm free chlorine. Also IZUMI (1999) found that a treatment with NEW containing 15 ppm of free chorine significantly decreased the aerobic plate count of trimmed spinach leaves, but 30 and 50 ppm of free chlorine did not increase the decontamination significantly. Moreover, they reported no significant effect on the aerobic plate count of carrot slices and cucumber slices treated at 15 and 50 ppm free chlorine.

Table 4 - Logarithmic reduction (log CFU/g ± SD) after washing several MPV with neutral electrolysed oxidising water with respect to raw material*. n=3.

MPV	[Free chlorine] (ppm)	Contact time (min) 1	5	10	20
Iceberg lettuce	0.09[1]	0.14±0.29[a]	0.50±0.23[abc]	0.22±0.42[ab]	0.29±0.45[ab]
	4.9[2]	0.88±0.34[bc]	1.12±0.27[c]	0.57±0.18[abc]	0.92±0.25[bc]
	39[3]	0.90±0.10[bc]	0.67±0.44[abc]	1.05±0.35[c]	1.05±0.70[c]
White cabbage	0.06[1]	0.36±0.05[a]	0.51±0.15[ab]	0.41±0.10[ab]	0.43±0.17[ab]
	5.0[2]	0.41±0.13[ab]	0.55±0.08[ab]	0.43±0.30[ab]	0.54±0.19[ab]
	38[3]	0.54±0.21[ab]	0.78±0.25[bc]	0.96±0.23[cd]	1.16±0.41[d]
Carrots	0.05[1]	0.39±0.05[a]	0.47±0.08[ab]	0.56±0.27[ab]	0.57±0.33[abc]
	5.1[2]	0.30±0.14[a]	0.26±0.35a[a]	0.37±0.26[a]	0.60±0.08[abc]
	43[3]	0.71±0.13[abc]	0.94±0.47[bcd]	1.08±0.10[cd]	1.23±0.06[d]

[a-d] For each MPV, values sharing a common letter are not significantly different (P=0.05).
[1] Tap water, [2] NEW produced from tap water, [3] NEW produced from a 0.05% NaCl solution.
* Raw material count: 5.71±1.17 log CFU/g for lettuce, 4.60±0.17 log CFU/g for cabbage and 5.02±0.79 log CFU/g for carrots.

There was a markedly slower depletion of free chlorine (Fig. 2) in the NEW used to treat MP lettuce in comparison with the decline observed after 1 min of treatment of MP cabbage or MP carrots. From the point of view of an industrial application, that means that NEW can be used to decontaminate relatively large amounts of MP lettuce without losing decontamination capacity. The larger cut area of MP cabbage and MP carrots in comparison with that of MP lettuce could explain this difference. Accordingly, OOMORI *et al.* (2000) reported that fine strips of chopped cabbage consumed 50 ppm free chlorine of acidic electrolysed water within 10 min, while a level above 40 ppm was maintained when the same experiment was carried out using larger strips.

The decontamination levels found in the present work agree with those reported by IZUMI (1999), although there were differences in the experimental set-ups. IZUMI (1999) rinsed five types of MPV with tap water (control) or NEW containing 20 ppm free chlorine for four min and reported between 0.2 and 0.4 log reduction except for spinach, whose reduction was 1.8. These results are similar to those obtained in this work (0.2-0.8), when dipping the MPV for 1 min in NEW containing 38-43 ppm free chlorine.

Although in the present work more that 1 logarithmic reduction was attained at a free chlorine concentration of 38-43 mg/L, part of this decontamination can be due to a simple water washing. When compared to water washing, the highest logarithmic reductions were between 0.7 and 0.8 for the different MPV, still good enough taking into consideration the simplicity of the production of the NEW.

The results of the sensory evaluation (Table 2) show that the panel was unable to detect differences between water washed MPV and samples washed with NEW with 40 mg/L of free chlorine ($P>0.05$), for 5 min, indicating that NEW does not impair the sensory quality of these foods. A strong off-odour was detected immediately after washing MP cabbage with NEW, but disappeared before starting the sensory evaluation, which took place 3-4 h after treatment. This time interval is short enough for an industrial application.

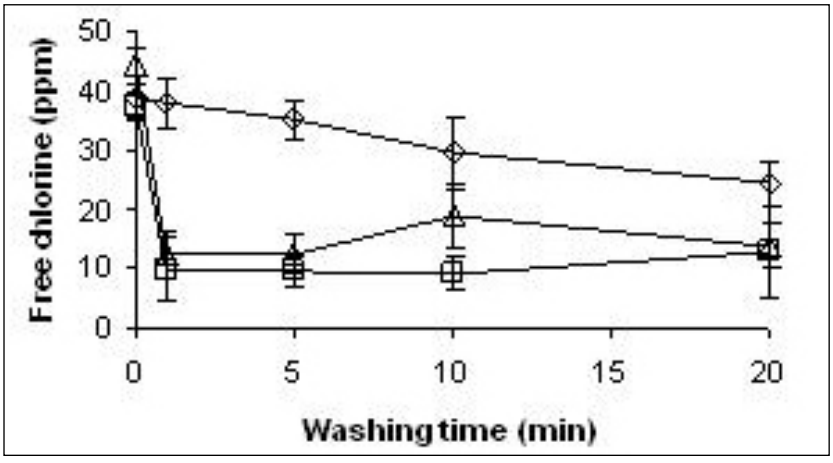

Fig. 2 - Concentration of free chlorine after treating several minimally processed vegetables with neutral electrolysed oxidising water originally containing 38-43 ppm of free chlorine concentration. Ratio MPV:water 1:20. Iceberg lettuce (◇), carrots (△), white cabbage (□).

In general, it seems that the simple production of NEW from tap water could be enough to decontaminate MP lettuce but not to decontaminate MP cabbage and MP carrots. Since electrolysis of sodium chloride solutions produce NEW with higher free chlorine concentrations than tap water electrolysis, it would therefore be necessary to add minimal amounts of sodium chloride to the water inlet of the electrolytic cell in order to achieve good levels of decontamination for MP cabbage and MP carrots.

In the three methods tested, the highest decontamination tended to be in shredded carrots. It has been proven that biofilms (NORWOOD and GILMOUR, 2000), attachment (LIAO and SAPERS, 2000) or internalisation (TAKEUCHI and FRANK, 2000) protect microorganisms from the action of sanitizers, all these processes require time. The microbial flora of MPV may reflect that of the vegetable in the growing field as well as contamination during processing (GARG *et al.*, 1990). Field contamination is reduced by the peeling step that is given to carrots (GARG *et al.*, 1990). Therefore, a substantial amount of microflora of MP carrots would likely come from processing and would consist in microorganisms that have not had time enough to attach or to form biofilms. Therefore, MP carrots should be easier to decontaminate. Moreover, microorganisms that could have been internalised into the carrot will get exposed by shredding.

In conclusion, fumigation with ClO_2 might be suitable to be tested to prolong the shelf-life of Iceberg lettuce and white cabbage if a suitable antibrowning agent is found. Liquid ClO_2 could be used for carrots and NEW for Iceberg lettuce, white cabbage and carrots. Since a reduction in initial counts does not necessarily lead to prolongation of the shelf-life of MPV, it is necessary to perform storage studies to test if these methods can indeed prolong the shelf-life of MPV.

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PAPER

TRADITIONAL ITALIAN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) CULTIVARS AND THEIR COMMERCIAL HOMOLOGUES: DIFFERENCES IN VOLATILE COMPOSITION

CULTIVAR ITALIANE DI POMODORO TRADIZIONALI E RISPETTIVI OMOLOGHI COMMERCIALI: DIFFERENZE NELLA COMPOSIZIONE DELLA FRAZIONE VOLATILE

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ABSTRACT

The volatile composition of four local varieties of tomato, originating from southern Italy (San Marzano, Vesuviano, Corbarino, Sorrento), was compared to that of their respective commercial homologues (Ranco F1, Principe Borghese, Faino F1, Cuore di Bue). Volatiles were sampled using Dynamic Headspace-Solid Phase Microextraction (DHS-SPME). Fifty-one volatile compounds comprising aldehydes, ketones, alcohols, esters, acids, volatile phenols, sulphur compounds, oxygen-

RIASSUNTO

La composizione della frazione volatile di quattro varietà tradizionali di pomodoro, originarie del Sud Italia (San Marzano, Vesuviano, Corbarino, Sorrento) è stata confrontata con quella dei rispettivi omologhi commerciali (Ranco F1, Principe Borghese, Faino F1, Cuore di Bue). La frazione volatile dei campioni è stata isolata mediante la tecnica DHS-SPME (Dynamic Headspace-Solid Phase Microextraction). Sono stati identificati cinquantuno componenti volatili appartenenti alle seguenti classi chi-

- Key words: commercial homologues, solid phase microextraction, tomato (*Lycopersicon esculentum* Mill.), traditional cultivars, volatile compounds -

containing cyclic compounds and an unsaturated hydrocarbon, were identified. Some of them have not previously been reported as being volatile compounds of tomato. Significant differences in the composition of the volatile fraction were detected between the homologues. The compound 2-hydroxybenzaldehyde was only detected in the four traditional tomato cultivars, and the sulphur compound 2-isobutylthiazole was specifically related to Faino F1. According to PCA analysis the homologues Corbarino and Faino F1 differed the most from each other.

miche: aldeidi, chetoni, alcoli, esteri, acidi, fenoli volatili, composti solforati, eterocicli ossigenati ed un idrocarburo insaturo. Alcuni di essi non sono stati precedentemente riportati come componenti della frazione volatile del pomodoro. Sono state rilevate differenze significative tra gli omologhi. In particolare la 2-idrossibenzaldeide è stata identificata esclusivamente nelle quattro cultivar tradizionali di pomodoro, mentre il 2-isobutiltiazolo è risultato essere specificatamente correlato al Faino F1. In accordo con la PCA, gli omologhi Corbarino e Faino F1 sono risultati essere quelli che maggiormente differiscono tra loro.

INTRODUCTION

Tomatoes (*Lycopersicon esculentum* Mill.) are an important dietary component in many parts of the world and are widely cultivated throughout the world. In addition to their external appearance, flavour, comprising aroma and taste, is one of the principal criteria for evaluating tomatoes. Tomato breeding often emphasizes yield, fruit size, lack of defects and resistance to diseases, while organoleptic quality is not always taken into account. This has resulted in the selection of cultivars which are highly productive and adapted to modern agricultural practices, but often with poor flavour.

Consumers have complained of the lack of characteristic taste and flavour of fresh tomatoes and they were willing to pay a higher price for tomatoes with better flavour and aroma (BRUHN *et al.*, 1991; AUERSWALD *et al.*, 1999). Although these surveys were not conducted with Italian consumers, these results can be reasonably applied to the Italian tomato market. Numerous local varieties of tomato originating in southern Italy (Campania region) and traditionally grown in this area have organoleptic attributes that are greatly appreciated by consumers and are considered to be typical Italian products. These local varieties are not readily available on the market because they have been replaced by selected commercial varieties that are morphologically similar to the traditional varieties and have the same market destination. The local varieties, produced in smaller quantities, are usually sold at a higher price than the selected commercial ones and can be considered as a niche market product. In the last few years, there has been some commercial protection of these traditional tomato cultivars.

In order to better understand how traditional cultivars differ from commercial ones, characterization of the volatile fraction is necessary because volatile compounds, along with non-volatile ones such as sugars and acids, contribute most to tomato flavour (BALDWIN *et al.*, 1998). Unlike some fruit or vegeta-

bles that have only one or a few odour-impact compounds, no single compound has been found that is responsible for a ripe tomato odour (RUIZ *et al.*, 2005). Tomato aroma is due to a complex mixture of volatile compounds and the levels of some of these volatiles change continuously as endogenous enzymes and substrates are mixed together in the homogenised tissue. The dynamic nature of the volatile fraction of tomato, together with the large number of volatiles, makes quantitative analysis very difficult (BUTTERY *et al.*, 1987).

More than 400 volatile compounds have been identified in tomato and tomato products (PETRÓ-TURZA, 1987), but several authors have found that among them, only a limited number of compounds play an outstanding role in tomato flavour (BUTTERY *et al.*, 1987; LANGLOIS *et al.*, 1996; RUIZ *et al.*, 2005; KRUMBEIN and AUERSWALD, 1998; TANDON *et al.*, 2000; STERN *et al.*, 1994). Tomato flavour is mostly formed during ripening and, as for all fruit and vegetables, it is influenced by several internal and external factors. Some internal factors are based on plant characteristics such as metabolism, maturity and genetic makeup, while the external factors are related to fruit growth and cultivation conditions such as climate, soil, fertilisation and storage (HEATH and REINECCIUS, 1986). Several studies on different tomato cultivars have shown significant differences in volatile flavour compounds among the cultivars (BALDWIN *et al.*, 1991a; LANGLOIS *et al.*, 1996) which confirms that flavour formation in tomato is under genetic control. In a recent study, significant differences were found among traditional and hybrid cultivars of Spanish tomato (CARBONELL-BARRACHINA *et al.*, 2006).

The aim of the present study was to compare the volatile composition of four traditional Italian tomato cultivars, originating from the Campania region (San Marzano, Vesuviano, Corbarino, Sorrento) to that of four selected commercial varieties that can be considered as their commercial homologues (Ranco F1, Principe Borghese, Faino F1, Cuore di Bue, respectively). Although the volatile tomato fraction has been widely studied and differences in the volatile composition of different tomato cultivars have been shown, no studies are available on the volatile composition of these traditional Italian tomato cultivars. Moreover, considering the efforts being made to protect the traditional Italian tomato varieties, it would be useful to be able to differentiate them from their commercial homologues on the basis of the volatile composition.

MATERIALS AND METHODS

Tomato samples

Four traditional cultivars originating from southern Italy (Campania region) (San Marzano, Corbarino, Vesuviano, Sorrento) along with their corresponding commercial homologues (Ranco F1, Faino F1, Principe Borghese, Cuore di Bue respectively) were analysed (Fig. 1). For the common consumer the commercial varieties chosen are almost indistinguishable from the corresponding traditional varieties. In 2001 tomatoes were grown in open fields at the Research Institute for Industrial Crops (ISCI), Battipaglia (SA), Italy, using a randomized block design with three replications. Tomatoes were harvested at the same stage of maturity. After harvesting, the fruit was dusted with clean tissues and stored at -20°C until analysed.

Sample preparation and SPME analysis

Three tomatoes (or six in the case of cherry tomatoes: Corbarino, Faino F1, Vesuviano and Principe Borghese) from each agronomic replicate, were homog-

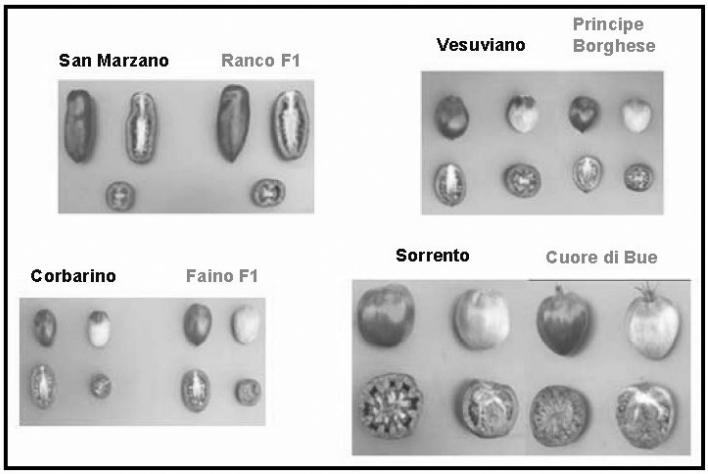

Fig. 1 - Traditional tomato cultivars (**black name**) and the corresponding commercial homologues (**grey name**) analysed (from TERMOLINO, 2006).

enised with liquid N_2 in order to avoid thawing of the tomato powder. Fifty g of frozen tomato powder was put in a 100 mL hermetically-sealed glass bottle. The samples were thawed in a thermostatic bath at 30°C for 10 min. After complete thawing, the tomato juice was stirred for 15 min at 30°C to allow the endogenous enzymes to produce volatile compounds. After this time, 50 mL of a saturated solution of $(NH_4)_2SO_4$ were added to inhibit endogenous enzymes in order to minimize further changes in volatile composition due to enzymatic activity. One hundred μL of a water solution of 2-octanone (5 mg/L) were then added as internal standard, the samples were then magnetically stirred in the closed bottle for 15 min at 30°C in order to equilibrate the headspace. The SPME holder for manual sampling and the fibres used in this study were purchased from Supelco (Bellefonte, PA). The SPME fibre used (85 μm, Carboxen/Polydimethylsiloxane, CAR/PDMS) was previously compared to other fibres tested on tomato (70 μm, Carbowax/Divinylbenzene, CW/DVB; 65 μm, Polydimethylsiloxane/Divinylbenzene, PDMS/DVB). It was selected because it performed best in our experiments, isolating the largest number of volatile compounds and had the lowest coefficients of variation % in three replicate extractions of the same tomato sample (standard deviation/mean x 100, data not shown). To collect the volatiles, the fibre was inserted through the cap septum into the sample headspace for 30 min at 30°C. During the fibre sampling, the samples were vigorously stirred so that the sample surface and headspace were also stirred. All of the fibres had been conditioned according to the indications given by Supelco: CAR/PDMS fibre was conditioned at 300°C for 2 h, CW/DVB fibre at 220°C for 30 min and PDMS/DVB fibre at 250°C for 30 min. To check possible carry-over effects, a blank test was performed before each analysis; the same chromatographic conditions of the sample analyses were used but with a desorption time of five min. If there were extraneous peaks in the chromatogram, the blank was repeated. If, after two blanks, the extraneous peaks persisted, the fibres were cleaned by thermal desorption. The volatile compounds were desorbed by inserting the fibre into the GC injector set at 250°C for 10 min. Three agronomic repetitions were analysed for each cultivar.

GC/FID analysis

A 4890D series gas chromatograph (Agilent Technologies, Avondale, PA, USA) provided with a DB-Wax fused-silica cap-

illary column (30 m, 0.32 mm i.d., 0.5 μm film thickness) (J&W Scientific, Folsom, CA, USA) was used. Injector port (splitless) and detector (FID) temperature was set at 250°C. The oven temperature was held at 35°C for 8 min, increased to 180°C at a rate of 2°C/min, then increased at a rate of 4°C/min up to 210°C where it was held for 15 min. Helium was used as carrier gas with a flow of 37 cm/s. Peak areas were calculated with an HP 3395 integrator (Hewlett-Packard, Palo Alto, CA, USA). Concentrations of the isolated compounds are expressed as ratios of the response of each compound against the response of the internal standard (2-octanone) according to the equation $C_{analyte} = (E_{analyte}/E_{2\text{-octanone}}) \times 1.016$, where $C_{analyte}$ is the concentration of the target compound, $E_{analyte}$ and $E_{2\text{-octanone}}$ are the peak areas of the target compound and the internal standard, respectively, and 1.016 is the correction to express concentrations as μg of internal standard per 100 g of frozen tomato powder.

GC/MS analysis

The volatile compounds were identified by using a 5973 Network quadrupole mass spectrometer (Agilent Technologies, Avondale, PA, USA), coupled with a 6890 series gas chromatograph (Agilent Technologies, Avondale, PA, USA) provided with a DB-Wax silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA 95630, USA). Electron impact mass spectra were recorded with ion-source energy of 70 eV. Chromatographic conditions were the same as for the GC/FID analysis. The identification of the volatile compounds was carried out by comparing retention times and mass spectra with those of pure chemical standards. Moreover, mass spectral data were compared with those of Wiley and NIST-98 libraries. Authentic reference chemical compounds were obtained from Aldrich (Steinheim, Germany).

Statistical analysis

For each pair of homologues the quantitative data were submitted to the Fisher's least significant difference test in order to evaluate significant differences between means. Quantitative data relative to those compounds that could be quantified in all samples were submitted to Principal Component Analysis (PCA) (Statgraphics 5.1).

RESULTS AND DISCUSSION

Fifty-one volatile compounds were identified in the volatile fraction of the tomato samples (Table 1) using Dynamic Headspace-Solid Phase Microextraction (DHS-SPME). Since the tomato matrix reduced the volatility of some volatile compounds in tomato (BEZMAN *et al.*, 2003), a headspace method was used to collect the volatiles, in order to better evaluate the volatile compounds that can reach the olfactory receptors. Since earlier studies on the composition of the volatile fraction of tomato, large quantitative differences in the volatile compounds have been found in individual tomatoes within the same variety (KAZENIAC and HALL, 1970). To minimize the effect of this source of variability, large samples consisting of three tomatoes for San Marzano, Ranco F1, Sorrento and Cuore di Bue or six tomatoes for Corbarino, Faino F1, Vesuviano and Principe Borghese from each agronomic replicate were homogenised. The appropriate 50 g aliquots were taken from these large samples.

The disruption of plant tissues causes major changes in the volatile composition, as enzymes, substrates and air are mixed together. Alcohols and aldehydes with 6 carbons derived from the oxidation of linolenic and linoleic acids are produced and undergo reduction and isomerisation reactions (SIESO *et al.*, 1976; STONE *et al.*, 1975; GALLIARD *et al.*, 1977). In order to standardize the

Table 1 - Volatile compounds detected in the tomato cultivars.

Peak no.	Compound °	Concentration μg I.S./100 g§			
		San Marzano	Ranco F1	Corbarino	Faino F1
	Aldehydes				
3	**hexanal** (a,b,c,d,e,f)	15.87±3.232	9.77±3.082	16.55±2.012	9.02±0.348*
4	**(E)-2-pentenal** (d,e)	3.82±0.140	2.39±0.500*	3.50±0.771	1.74±0.290
7	heptanal	0.34±0.002	0.57±0.177	0.42±0.103	0.23±0.078
8	**(E)-2-hexenal** (a,b,d,e)	0.75±0.052	0.59±0.009*	1.04±0.092	0.68±0.092*
14	**(E)-2-heptenal** (d)	3.26±1.014	1.84±0.342	1.44±0.338	1.47±0.310
19+20+21	**(Z)-3-hexen-1-ol** (a,b,d)+ (E,E)-2,4-hexadienal+ NI	0.47±0.087	0.49±0.042	0.73±0.239	0.49±0.118
24	(E)-2-octenal	2.54±1.020	2.37±0.434	1.78±0.662	1.90±0.533
29	(E,E)-2,4-heptadienal	0.24±0.060	0.17±0.042	0.30±0.082	0.06±0.033*
31	**benzaldehyde** (d)	0.21±0.011	0.15±0.043	0.17±0.032	0.11±0.028
35	(E,E)-2,4-octadienal	0.22±0.040	0.21±0.052	0.14±0.026	0.20±0.057
37	**β-cylocitral** (d)	0.11±0.013	0.08±0.018	0.14±0.032	0.07±0.017
38	2-hydroxybenzaldehyde	0.07±0.004	nd	tr	nd
40	**(Z)-citral (neral)** (d)	0.02±0.003	0.03±0.011	0.06±0.012	0.03±0.004
41	**(E)-citral (geranial)** (d)	0.10±0.006	0.09±0.020	0.22±0.059	0.05±0.001*
44	**(E,E)-2,4-decadienal** (g)	0.08±0.016	0.07±0.010	0.07±0.017	0.04±0.014
	Total aldehydes	28.08±0.915	18.79±0.849	26.55±0.615	16.09±0.210
	Ketones				
1	**1-penten-3-one** (b,d,f,e)	18.83±1.902	10.84±3.288	12.83±3.034	5.84±1.094
13	**1-octen-3-one** (f)	0.34±0.012	0.60±0.163	0.19±0.004	ne
16	**6-methyl-5-hepten-2-one** (b,c,d)	36.24±3.978	25.07±4.765	55.35±13.123	27.36±2.065
36	6-methyl-3,5-heptadien-2-one	0.21±0.015	0.15±0.012*	0.40±0.088	0.18±0.000*
46	**geranylacetone** (d)	0.06±0.001	0.07±0.013	0.04±0.006	0.03±0.001
50	**β-ionone** (b,d,g)	0.03±0.001	0.02±0.003*	0.02±0.001	tr
	Total ketones	55.70±1.800	36.75±2.365	68.81±5.499	33.40±1.168
	Alcohols				
5	1-butanol	0.46±0.155	ne	nd	nd
6	**1-penten-3-ol** (d)	0.81±0.157	0.34±0.076*	0.50±0.092	0.29±0.006
9	**2+3-methyl-1-butanol** (b,e)	0.99±0.052	0.40±0.125	2.78±0.163	1.24±0.042*
12	**1-pentanol** (d)	3.31±0.670	1.72±0.431*	2.01±0.418	1.57±0.019
15	(Z)-2-penten-1-ol	nd	nd	nd	nd
17	**1-hexanol** (a,d)	0.25±0.061	0.08±0.021	0.36±0.021	0.23±0.047*
28	**6-methyl-5-hepten-2-ol** (d)	0.45±0.225	0.20±0.013	0.36±0.106	0.57±0.057
30	2-ethyl-1-hexanol	nd	nd	nd	nd
48	benzyl alcohol	0.05±0.001	0.04±0.006*	0.06±0.012	0.06±0.019
49	**2-phenylethyl alcohol** (e,d,c)	0.11±0.030	0.14±0.038	0.10±0.043	0.05±0.001
	Total alcohols	6.44±0.264	2.93±0.173	6.16±0.179	4.00±0.034
	Esters				
2	ethyl 3-methylbutyrate	0.31±0.011	ne	0.77±0.101	0.32±0.022**
11	ethyl hexanoate	nd	ne	ne	nd
18	methyl octanoate	nd	nd	nd	nd
25	ethyl octanoate	nd	nd	nd	nd
42	**methyl salicylate** (d)	3.84±1.271	3.18±0.599	1.61±0.420	1.66±0.671
43	ethyl salicylate	3.08±1.530	2.57±1.266	1.21±0.138	0.60±0.116*
	Total esters	7.23±1.148	5.75±0.990	3.58±0.262	2.58±0.393

(continued Table 1)

Peak no.	Compound °	Concentration μg I.S./100 g§			
		San Marzano	Ranco F1	Corbarino	Faino F1
	Acids				
26	acetic acid	0.05±0.007	0.03±0.004	0.06±0.011	0.11±0.032
39	isovaleric acid	nd	ne	tr	nd
45	hexanoic acid	0.02±0.006	0.03±0.008	0.06±0.020	0.03±0.011
	Total acids	0.07±0.007	0.05±0.006	0.11±0.016	0.14±0.024
	Phenols				
47	guaiacol	1.11±0.208	0.70±0.123	0.40±0.192	0.28±0.066
51	phenol	0.19±0.025	0.16±0.038	0.16±0.030	0.15±0.025
	Total phenols	1.31±0.148	0.87±0.091	0.56±0.137	0.44±0.050
	Sulphur compounds				
22	2-isobutylthiazole	1.16±0.609	3.44±0.738	6.68±1.213	18.8±3.685*
32	3-methylisothiazole	nd	0.05±0.004	0.04±0.009	0.05±0.003
34	dimethylsulfoxide	nd	nd	0.07±0.059	nd
	Total sulphur compounds	1.16±0.609	3.49±0.522	6.80±0.701	18.87±2.605
	Oxygen-containing compounds				
10	2-pentylfuran	0.37±0.037	0.36±0.054	0.31±0.080	0.38±0.056
27	furfural	nd	nd	nd	0.18±0.061
33	5-methylfurfural	nd	0.06±0.005	nd	0.07±0.026
	Total oxygen-containing compounds	0.37±0.037	0.42±0.039	0.31±0.080	0.63±0.050
	Unsaturated hydrocarbons				
23	3-ethyl-2-methyl-1,3-hexadiene[t]	nd	nd	nd	nd
	Aldehydes				
3	**hexanal** (a,b,c,d,e,f)	10.01±2.414	17.03±1.570*	6.58±1.907	12.27±3.250
4	**(E)-2-pentenal** (d,e)	2.71±0.378	4.97±0.819*	4.61±0.051	4.17±1.135
7	heptanal	ne	0.52±0.070	0.13±0.069	ne
8	**(E)-2-hexenal** (a,b,d,e)	0.76±0.214	1.39±0.199*	1.11±0.285	1.42±0.598
14	**(E)-2-heptenal** (d)	1.10±0.271	1.22 ±0.434	2.63±0.088	1.98±0.131**
19+20+21	**(Z)-3-hexen-1-ol** (a,b,d)+ (E,E)-2,4-hexadienal+ NI	0.37±0.130	0.49±0.111	0.39±0.147	0.38±0.038
24	(E)-2-octenal	1.08±0.211	1.45±0.262	0.75±0.037	1.58±0.258*
29	(E,E)-2,4-heptadienal	0.22±0.039	0.30±0.094	0.11±0.026	0.35±0.087*
31	**benzaldehyde** (d)	0.14±0.020	0.22±0.057	0.11±0.032	0.27±0.069
35	(E,E)-2,4-octadienal	0.12±0.029	tr	0.03±0.012	0.20±0.058*
37	**β-cyclocitral** (d)	0.08±0.016	0.09±0.016	0.08±0.029	0.10±0.023
38	2-hydroxybenzaldehyde	0.05±0.010	nd	tr	nd
40	**(Z)-citral (neral)** (d)	0.03±0.002	tr	0.03±0.001	nd
41	**(E)-citral (geranial)** (d)	0.09±0.026	0.05±0.004	0.10±0.001	tr
44	**(E,E)-2,4-decadienal** (g)	0.03±0.004	nd	0.06±0.018	ne
	Total aldehydes	16.81±0.663	27.71±0.561	16.70±0.518	22.73±1.110
	Ketones				
1	**1-penten-3-one** (b,d,f,e)	13.56±2.438	20.60±3.946	23.37±0.702	19.74±4.765
13	**1-octen-3-one** (f)	ne	0.61±0.065	0.46±0.019	1.08±0.218**
16	**6-methyl-5-hepten-2-one** (b,c,d)	27.37±7.085	51.48±13.50	25.18±4.676	20.82±6.128
36	6-methyl-3,5-heptadien-2-one	0.23±0.045	tr	0.14±0.044	0.11±0.029
46	**geranylacetone** (d)	0.05±0.010	nd	0.01±0.006	0.03±0.008
50	**β-ionone** (b,d,g)	nd	nd	nd	nd
	Total ketones	41.22±3.746	72.69±8.121	49.16 ±2.115	41.79±3.473

(continued Table 1)

Peak no.	Compound °	Concentration μg I.S./100 g§			
		San Marzano	Ranco F1	Corbarino	Faino F1
	Alcohols				
5	1-butanol	nd	0.66±0.137	nd	ne
6	**1-penten-3-ol** (d)	0.49±0.029	0.96±0.085**	0.52±0.046	1.42±0.350
9	**2+3-methyl-1-butanol** (b,e)	2.88±0.816	2.25±0.177	3.06±0.647	2.58±0.633
12	**1-pentanol** (d)	1.63±0.146	2.87±0.411*	1.47±0.354	3.29±0.830
15	(Z)-2-penten-1-ol	nd	nd	nd	0.68±0.111
17	**1-hexanol** (a,d)	0.33±0.110	0.36±0.126	0.12±0.034	0.31±0.057
28	**6-methyl-5-hepten-2-ol** (d)	0.24±0.017	0.22±0.045	0.27±0.040	0.36±0.001
30	2-ethyl-1-hexanol	nd	nd	0.18±0.048	0.20±0.046
48	benzyl alcohol	0.03±0.010	0.04±0.006	0.05±0.018	0.05±0.009
49	**2-phenylethyl alcohol** (e,d,c)	tr	0.08±0.000	0.18±0.042	0.05±0.011*
	Total alcohols	5.61±0.342	7.44±0.175	5.85±0.263	8.95±0.370
	Esters				
2	ethyl 3-methylbutyrate	0.52±0.112	0.87±0.002*	1.11±0.532	1.26±0.183
11	ethyl hexanoate	ne	nd	nd	nd
18	methyl octanoate	nd	nd	nd	0.09±0.015
25	ethyl octanoate	nd	nd	nd	0.21±0.044
42	**methyl salicylate** (d)	0.66±0.062	4.39±0.573*	2.83±0.347	2.40±1.337
43	ethyl salicylate	0.80±0.244	2.49±0.028*	0.42±0.122	1.66±0.692
	Total esters	1.98±0.159	7.75±0.331	4.36±0.373	5.62±0.678
	Acids				
26	acetic acid	0.13±0.021	0.15±0.039	0.12±0.046	0.12±0.009
39	isovaleric acid	tr	0.03±0.008	0.03±0.008	0.02±0.006
45	hexanoic acid	0.02±0.007	0.04±0.013	0.02±0.003	0.04±0.009*
	Total acids	0.16±0.015	0.23±0.024	0.17±0.027	0.18±0.008
	Phenols				
47	guaiacol	0.21±0.051	1.01±0.119**	0.60±0.170	0.98±0.018
51	phenol	0.13±0.015	0.19±0.009*	0.13±0.024	0.24±0.019**
	Total phenols	0.34±0.038	1.20±0.084	0.73±0.121	1.22±0.019
	Sulphur compounds				
22	2-isobutylthiazole	7.02±2.234	8.02±1.548	2.08±0.864	1.96±0.719
32	3-methylisothiazole	0.05±0.004	nd	ne	0.04±0.010
34	dimethylsulfoxide	nd	nd	nd	nd
	Total sulphur compounds	7.07±1.580	8.02±1.548	2.08±0.864	1.99±0.508
	Oxygen-containing compounds				
10	2-pentylfuran	0.10±0.043	0.26±0.027*	0.19±0.074	0.60±0.117*
27	furfural	ne	0.14±0.013	nd	nd
33	5-methylfurfural	0.06±0.015	0.11±0.031	nd	0.07±0.019
	Total oxygen-containing compounds	0.16±0.032	0.50±0.025	0.19±0.074	0.67±0.084
	Unsaturated hydrocarbons				
23	3-ethyl-2-methyl-1,3-hexadiene[t]	nd	nd	nd	0.31±0.063

° Bold type indicates characteristic impact flavour or aroma compounds in tomato, as reported in the literature in brackets. References: a) RUIZ *et al.*, 2005; b) BUTTERY *et al.*, 1987; c) LANGLOIS *et al.*, 1996; d) STERN *et al.*, 1994; e) TANDON *et al.*, 2000; f) KRUMBEIN and AUERSWALD, 1998; g) BUTTERY *et al.*, 1971.

§ Means of triplicate samples; * Single asterisk indicates significant difference between the homologues at $p<0.05$; ** Double asterisks indicate significant difference between the homologues at $p<0.01$; ne=not evaluated. nd=not detected. tr=traces. [t]=compound tentatively identified by the comparison of mass spectral data with those of Wiley and NIST-98 libraries.

time of enzymatic action and improve the reproducibility of the analysis, some precautions had to be taken during sample preparation. The tomatoes were homogenised in the presence of liquid nitrogen in order to avoid thawing of the sample during homogenisation and transferring of the sample to the bottle. After 25 min at 30°C, the enzymes were deactivated by adding a saturated solution of $(NH_4)_2SO_4$ (Saliba-Colombani *et al.*, 2001). In this way, the time during which endogenous enzymes could act was the same for all the samples.

The volatiles identified belonged to the following chemical classes: aldehydes (15 compounds), ketones (6 compounds), alcohols (12 compounds), esters (6 compounds), acids (3 compounds), phenols (2 compounds), sulphur compounds (3 compounds), oxygen-containing compounds (3 compounds) and one unsaturated hydrocarbon. Some volatile compounds were identified that, to the best of our knowledge, have not been previously mentioned as volatile compounds of tomato: (E,E)-2,4-octadienal, 2-ethyl hexanol, ethyl 3-methylbutyrate, ethyl octanoate and 3-methylisothiazole. The qualifier ions considered for the identification of these compounds were: m/z 81-82-95-124 for (E,E)-2,4-octadienal, m/z 57-69-70-83 for 2-ethyl hexanol, m/z 57-85-88-102 for ethyl 3-methylbutyrate, m/z 88-101-127 for ethyl octanoate and m/z 72-73-98-99 for 3-methylisothiazole. The relative abundances of these qualifier ions corresponded to those of the reference chemical standards. Moreover, the analytes eluted at the same retention time as the pure chemical standards (43.72 min, (E,E)-2,4-octadienal; 38.69 min 2-ethyl hexanol; 10.06 min, ethyl 3-methylbutyrate; 34.61 min, ethyl octanoate and 39.80 min, 3-methylisothiazole). The percent identity of mass-spectra to the NIST-98 and Wiley libraries was greater than 83% for all the compounds. Of the volatile compounds identified, 23 were previously reported by several authors as odour-active compounds for tomato (Table 1). In spite of morphological similarity, there were significant qualitative and quantitative differences in the composition of the volatile fraction for all of the pairs of homologues analysed.

San Marzano and Ranco F1 tomatoes are long and cylindrical in shape but the average weight of Ranco F1 is greater than San Marzano (45-60 g) (Fig. 1). The

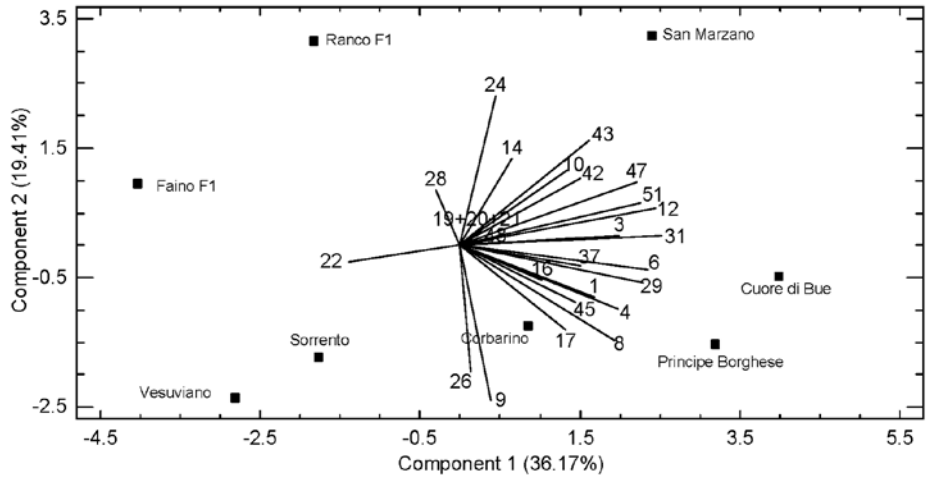

Fig. 2 - Principal Component Analysis (PCA) of quantitative data relative to volatile compounds detected in the different tomato cultivars analysed (the compound numbers refer to Table 1).

latter probably derived from very old local varieties and was selected by breeders over time by the hybridisation or selection of spontaneous mutations within the ancient varieties ("Fiaschella", "Fiascona", "Lampadina"). In 1996 San Marzano obtained the PDO (Protected Designation of Origin) status with the denomination of "Pomodoro San Marzano dell'Agro Sarnese-Nocerino". Although Ranco F1 is grown in the same areas as San Marzano, it cannot be labelled with the PDO.

Even though the number of identified compounds was about the same, San Marzano had about a 1.5 times more concentrated volatile fraction than that of its commercial homologue. San Marzano had higher total amounts of all the chemical classes than Ranco F1 except sulphur compounds and oxygen-containing compounds. The most abundant chemical class in both varieties was ketones followed by aldehydes. This was true for all the tomato varieties analysed, except Faino F1, in which the amount of sulphur compounds was higher than that of aldehydes. Twelve volatile compounds differed significantly between San Marzano and Ranco F1. Among the aldehydes, (E)-2-pentenal and (E)-2-hexenal were detected in higher amounts in San Marzano. Several authors have reported that (E)-2-hexenal is a key compound in tomato flavour; it belongs to the six-carbon (C6) volatile compounds formed upon breakdown of the fruit (GALLIARD *et al.*, 1977). During tomato maceration, linoleic acid and α–linolenic acid that are derived from glycerolipid are converted into hexanal and (Z)-3-hexenal, respectively. The latter can be isomerised *in situ* to the much more stable (E)-2-hexenal isomer. These aldehydes and the corresponding alcohols that are formed from the aldehydes due to the action of alcohol dehydrogenase (ADH), generally coexist. Considering that the average weight of Ranco F1 is greater than San Marzano, our results seem to agree with those reported by GRAY *et al.* (1999), who found that the concentration of both hexenal isomers, (Z)-3-hexenal and (E)-2-hexenal, in the headspace of 16 tomato varieties, increased as the average weight decreased. In the present study neither (Z)-3-hexenal, nor the corresponding alcohol (Z)-3-hexenol, were detected. Since (Z)-3-hexenal is an unstable compound, it is possible that the amount of (Z)-3-hexenal after its isomerisation to (E)-2-hexenal was undetectable under our conditions. In previous studies (Z)-3-hexenal was not detected (CHUNG *et al.*, 1983; DIRINCK *et al.*, 1976) and the authors supposed that (Z)-3-hexenal was probably isomerised to (E)-2-hexenal during sample preparation. However, preliminary analysis of fresh tomatoes using the same method as in this study allowed both (Z)-3-hexenal and (Z)-3-hexenol to be identified and quantified. This evidence suggests that the isomerization took place principally during storage at -20°C and not during sample preparation. Unfortunately, the number of samples, the length of the analysis and the perishability of tomatoes did not allow the analysis of fresh tomatoes, so frozen storage was necessary. This choice seemed reasonable considering the results of BALDWIN *et al.* (1991b) in which the levels of volatile compounds in tomatoes remained constant for up to 3 months of frozen storage at -20°C. Storage seems to be a critical point in the study of fresh vegetables and further study on how sample storage affects volatile composition would be very useful.

The compound 2-hydroxybenzaldehyde was only detected in the local San Marzano variety and not in Ranco F1. The same was true for the other three pairs of homologues in which 2-hydroxybenzaldehyde was only detected in the local Corbarino, Vesuviano and Sorrento varieties but not in the corresponding commercial varieties. It was demonstrated that 2-hydroxybenzaldehyde, also called salicylal-

dehyde, is a plant repellent volatile compound. It is active against *Frankliniella occidentalis* (Pergande), a thrip that causes serious damage to tomato plants, by directly damaging the leaves and fruit, and by transmitting the tomato spotted wilt virus (TSWV) (GHIDIU *et al.*, 2006; KOSCHIER *et al.*, 2007; NAGATA *et al.*, 1999). To our knowledge this compound has been reported as a compound of the volatile fraction of tomato in only two other studies (VIANI *et al.*, 1969; SERVILI *et al.*, 2000). Since 2-hydroxybenzaldehyde does not seem to be a common constituent of the volatile fraction of tomatoes, it can be supposed that it is only synthesized in detectable amounts by some cultivars; in this case it is a volatile marker for the four traditional ones. In a recent study on wild tomato species (*Solanum pennellii*), an allele was identified to which the formation of cucumber-like flavour, due to C9-aldehydes, was attributed and that was inactivated during domestication (MATSUI *et al.*, 2007). This could suggest that the capacity to synthesize 2-hydroxybenzaldehyde has been lost in the selected cultivars, while it may have been preserved by the ecotypes. Further studies on the genetics of the ecotypes and their commercial homologues could explain this result. Among the ketones significantly higher amounts of 6-methyl-3,5-heptadien-2-one and β-ionone were found in San Marzano. The compound β-ionone was not detected in the Vesuviano-Principe Borghese and Sorrento-Cuore di Bue pairs. β-Ionone is derived from the biological or chemical degradation of β-carotene (BUTTERY *et al.*, 1988). Several authors have indicated that this is as an odour-active compound for tomato, considering its Number of Odour Units (NOU, concentration/odour threshold) (RUIZ *et al.*, 2005; BUTTERY *et al.*, 1987; STERN *et al.*, 1994); moreover it was positively correlated with ripe tomato aroma (Maul *et al.*, 2000). Considering the very low odour threshold of β-ionone (0.007 ppb, (BUTTERY *et al.*, 1987)), with its floral odorous note (Flavours & Fragrances catalogue 2003-2004, Sigma-Aldrich) it could contribute to the odour profiles of the varieties in which it was detected. The difference in concentration detected between San Marzano and Ranco F1, although statistically significant, was too small to deduce that this compound could determine perceptible differences between the two homologues. The three alcohols, 1-penten-3-ol, 1-pentanol and benzyl alcohol were detected in significantly higher amounts in San Marzano than Ranco F1. The first two compounds have been considered odour-active for tomato (Table 1), however the small concentration differences detected could not to be nasally perceived. The ethyl hexanoate, isovaleric acid, 3-methylisothiazole and 5-methylfurfural compounds were detected in Ranco F1 but not in San Marzano.

The two varieties, Corbarino and Faino F1, have small oval tomatoes and an average weight between 13 and 20 g (Fig. 1). The local variety Corbarino originated from the hilly area surrounding the town of Corbara (SA, Campania region, Italy). For many years it has been selected by farmers from the old varieties ("Fiaschella", "Lampadina", "Principe Borghese", "Re Umberto") and grown there since the early 1900s. A committee has been set up to request PDO (Protected Designation of Origin) status for Corbarino. The hybrid Faino F1 is morphologically very similar to Corbarino, and is also grown in several areas other than in the Campania region.

The traditional cultivar Corbarino had a volatile fraction that was almost 1.5 times more concentrated than its commercial homologue Faino F1. Five aldehydes differentiate Corbarino and Faino F1. The two aldehydes (E)-2-hexenal and (E)-citral are related, respectively, to lipids and lignin metabolism and are key compounds in tomato flavour (Table 1). Hexanal, (E)-2-hexenal, (E,E)-2,4-heptadienal and (E)-citral were detected in higher amounts in Corbari-

no, while 2-hydroxybenzaldhehyde was only detected in the local Corbarino variety. Hexanal is derived from the oxidative degradation of linoleic acid. It is one of the major aldehydes in tomato (PETRÓ-TURZA, 1987), as confirmed in the present study; it was the most abundant aldehyde in all the samples, and is characterized by a fresh grass odour. The large amounts in which it generally occurs in tomato and its relatively low odour threshold (4.5 ppb, (BUTTERY *et al.*, 1971)), make it one of the most important volatile compounds in the fresh tomato flavour, as assessed by several authors (Table 1). According to Kazeniak and Hall (1970), a concentration range of 0.1-0.5 ppm improved the aroma of tomato juice with its "green" flavour; at higher concentrations its off-flavour notes, typical of rancid vegetable fats, became evident. The alcohols 2+3-methyl-1-butanol and 1-hexanol were present in higher concentrations in Corbarino. The 2+3-methyl-1-butanol is derived from the catabolism of isoleucine and leucine, while 1-hexanol is derived from the oxidation of linoleic acid by the reduction of hexanal. In a previous study (RUIZ *et al.*, 2005) hexanal and hexanol were able to discriminate between two closely related traditional Spanish cultivars of the "Muchamiel" type, although the differences in concentration were relatively small (not even a factor of 2) as in the present study. Among the esters, ethyl 3-methylbutyrate and ethyl salicylate were detected in higher amounts in Corbarino, while ethyl hexanoate was not detected in Faino F1. Ethyl 3-methylbutyrate has not been detected in the volatile fraction of tomato in previous studies. This ester, characterized by a fruity odour, has an extremely low odour threshold (0.01 μL/103 L, (TAKEOKA *et al.*, 1991)) so it could be an odour-active compound in tomato even at low concentrations. In the present study it was quantified in all samples, except Ranco F1. An interesting result was found concerning the compound 2-isobuthylthiazole. Among the tomatoes analysed, the Corbarino-Faino F1 and Vesuviano-Principe Borghese pairs had the highest concentrations of 2-isobutylthiazole. This compound allowed the commercial homologue Faino F1 to be distinguished from the traditional Corbarino variety as it was detected in a significantly higher amount in Faino F1 and it was the second most abundant volatile compound for this variety. Moreover, Tukey's test performed on all cultivars showed that a significantly higher amount of 2-isobutylthiazole was found in Faino F1 ($p<0.05$, results not shown). This compound is only found in the tomato fruit and is derived from amino acid metabolism (Kazeniac and Hall, 1970). In contrast to other compounds in which the concentration increases after the crushing procedures (such as C6 alcohols and aldehydes), the amounts of 2-isobutylthiazole did not appear to be dependent on the maceration time after crushing, suggesting that it is pre-formed in fruit (BOUKOBZA *et al.*, 2001). 2-Isobutylthiazole is characterized by a tomato leaf-like odour (Flavours & Fragrances catalogue 2003-2004. Sigma-Aldrich) and several authors have found it is a key-component of tomato aroma (Table 1). It seems however that its contribution to tomato aroma can be positive or negative depending on the concentration. According to Kazeniac and Hall (1970) in a concentration range from 25 to 50 ppb this compound positively contributes to fresh tomato flavour while at higher levels, its flavour becomes objectionable, described as rancid, medicinal or metallic. In a sensory study conducted on the same tomato samples as the present work, both Faino F1 and Vesuviano were characterized by a flavour described as "watermelon rind" (SINESIO *et al.*, 2003). Considering its low odour threshold (3.5 ppb, (BUTTERY *et al.*, 1971) and the fact that higher levels of this compound were detected in Faino F1, 2-isobutylthiazole

could be responsible for this flavour in Faino F1. However, it must be considered that the olfactory impact of a compound is not only dependent on the concentration of that compound but also on the complex equilibrium with the other volatiles. Although the amount of 2-isobutylthiazole detected in Vesuviano was not significantly different with respect to its homologue Principe Borghese, it had a lower concentrated volatile fraction, so in the complex equilibrium among volatiles the odorous contribution of 2-isobutylthiazole could have been perceptible. Considering that previous studies have also shown that the concentration of 2-isobutylthiazole varied significantly from one tomato variety to another (BALDWIN *et al.*, 1991a; LANGLOIS *et al.*, 1996; KAZENIAC and HALL, 1970) and that its levels do not depend on sample preparation procedure, it is reasonable that 2-isobutylthiazole could specifically differentiate Faino F1 from the other cultivars under investigation. It would be extremely interesting to study the genetic and molecular mechanisms that result in this specificity. The compound dimethylsulfoxide was only detected in Corbarino. This compound is derived from the oxidation of dimethyl sulphide, and the amount increases in tomato pastes. It was previously detected in fresh tomatoes at levels between 0.08 and 0.16 ppm (PEARSON *et al.*, 1981). Finally, furfural and its derivative 5-methylfurfural were detected in Faino F1 but not in Corbarino.

Vesuviano and Principe Borghese are cherry tomatoes (Fig. 1). Vesuviano is mainly grown along the slopes of the Vesuvius volcano and it is derived from old varieties grown in the same area in the absence of irrigation. The average weight of the fruit is 21 g and it grows in bunches. Traditionally, the bunches were hung up and the tomatoes, after a light drying, were consumed throughout the winter. The registration of its PDO status with the denomination "Pomodorino del piennolo del Vesuvio" is nearly completed. Principe Borghese is almost morphologically identical to Vesuviano and it seems to have derived from a selection made on a population of Vesuviano. Forty-one volatile compounds were detected in Vesuviano and 37 in Principe Borghese. In contrast to the results found for the two pairs of homologues just discussed, the commercial homologue Principe Borghese had a volatile fraction that was more concentrated than the traditional Vesuviano variety. Unlike what was found for the San Marzano-Ranco F1 and Corbarino-Faino F1 pairs, all of the compounds detected in significantly different amounts were more concentrated in the commercial homologue Principe Borghese. The aldehydes hexanal, (E)-2-pentenal and (E)-2-hexenal differentiated the two cultivars. Among the ketones, only geranylacetone differentiated the two cultivars, it was detected in Vesuviano but not in the volatile fraction of Principe Borghese. Geranylacetone is a product of the degradation of lycopene (PETRÓ-TURZA, 1987) and is characterized by an odour of rose and magnolia (FLAVOURS & FRAGRANCES catalogue 2003-2004, Sigma-Aldrich). Its role in tomato flavour is not clear, BUTTERY *et al.* (1987) reported a NOU (Number of Odour Units) less than one and STERN *et al.* (1994) reported 0.9, while KRUMBEIN and AUERSWALD (1998), using gas chromatography-olfactometry (aroma extract dilution analysis), included it among the 34 most powerful aroma compounds for tomato but with a dilution factor of only 4. In a previous comparative study on six tomato cultivars (BALDWIN *et al.*, 1991a) the amounts of geranylacetone were significantly higher in Solar Set fruit than in the other cultivars analysed. The alcohols 1-penten-3-ol and 1-pentanol were present in higher amounts in Principe Borghese, while 1-butanol was not detected in Vesuviano; the first two were reported to be critical to fresh tomato flavour (STERN *et*

al., 1994). Three of the six esters identified, ethyl-3-methylbutyrate, methyl salicylate and ethyl salicylate, were detected in higher amounts in Principe Borghese, while ethyl hexanoate was only detected in the traditional Vesuviano variety. The lignin-related methyl salicylate has an odour threshold of 40 ppb (BUTTERY *et al.*, 1987) and is characterized by a minty, spicy odour (FLAVOURS & FRAGRANCES catalogue 2003-2004, Sigma-Aldrich). The greatest difference in concentration of this compound was found between the Vesuviano-Principe Borghese homologues and this significant concentration difference could be perceived nasally. The two phenolic compounds detected, guaiacol and phenol, were present in significantly higher amounts in Principe Borghese, such as 2-pentylfuran, while 3-methylisothiazole, a compound not previously detected in tomato, was not detected in Principe Borghese.

The tomato "Sorrento" has very big fruit (average weight 150-160 g, but up to 200 g), with a round shape and ribs (Fig. 1). It is grown in almost all areas of the Campania region and was probably derived from selection on the Cuore di Bue tomato. Another hypothesis is that this variety was imported from America at the beginning of the twentieth century. Because of its characteristics (short durability, high weight, good flavour) it is particularly suitable for the fresh market. There is great interest in the request for Protected Geographical Indication (PGI) status for the Sorrento tomato and the procedures have been started. Cuore di Bue tomato is morphologically very similar to Sorrento but is heart-shaped and without ribs, its average weight (almost 80 g) is about half that of Sorrento. Even if the morphological characteristics of the homologues are not identical, as in this case, the differences are minimal and can only be noted by experts. The common consumer can hardly distinguish the traditional variety from its commercial homologue. As for the Vesuviano-Principe Borghese pair, the commercial homologue Cuore di Bue had a slightly more concentrated volatile fraction. As for the Vesuviano-Principe Borghese pair, the commercial homologue Cuore di Bue had a more concentrated volatile fraction than the traditional Sorrento variety. The aldehyde (E)-2-octenal was detected in significantly higher amounts in Cuore di Bue, while greater amounts of (E)-2-heptenal were found in the traditional Sorrento variety. According to KAZENIAC and HALL (1970), (E)-2-octenal in both tomato juice and diluted paste produced cardboard-type flavours in the 0.1 ppm range. The amounts of the conjugated dienals, (E,E)-2,4-heptadienal and (E,E)-2,4-octadienal, were two- and seven-times greater, respectively, in Cuore di Bue. (E,E)-2,4-Octadienal was detected for the first time in tomato. Conjugated dienals seem to have rather unique flavour properties. In a previous study it was found that (E,E)-2,4-decadienal produced very desirable mouth-feel properties, smoothing out harsh, acidic-type notes of tomato juice and diluted paste at small concentrations, in a range of 2.5-10 ppb (KAZENIAC and HALL, 1970). This flavour property appears to be characteristic of conjugated dienals, not only in tomatoes but in other food as well. While the range of concentration of (E,E)-2,4-heptadienal and (E,E)-2,4-octadienal in which such effect is noted is unknown, it can be supposed that the positive effect of the conjugated dienals may be more perceptible in the flavour of Cuore di Bue than in that of Sorrento. The only ketone that showed significant differences between these two homologues was 1-octen-3-one. This compound is characterized by a strong mushroom odour and is one of the impact odorants of mushroom (BERGER *et al.*, 1992). In previous studies, it was also found to be an impact odorant for tomato (BUTTERY *et al.*, 1990; KRUMBEIN & AUERSWALD, 1998)

and was found to have an extremely low odour threshold (0.005 ppb, (BUTTERY *et al.*, 1990)). The difference in concentration between Sorrento and Cuore di Bue, although statistically significant, is small and probably barely perceptible. The alcohols 1-butanol and (Z)-2-penten-ol determined a qualitative difference between the two homologues, being detected only in Cuore di Bue. 2-Phenylethyl alcohol, characterized by a rose-like odour, is derived from the metabolism of the amino acid 2-phenylalanine and it was detected in a significantly higher amount in Cuore di Bue. Two tomato enzymes were recently identified that catalyze the conversion of 2-phenylacetaldehyde to 2-phenylethanol (TIEMAN *et al.*, 2007). Two esters, methyl and ethyl octanoate, were only detected in Cuore di Bue. This is the first report of the latter being detected in tomato volatile fraction. The role of esters in tomato flavour does not seem to be determinant, in fact, only methyl salicylate seems to be an impact odorant (see literature cited in Table 1). However it would be interesting to understand the metabolic pathway that led to these differences in the ester composition between the homologues. Hexanoic acid, phenol and 2-pentylfuran were detected in higher amounts in Cuore di Bue, while 5-methylfurfural was only detected in the commercial homologue Cuore di Bue. Finally, the only unsaturated hydrocarbon, tentatively identified as 3-ethyl-2-methyl-1,3-hexadiene, was only detected in Cuore di Bue. This compound had already been tentatively identified in the volatile fraction of tomato (SERVILI *et al.*, 2000).

The quantitative data relative to all the samples was statistically analysed in order to better visualize what compounds were specifically related to the different cultivars. A Principal Component Analysis (PCA) was performed and included the compounds that were quantified in all the samples (Fig. 2). In a previous study the use of PCA allowed the commercial tomato varieties to be characterized on the basis of volatile composition (LANGLOIS *et al.*, 1996). The first two components account for 55.58% of the total variability in the original data. The first component discriminates two groups of varieties: the first group situated along the positive semiaxis is formed by San Marzano, Cuore di Bue, Principe Borghese and Corbarino; the second group situated along the negative semiaxis is formed by Ranco F1, Faino F1, Sorrento and Vesuviano. The two varieties that constituted each pair of homologues were all separated by the first component. This result seems to confirm that, in spite of a great similarity of the homologues for morphology and market destination, the composition of the volatile fraction is quite different. The tomatoes positively correlated with the first principal component were principally Cuore di Bue and Principe Borghese. The volatile compounds that seemed to characterize these varieties were guaiacol (47), phenol (51), 1-pentanol (12), benzaldehyde (31), 1-penten-3-ol (6), (E,E)-2,4-heptadienal (29) and (E)-2-pentenal (4). The correlation between the compound 2-isobutylthiazole (22) and the commercial variety Faino F1 was evident from the PCA analysis. 2-Isobutylthiazole was the only compound that correlated principally with the negative semiaxis of the first component as the sample Faino F1. Considering the second principal component, the homologues San Marzano and Ranco were both characterized by (E)-2-octenal (24), while Vesuviano and weakly Sorrento, Principe Borghese and Corbarino were correlated to acetic acid (26) and 2+3-methylbutanol (9). The analysis of the second component leads to an interesting observation: for three pairs of homologues the traditional variety and the commercial one showed the same type of correlation with the second principal component, with San Marzano-Ranco F1 having a positive correlation and Vesuviano-Principe Borghese, Sor-

rento-Cuore di Bue, a negative correlation. In contrast, in the case of the Corbarino-Faino F1 pair, the former showed a negative and the latter a positive correlation with the second component. Considering that all the homologues were discriminated by the first component, it seems that Corbarino and Faino F1 were the homologues that differed the most and the compound principally involved in this differentiation was probably 2-isobutylthiazole.

CONCLUSIONS

Several significant differences in the volatile composition, both qualitative and quantitative, were able to discriminate between traditional tomato varieties and their correspondent commercial homologues. The most interesting results were those found for the compounds 2-hydroxybenzaldheyde and 2-isobutylthiazole. The compound 2-hydroxybenzaldheyde was only detected in the four traditional cultivars of tomato and not in their commercial homologues. The sulphur compound 2-isobutylthiazole seems to be specifically related to Faino F1 and is probably responsible for the watermelon rind olfactory note detected in a sensory study conducted on the same samples (SINESIO *et al.*, 2003). Moreover, the analysis allowed some volatile compounds to be identified that, to the best of our knowledge, have not been reported as volatile compounds of tomato: (E,E)-2,4-octadienal, 2-ethyl hexanol, ethyl 3-methylbutyrate, ethyl octanoate and 3-methylisothiazole. PCA analysis of the quantitative data clearly showed differences between all the homologues, and Corbarino and Faino F1 were the homologues that differed the most. The compound principally involved in this differentiation was probably 2-isobutylthiazole. Further sensory studies could verify if some of these differences have a perceptible effect on the flavour of these cultivars. Moreover, a greater understanding of the genetic differences that determine the different volatile composition could allow the commercial protection of the traditional cultivars to be based on scientific evidence and help to preserve their biodiversity.

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PAPER

EVALUATION OF THE AROMATIC AND POLYPHENOLIC COMPOSITION OF COLA AND COLA GELATO APPLES GROWN IN THE AREA OF THE ETNA VOLCANO

VALUTAZIONE DELLA COMPONENTE AROMATICA
E POLIFENOLICA DI MELE
"COLA" E "COLA GELATO" PRODOTTE NEL TERRITORIO DELL'ETNA

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ABSTRACT

The composition of the volatile and polyphenolic fractions was determined in the peel and pulp of two apple cultivars (Cola and Cola Gelato) grown in the area of the Etna Volcano (Catania, Italy) and compared with Golden Delicious. Volatile compounds were extracted by SPME and with solvent, identified by GC/MS and quantified by GC/FID using internal standards. The total volatile content in peel extracts was much greater than in the pulp. The main factors contributing to the aro-

RIASSUNTO

La composizione aromatica e polifenolica di mele Cola e Cola Gelato, tipiche del territorio etneo, e di mele Golden Delicious è stata determinata separatamente per bucce e polpe. I componenti aromatici sono stati estratti con solvente, separati ed identificati mediante GC/MS e quantificati mediante GC/FID con il metodo dello standard interno. Il contenuto complessivo di aromi è notevolmente superiore nelle bucce rispetto alle polpe. Gli odoranti dominanti delle bucce sono l'α-farnesene, l'esil

- Key words: apples, aroma components, Cola variety, Cola Gelato variety, Golden delicious variety, polyphenols -

matic impact were determined by calculating the odor units of those compounds having the lowest threshold values. Hexyl ethanoate, ethyl hexanoate and α-farnesene were the most powerful odorants in the peel, while hexyl ethanoate, hexanal and (*E*)-2-hexenal were dominant in the pulp odor. The Cola Gelato variety had the highest aromatic value, while Cola was very similar to Golden Delicious. Polyphenols were extracted with methanol and identified and quantified by HPLC/PDA using external standards. The fraction included flavanols (procyanidins and epicatechins), 5-caffeoylquinic acid, dihydrochalcones and quercetin 3-glycosides. The last class was found almost exclusively in the peel. The content of total polyphenols in each variety was appreciably higher in the peel than in the corresponding pulp. Cola apples showed the highest polyphenol levels in both peel and pulp. When compared with Golden Delicious, the Cola apples had more flavanols and 5-caffeoylquinic acid, but fewer flavonol glycosides.

etanoato e l'etil esanoato, mentre nelle polpe prevalgono l'esil etanoato, l'esanale e l'(*E*)-2-esenale. Le mele Cola Gelato possiedono un corredo di sostanze aromatiche più ricco e vario rispetto alle Cola e alle Golden Delicious. Tra i componenti polifenolici, identificati e quantificati mediante HPLC/DAD, sono presenti diversi flavanoli (procianidine e catechine), l'acido 5-caffeoil chinico, due diidrocalconi e vari glicosidi della quercetina, questi ultimi presenti soltanto nelle bucce. Le mele Cola sono più ricche di flavanoli e di acido 5-caffeoilchinico rispetto alle altre varietà, ma più povere di flavonoli glicosidi rispetto alle Golden Delicious.

INTRODUCTION

The natural flavours in apples have been extensively documented. Over a hundred papers have been published since 1991 (BERGER, 1991). Many have appeared more recently in the literature, and have reported the chemical composition of different varieties, storage effects, sensory and biological properties. Apple aroma is due to a combination of several volatile metabolites, including low-molecular-weight esters, alcohols, aldehydes and α-farnesene. A great variability is observed in the distribution of compounds, depending on the variety and geographic origin (KARLSEN *et al.*, 1999; LO SCALZO *et al.*, 2001; YOUNG *et al.*, 2004), storage conditions (AABY *et al.*, 2002; DE FILIPPI *et al.*, 2004), pre-storage heat treatment (FALLIK *et al.*, 1997) and the kind and amount of skin coating applied (BAI *et al.*, 2002). Among the reported compounds, some esters of ethanoic, butanoic and 2-methylbutanoic acids are mainly responsible for the aromatic impact.

Many other studies have investigated polyphenol constituents, which are considered effective scavengers of reactive oxygen species. The potential oxidative damage of large biomolecules that cause different pathologies in humans, could be prevented by dietary antioxidants,

such as polyphenols found in fruit and vegetables. There is a relationship between polyphenol content and total antioxidant activity *in vitro* (VAN DER SUIS *et al.*, 2000; VINSON *et al.*, 2001; SUN *et al.*, 2002; WOLFE *et al.*, 2003); procyanidins and quercetin glycosides have a great antioxidant capacity (LU and FOO, 2000). Moreover, *in vivo* studies have demonstrated that apple phenolics can prevent LDL oxidation (PEARSON *et al.*, 1999) and inhibit or delay arteriosclerosis (KNEKT *et al.*, 1996*)* and other diseases (KNEKT *et al.*, 2000; GRAZIANI *et al.*, 2005), including cancer (KNEKT *et al.*, 1997; LE MARCHAND *et al.*, 2000).

Apples contain high levels of polyphenols, including flavanols (procyanidins and epicatechins), hydroxycinnamic acid derivatives, flavonol glycosides and dihydrochalcones. The quality and quantity of these constituents depend on the variety (TSAO *et al.*, 2003) and can be modified by post-harvest factors (SPANOS and WROLSTAD, 1992; ESCARPA and GONZALES, 1998; CHINNICI *et al.*, 2004; NAPOLITANO *et al.*, 2004). In this paper the aromatic and polyphenolic components of two apple varieties, Cola and Cola Gelato, which are grown on the mountainsides of the Etna Volcano (Catania, Italy), are reported. Their chemical composition has never been characterised.

Cola and Cola Gelato apples are ancient varieties that are typical of the Etnean territory (SAVASTANO, 1926); they grow at an altitude between 600 and 1600 m often in organic production. Cola apples have a yellow skin dotted with small brown spots, like freckles, and white slightly acidulous pulp. Cola looks like the Neapolitan apple variety, "Limoncella". Cola Gelato originated from cross breeding of Cola with "Gelato", another Etnean apple variety that is very sporadic now. At harvest, it has a pale green skin that changes to a straw-yellow colour over time. It is sweet and has aromatic pulp. Both varieties produce small-medium size fruit of about 70 g having an almost unitary polar ratio and a mean number of 7 and 4 seeds for Cola and Cola Gelato, respectively (CONTINELLA *et al.*, 2006). They ripen at the beginning of autumn or later depending on the altitude.

Volatile compounds were extracted from the peel and pulp by using two complementary techniques: solid phase micro-extraction (SPME) and solvent extraction. The SPME provides a rapid screening by GC/MS (SONG *et al.*, 1997; YOUNG *et al.*, 2004), while solvent extraction provides a quantitative analysis by GC/FID using internal standards. Polyphenols were extracted from the peel and pulp using methanol as a solvent, and identified and quantified by HPLC/PDA using external standards. These local varieties were evaluated because consumers appreciate their crispy, juicy and strongly aromatic characteristics. The volatile and polyphenolic components of the well known Golden Delicious apples were also determined and the data were used for comparison purposes.

MATERIALS AND METHODS

Apple fruit samples and chemicals

Six samples of the Cola variety and four of Cola Gelato (~ 2 kg for each lot) were harvested in October 2004 and October 2005 on four different farms located in the western and south-eastern foothills of Mount Etna (Catania, Italy) at an altitude between 800-1200 m. Four samples of Golden Delicious variety (Melinda™, Val di Non, Trentino, Italy) were purchased in local markets in November 2005. Four apples per sample were collected and the peels were carefully separated from the pulp. The same potato peeler was always used to perform thin and reproducible peel strips, and the seeds and core were removed and then the pulp was chopped into small

cubes. All samples were extracted immediately.

HPLC solvents and the standard volatile compounds indicated in footnote *a* of Tables 1 and 2, were from Sigma-Aldrich (Milan, Italy). The pure standard polyphenols indicated in footnote *a* of Table 5, were from Extrasynthèse (Génay, France).

SPME and solvent extractions of volatile components

SPME was performed on 5 g of cut peel or 25 g of pulp, put into a 40 mL vial sealed with a PTFE-lined screw cap, using a SPME fibre 50/30 µm DVB/CAR/PDMS (Supelco, Milan, Italy). The sample was heated to 35°C for 30 min be-

Table 1 - Volatile compounds in peel and pulp of Cola, Cola Gelato and Golden Delicious apple varieties detected by SPME-GC/MS.

Volatile components*		Rt (min)	Cola		Cola Gelato		Golden Del.	
			Peel	Pulp	Peel	Pulp	Peel	Pulp
1.	Ethyl ethanoate[a]	4.5	+	+	+	–	+	–
2.	Ethyl hydroxyethanoate[b]	4.7	+	+	+	+	+	+
3.	Ethyl propanoate[a]	4.9	+	–	+	–	tr	+
4.	Propyl ethanoate[a]	5.0	–	+	tr	+	tr	+
5.	Ethyl butanoate[a]	5.4	+	+	+	+	+	+
6.	Propyl propanoate[a]	5.5	–	+	+	+	–	+
7.	Butyl ethanoate[a]	5.9	++	++	+	++	++	++
8.	Hexanal[a]	6.1	–	+	–	–	–	+
9.	2-Methylbutyl ethanoate[a]	6.5	+	++	+	++	+	+
10.	1-Butanol[a]	6.7	+	+	+	+	+	+
11,	Butyl propanoate[a]	6.8	+	–	+	+	+	+
12.	2-Methyl 1-butanol[a]	7.7	+	+	+	+	tr	+
13.	Butyl butanoate[a]	8.2	+	++	+	++	+	++
14.	(*E*)-2-Hexenal[a]	8.4	–	+	–	–	–	+
15.	Butyl 2-methylbutanoate[a]	8.5	+	++	+	++	+	+
16.	2-Methylbutyl butanoate[a]	9.1	+	+	tr	+	tr	+
17.	Ethyl hexanoate[a]	9.3	tr	+	+	+	–	+
18.	Hexyl ethanoate[a]	10.7	+	++	+	++	++	++
19.	Hexyl propanoate[a]	10.9	+	–	+	+	+	+
20.	1-Hexanol[a]	11.1	+	++	+	+	+	++
21.	(*E*)-2-Hexenol[a]	12.6	tr	+	+	+	–	+
22.	Butyl hexanoate[a]	13.0	+	tr	++	–	++	+
23.	Hexyl 2-methylbutanoate[b]	13.4	++	+	++	+	++	++
24.	2-Methylbutyl hexanoate[a]	14.2	+	+	+	+	tr	+
25	(*E*)-2-Hexenyl butanoate[b]	14.7	tr	–	+	–	+	–
26.	Butyl heptanoate[a]	15.8	+	–	+	–	+	–
27.	1-Octanol[a]	17.0	–	–	+	–	+	–
28.	Hexyl hexanoate[a]	18.7	+	+	++	+	++	++
29.	Butyl octanoate[b]	18.8	–	–	+	–	+	–
30.	β-Farnesene[b]	22.1	+	+	+	+	+	+
31.	α-Farnesene[a]	22.9	+++	+	+++	+	+++	+
Total Ionic Current x 10^8			9.25	5.47	15.34	5.80	9.77	4.40

*[Symbol, TIC %]: –, Undetected compound; tr (traces), <0.1%; +, 0.1-5.0%; ++, 5-10%; +++, >10%.
[a] Identified by correspondence of retention time and/or mass spectra with standard.
[b] Identified by fragmentation pattern recognition.

Table 2 - Mean content of volatile compounds of peel and pulp of Cola, Cola Gelato and Golden Delicious apple varieties by L/L extraction and GC/FID analysis.

Volatile compounds[1]	Peel			Pulp		
	Cola[2]	Cola Gelato[3]	Golden Delicious[3]	Cola[2]	Cola Gelato[3]	Golden Delicious[3]
Butyl ethanoate[a]	13.1 (2.6) b	1.8 (0.4) a	1.2 (0.1) a	4.1 (0.8) a'	1.6 (0.3) a'	0.4(0.1)a'
Hexanal[a]	0.6 (0.1)	-	-	1.0 (0.0) a'	1.6 (0.3) a'	2.8(0.5)a'
2-Methylbutyl ethanoate[a]	4.5 (1.1) a	2.0 (0.1) a	1.0 (0.1) a	0.8 (0.0) a'	2.3 (0.4) a'	0.1(0.0)a'
1-Butanol[a]	20.7 (2.4) b	4.1 (0.8) a	1.9 (0.2) a	20.5 (3.6) b'	7.5 (1.5) a'	2.1(0.5)a'
2-Methyl-1-butanol[a]	2.9 (0.9) b	1.2 (0.2) ab	0.5 (0.1) a	4.4 (0.7) c'	2.2 (0.5) b'	0.4(0.1)a'
Butyl butanoate[a]	1.0 (0.1) ab	2.5 (0.6) b	0.5 (0.1) a	-	-	-
(*E*)-2-Hexenal[a]	-	-	-	1.8 (0.5) b'	5.6 (0.9) c'	0.9(0.1)a'
Butyl 2-methylbutanoate[a]	-	1.0 (0.1)	0.9 (0.1)	-	-	-
Ethyl hexanoate[a]	-	1.3 (0.1)	1.2 (0.2)	-	-	-
Hexyl ethanoate[a]	3.9 (0.7) a	2.9 (0.4) a	2.8 (0.1) a	0.6 (0.0) c'	0.5 (0.1) b'	0.3(0.1)a'
1-Hexanol[a]	5.5 (0.9) b	0.8 (0.0) a	0.7 (0.0) a	4.3 (0.8) b'	1.4 (0.2) a'	1.1(0.1)a'
Nonanal[a]	1.3 (0.1) a	5.1 (1.1) a	0.9 (0.1) a	-	-	-
Butyl hexanoate[a]	1.6 (0.7) a	8.9 (1.5) b	2.8 (0.1) ab	-	-	-
Hexyl 2-methylbutanoate[b]	5.8 (1.0) a	7.9 (1.1) a	1.4 (0.0) a	-	-	-
2-Methylbutyl hexanoate[a]	4.9 (0.9) b	5.0 (1.8) b	2.4 (0.0) a	-	-	-
Hexyl hexanoate[a]	4.9 (0.6) a	20.0 (1.6) b	7.6 (0.5) a	-	-	-
Butyl octanoate[b]	0.6 (0.3) a	2.3 (0.3) b	0.9 (0.1) a	-	-	-
β-Farnesene[b]	1.0 (0.2) a	0.7 (0.0) a	1.2 (0.1) a	-	-	–
α-Farnesene[a] (+ 2-Ethylhexyl 2-ethylhexanoate)	202.1 (18.4) a	223.0 (16.1) a	229.3 (17.4) a	-	-	-
2-Ethylhexyl 2-ethylhexanoate[b] (+ α-Farnesene)	-	-	-	4.7 (0.7) a'	2.8 (0.5) a'	3.7(0.6)a'
Total aroma components (mg/kg)	274.4	290.5	257.2	42.2	25.5	11.8

[1] In mg/kg of fresh weight (standard deviation in parentheses). Means in the same line followed by common letter are not significantly different ($p<0.05$), for peel and pulp, respectively.
[2] Mean value of six different samples, each analysed in duplicate.
[3] Mean value of four different samples, each analysed in duplicate.
[a] Identified by correspondence of retention time and/or mass spectra with standard.
[b] Identified by fragmentation pattern recognition.

fore introducing the SPME syringe into the vial headspace. Volatile compounds were adsorbed onto the SPME coating for 15 min and then desorbed in the GC/MS injector at 250°C for 5 min.

Solvent extraction was carried out using 30 g of cut peel strips (about 5 mm wide and 1-2 mm of thick) or 50 g of pulp homogenised in a domestic blender (Moulinette D56 with Bol mixer mod. R62, Moulinex, SEB Italia s.p.a, Milan, Italy) for 10 s. Five mL of a pentane/dichloromethane solution (2/1, v/v) containing three internal standard for quantitative purposes (see later) were added to the peel and pulp and then extracted using 100 mL of a pentane/dichloromethane solution (2/1 v/v) for 15 h at 40°C in a glass apparatus equipped with a vapour refrigerator at –20°C (MACCARONE *et al.*, 1998; ARENA *et al.*, 2001). The organic layer was

Table 3 - Distribution of the volatile compounds of peel and pulp of Cola, Cola Gelato and Golden Delicious apple varieties, in terms of functional classes.

Cultivar			Esters	Alcohols	Aldehydes	Sesqui terpenes	Total
Cola	Peel	Number of compounds	9	3	2	2	16
		Total content: mg/kg	40.3	29.2	1.9	203.1	274.4
		%	14.7	10.6	0.7	74.0	100.0
	Pulp	Number of compounds	4	3	2	-	9
		Total content: mg/kg	10.2	29.2	2.8	-	42.2
		%	24.2	69.2	6.6	-	100.0
Cola Gelato	Peel	Number of compounds	11	3	1	2	17
		Total content: mg/kg	55.6	6.1	5.1	223.7	290.5
		%	19.1	2.1	1.8	77.0	100.0
	Pulp	Number of compounds	4	3	2	-	9
		Total content: mg/kg	7.2	11.1	7.2	-	25.5
		%	28.2	43.5	28.2	-	99.9
Golden Delicious	Peel	Number of compounds	11	3	1	2	17
		Total content: mg/kg	22.7	3.1	0.9	230.5	257.2
		%	8.8	1.2	0.3	89.6	99.9
	Pulp	Number of compounds	4	3	2	-	9
		Total content: mg/kg	4.5	3.6	3.7	-	11.8
		%	38,1	30.5	31.3	-	99.9

Table 4 - Aromatic values (odour units) of some volatile compounds of Cola, Cola Gelato and Golden Delicious apple varieties.

Volatile compounds	Odour threshold	Cola		Cola Gelato		Golden Delicious	
	(ppm in water)	Peel	Pulp	Peel	Pulp	Peel	Pulp
Butyl ethanoate	0.066[b]	198	62	27	24	18	6
Hexanal	0.005[c]	120	200	-	320	-	560
2-Methylbutyl ethanoate	0.011[d]	409	73	182	209	91	9
1-Butanol	0.5[e]	41	41	8	15	4	4
(*E*)-2-Hexenal	0.017[d]	-	106	-	329	-	53
Ethyl hexanoate	0.001[d]	-		1300	-	1200	-
Hexyl ethanoate	0.002[f]	1950	300	1450	250	1400	150
Nonanal	0.0043[g]	302	-	1186	-	209	-
α-Farnesene	0.16[h]	1263	-	1394	-	1433	-
Total odour units		4283	782	5547	1147	4335	782

[a] Odour units = (analytical concentration/odour threshold).
[b] FLATH *et al.*, 1967. [c] BUTTERY *et al.*, 1987. [d] TAKEOKA *et al.*, 1992. [e] BELITZ and GROSCH, 1999. [f] BUTTERY *et al.*, 1982. [g] AHMED *et al.*, 1987. [h] Odour threshold in deodorized beer (BERGER, p. 610).

Table 5 - Polyphenol content in peel and pulp of Cola, Cola Gelato and Golden Delicious apple varieties.

Polyphenol[1]	R.t. (min)	λ_{max} (nm)	Peel			Pulp		
			Cola[2]	Cola Gelato[3]	Golden Delicious[3]	Cola[2]	Cola Gelato[3]	Golden Delicious[3]
Procyanidin B1[a]	21.6	277	10.5 (4.4)	-	-	12.3 (5.4) b'	3.1 (0.5) a'b'	-
(+)-Catechin[a]	23.8	277	29.7 (7.1)	-	-	14.6 (9.1)	-	-
5-Caffeoylquinic acid[a]	24.5	325	16.8 (5.8) b	6.1 (0.2) a	6.8 (0.6) a	13.9 (4.9) a'	10.9 (1.3) a'	9.3 (4.6) a'
Procyanidin B2[a]	25.8	277	30.9 (2.5) a	19.6 (6.4) a	26.4 (21.6) a	8.9 (2.7) a'	11.3 (1.9) a'	7.4 (3.2) a'
(-)-Epicatechin[a]	27.1	277	26.7 (3.9) b	9.5 (4.6) a	19.9 (12.9) ab	7.6 (2.0) a'	9.7 (0.4) a'	5.4 (1.9) a'
Quercetin-3-rutinoside[a]	32.5	250,358	1.4 (0.9)	-	-	-	-	-
Quercetin-3-galactoside[a]	33.2	250,360	2.6 (1.4) a	1.3 (1.5) a	10.4 (3.8) a	-	-	-
Quercetin-3-glucoside[a]	33.4	250,352	3.3 (0.6) b	0.8 (0.9) a	2.7 (0.5) b	-	-	-
Quercetin-3-xyloside[b]	34.5	250,352	3.2 (0.3) b	1.9 (1.1) a	4.2 (0.4) b	-	-	-
Phloretin glucoside[c]	35.2	280	3.4 (0.8) a	5.1 (0.5) a	3.9 (1.0) a	0.6 (0.1) a' b'	0.9 (0.1) a'	5.1 (4.5) b'
Quercetin-3-arabinoside[b]	35.5	250,352	7.7 (1.6) b	2.3 (1.8) a	7.7 (1.3) b	-	-	-
Quercetin-3-rhamnoside[a]	36.0	250,358	4.6 (0.8) b	2.6 (1.3) a	6.9 (0.7) c	-	-	-
Phloridzin[c]	38.0	280	3.7 (0.6) a	2.5 (0.9) a	6.1 (0.4) b	0.6 (0.1) a'	0.4 (0.1) a'	0.6 (0.2) a'
Total polyphenols			139.8	58.6	59.3	36.3	95.4	27.4

[1] In mg/100 g of fresh weight (standard deviation in parentheses). Means in the same line followed by common letter are not significantly different ($p<0.05$), for peel and pulp, respectively. [2] Mean value of six different samples, each analysed in duplicate. [3] Mean value of four different samples, each analysed in duplicate.
[a] Identified by correspondence of retention time and UV spectra with standard.
[b] Identified by fragmentation pattern recognition and quantified as quercetin 3-galactoside.
[c] Identified by fragmentation pattern recognition and quantified as phloridzin.

dried by rotary evaporator under vacuum at 20°C and made up to 5 mL with a pentane/dichloromethane solution. Such comminution and extraction allow an almost quantitative removal of volatile compounds from peel and pulp, as verified by preliminary trials.

GC/MS and GC/FID analysis of volatile components

GC/MS analysis was performed with a Shimadzu QP 5050 A (Shimadzu, Milan, Italy) equipped with a column CP-WAX 57 CB (50 m, 0.25 mm i.d., 0.25 μm film thickness), under the following conditions: helium as carrier at 2 mL/min; injector temperature 250°C; oven temperature: initial 70°C, raised to 220°C with a gradient of 4°C/min, final 220°C held for 15 min; transfer line 200°C; split ratio 1:10; EI source at 70 eV. Structures were assigned based on the correspondence of peak retention times and/or mass spectra with those of standard compounds, as indicated in Table 1.

A GC Shimadzu GR-17 AAF equipped with a Supelcowax column (30 m, 0.32 mm i.d., 0.5 μm film thickness-Sigma Aldrich, Milan, Italy) was used for quantitative analysis of the components in the solvent extracts under the following conditions: helium as carrier at a linear velocity of

35 cm/sec; split ratio 1:10; injector and oven temperatures were those of GC/MS analysis. The component concentrations were calculated from the peak areas through the use of three internal standards (1-pentyl ethanoate, 1-heptanol and β-citronellol, 87, 82 and 85.9 ppm, respectively). The areas of these three standards were used to calculate the concentrations of the aroma components with the lowest, medium and highest molecular weights, respectively. Since the Response Factors (RF) were about 1.00, the quantification was performed based on the proportion between the observed area and the known concentration of referring standard. Analyses were carried out in duplicate.

Extraction of polyphenols

Weighed samples of about 3 g of cut peel strips (about 5 mm wide and 1 mm thick) or 7 g of homogenised pulp (has indicated for volatile extraction) were extracted with five aliquots of methanol (25 mL for peel or 50 mL for pulp) using an ultrasonic bath in the dark (ESCARPA and GONZALES, 1998; CHINNICI *et al.*, 2004). The first extraction was performed with a methanol solution containing 150 or 250 mg/L of sodium bisulphite for peel or pulp, respectively, to avoid enzymatic browning. The five extracts were combined and concentrated in a rotary evaporator under vacuum at 20°C and then made up to 5 mL with methanol. The solution was centrifuged at 9000 rpm for 15 min at 4°C and supernatant was passed through a hydrophobic PTFE 0.45 µm filter (Albet), before HPLC analysis.

HPLC analysis of polyphenols

Separation of polyphenols was carried out on a Varian 9012 Q liquid chromatograph equipped with a binary gradient pump and a photo diode array detector. The column was a Phenomenex Luna 3 µm C18(2) 100 Å (15 cm, 4.6 mm i.d. – Chemtek Analytica, Anzola Emilia, BO, Italy) operating at 25°C. The mobile phase and the gradient program were those suggested by SCHIEBER *et al.* (2001) with slight changes. Eluent A was 2% (v/v) ethanoic acid in water and eluent B was 85/15 acetonitrile/water; solvent B varied from 0% to 55% in 45 min, then from 55% to 100% in 15 min and finally from 100% to 0% B in 5 min, at a constant flow rate of 0.6 mL/min. The injection volume was 20 µL. Spectra were recorded from 200 to 450 nm. The polyphenols were identified by comparing retention times and UV spectra with those of the standards.

The polyphenols for which the standards were lacking were identified by LC-MS using a Thermo liquid chromatograph consisting of a Finningan Surveyor MS-pump, a Finningan Surveyor autosampler, a Finnigan Surveyor PDA detector and a Finnigan LCQ DECA XP MAX detector. The analytical column was a Phenomenex Luna 5 µm C18 (25 cm, 4,6 mm i.d. – Chemtek Analytica, Anzola Emilia, BO, Italy). Eluent A was acetonitrile and eluent B was 1% (v/v) formic acid in water. Separation was carried out at 30°C in 80 min, under the following conditions: the flow rate was 1 mL/min and the injection volume was 20 µL; split 1/10; solvent A changes from 5% to 28% in 50 min, then to 57% until 60 min, isocratic to 65 min and finally it changes to 5% in 80 min. Scan range PDA 190-700 MM; scan range *m/z* 100-800, sheath gas 18 units, auxiliary gas 16 units, capillary temperature 220°C, spray voltage 4.00 KV, negative mode.

Quantification was carried out using external standards. Flavanols and dihydrochalcones were monitored at 280 nm, 5-caffeoylquinic acid at 325 nm and quercetin glycosides at 370 nm, i.e. at the wavelengths near the maximum absorption of each component. For the compounds lacking standards, quanti-

fication was carried out using structurally related compounds; thus quercetin 3-xyloside and quercetin 3-arabinoside were quantified as quercetin 3-galactoside and the phloretin derivative was quantified as phloridzin. Analyses were carried out in duplicate.

RESULTS AND DISCUSSION

Aromatic composition

In preliminary experiments, the SPME/GC/MS methodology was applied to identify the volatile compounds of separated peel and pulp of Cola, Cola Gelato and Golden Delicious varieties. The chromatograms show about 50 peaks, many of these in traces or lacking in some samples. By comparing peak retention times and mass spectra with those of the standards, 31 compounds were identified as esters, alcohols, aldehydes and sesquiterpenes. Peak areas as % of total ion current (TIC), were used to compare the approximate amounts of each component. Table 1 lists the volatile compounds in the order of retention times: the most abundant are butyl, 2-methylbutyl and hexyl esters of ethanoic acid, butyl butanoate, butyl and hexyl esters of 2-methylbutanoic acid, hexyl hexanoate, 1-hexanol and α-farnesene. TIC values indicate that the Cola Gelato variety had the highest content of volatiles in the peel, whereas Golden Delicious had the lowest content in the pulp. These data are only a rough indicator, because both SPME and GC/MS are unreliable for quantitative analysis. Therefore, to determine the actual content of volatile metabolites, extraction with solvent and GC/FID quantification with three internal standards were used.

The concentrations of 20 representative flavour metabolites, expressed in mg/kg of fresh peel and pulp, are reported in Table 2. Eighteen were already identified in the SPME extracts, 2 are new: nonanal, found in the peel, and 2-ethylhexyl 2-ethylhexanoate found only in the pulp. The retention time of the latter ester is almost the same as that of α-farnesene which greatly exceeds its concentration in the peel. MS scanning of the α-farnesene peak clearly indicates the presence of the ester. In turn, the 2-ethylhexyl 2-ethylhexanoate peak in the pulp includes a small amount of α-farnesene. The dominant amount of this sesquiterpene in the peel extracts (from 74 to 89% of total volatiles) agrees with the findings of YOUNG *et al.* (2004). This demonstrates that green apples produce the highest α-farnesene levels, whereas esters prevail in red apple varieties.

The total amount of volatiles in peel extracts is much higher than in pulp extracts. The overall content is similar in Cola and Gelato peels, and slightly less in Golden Delicious; the situation is different in pulps, going from 42.2 mg/kg in Cola to 25.5 mg/kg in Cola Gelato and 11.8 mg/kg in Golden Delicious.

The distribution of the components grouped into functional classes is reported in Table 3. The chemical profile is quite different between peel and pulp. In peel, after α-farnesene is always in excess, and esters prevail over alcohols and aldehydes. Alcohols are distributed quite equally in both peel and pulp, while hexanal and (*E*)-2-hexenal are found in the pulp and nonanal in the peel. The alcohols predominate in Cola pulp and compete with the aldehydes in Cola Gelato and Golden Delicious.

Apple aroma is the result of a combination of the odour intensities of each volatile component, which in turn depend on the ratio between their analytical and threshold concentrations (odour units), also known as aromatic values (GUADAGNI *et al.*, 1966)). Table 4 lists the odour threshold and corresponding odour units of the compounds that have the lowest

odour threshold. The main contributors to peel aroma are hexyl ethanoate and α-farnesene. These esters and hexanal prevail in the pulp of Cola and Golden Delicious, while hexanal and (*E*)-2-hexenal characterize the pulp aroma of Cola Gelato. In spite of its high analytical concentration, 1-butanol does not contribute significantly to the aroma because of its high threshold concentration. GC/Olfactometry measurements indicated that butyl, 2-methylbutyl and hexyl ethanoates and ethyl hexanoate are characterized by a fruity/sweet/fresh odour, while that of α-farnesene is more greenish and herbaceous (data not reported).

FALLIK *et al.* (1997) showed that the prominent aliphatic esters detected in non-heat-treated Golden Delicious apples were butyl acetate, 2-methylbutyl acetate, hexyl acetate, butyl hexanoate/ hexyl butanoate and hexyl-2-methylbutanoate. Most of these were the most powerful odorants in Cola and Cola Gelato apples (Table 4).

YOUNG *et al.* (2004), reported the level of total esters in 13 apple cultivars using the MS ion count abundance: McIntosh and Granny Smith had low levels of total esters, Golden and Red Delicious had medium levels, while Spartan, Ida Red and Jonagold had high levels. Using Golden Delicious as a reference for a relative comparison with the HPLC results (Table 3) Cola and Cola Gelato apples are among the varieties with high levels of total esters.

Polyphenolic composition

Thirteen compounds were identified in the HPLC profile of polyphenols extracted from the peel of Cola apple (Table 5). As expected, these were fla-

Table 6 - Distribution of polyphenols in peel and pulp of Cola, Cola Gelato and Golden Delicious apple varieties.

Cultivar			Flavanols	5-Caffeoyl-quinic acid	Quercetin glycosides	Dihydro-chalcones	Total
Cola	Peel	Number of compounds	4	1	6	2	13
		Total content: mg/100g	93.7 (14.8)	16.8 (5.8)	22.6 (2.2)	7.1 (1.3)	139.8 (30.7)
		%	66.7	12.0	16.2	5.1	100.0
	Pulp	Number of compounds	4	1	-	2	7
		Total content: mg/100g	43.5 (14.7)	13.9 (4.9)	-	1.2 (0.1)	58.6 (24.3)
		%	74.2	23.7	-	2.0	99.9
Cola Gelato	Peel	Number of compounds	2	1	5	2	11
		Total content: mg/100g	36.6 (8.9)	6.1 (0.2)	9 (6.4)	7.6 (0.4)	59.3 (15.9)
		%	61.7	10.3	15.2	12.8	100,0
	Pulp	Number of compounds	3	1	-	2	6
		Total content: mg/100g	24.0 (2.1)	10.9 (1.3)	-	1.4 (0.1)	36.3 (4.2)
		%	66.1	30.1	-	3.9	100.0
Golden Delicious	Peel	Number of compounds	2	1	5	2	10
		Total content: mg/100g	46.5 (34.5)	6.8 (0.6)	32.1 (6.1)	10.0 (0.6)	95.4 (33.2)
		%	48.7	7.1	33.6	10.5	99.9
	Pulp	Number of compounds	2	1	-	2	5
		Total content: mg/100g	12.8 (5.2)	9.3 (4.6)	-	5.7 (4.2)	27.8 (14.4)
		%	46.0	33.5	-	20.5	100.0

Table 7 - Polyphenol content in peel (mg/kg) of the apple varieties.

Polyphenol	Cola[a]	Cola Gelato[a]	Golden Delicious[a]	Golden Delicious[b]	Empire[b]	McIntosh[b]	Cortland[b]	Mutsu[b]	Red Delicious[b]	Northern Spy[b]	Ida Red[b]
Total hydroxycinnamics	168	61	68	158.6	182.3	169.2	33.5	141.8	50.1	247.4	204.6
Total flavanols	978	291	463	708.7	151.3	1015.3	1065.3	574.9	1654.8	1456.4	1037.9
Total anthocyanins	-	-	-	-	208.2	42.9	159.8	-	148.9	17.4	111
Total flavonols	228	89	319	220.3	349.9	300.8	333.8	273.4	244.1	273.4	308.9
Total dihydrochalcones	71	76	100	161	124.8	108.3	66	99.2	252.5	78.1	100.1
Total polyphenols	1445	517	950	1248.5	1016.5	1636.4	1658.5	1089.4	2350.4	2072.7	1762.6

[a] This work.
[b] TSAO *et al.*, (2003).

vanols (procyanidins and epicatechins), 5-caffeoylquinic acid, quercetin 3-glycosides and dihydrochalcones. The HPLC pattern of flavanols, 5-caffeoylquinic acid and dihydrochalcones in the pulp was qualitatively similar to that of the peel, but quercetin glycosides were lacking. Most of these compounds were quantified by using the external standard calibration lines, and are expressed as mg/100 g of fresh peel and pulp (Table 5).

The polyphenol levels in each variety were appreciably higher in the peel than in the corresponding pulp, mainly due to the contribution of procyanidin B2 and quercetin derivatives. Cola apples had more procyanidins, (+)-catechin and 5-caffeoylquinic acid than the Golden Delicious and Cola Gelato varieties, in both peel and pulp.

The mean distribution of different classes of polyphenols (Table 6) was quite different between peel and pulp, but the flavanols were dominant in both parts of the fruit, and 5-caffeoylquinic acid was distributed comparably. Golden Delicious was is the richest in quercetin glycosides (in peel) and dihydrochalcones (in peel and pulp).

The polyphenol content of Cola and Cola Gelato was compared with that of other apple varieties using the results of TSAO *et al.* (2003), in which the polyphenolic profiles of 8 different apple cultivars were reported. Tables 7 and 8 report the content of different classes of polyphenols in peel and pulp, respectively. The peel of Cola had a higher level of total polyphenols than the other green varieties (Golden Delicious and Mutsu), but a lower level than the red ones, because the red apple peel contains an additional content of anthocyanins. The polyphenolic level in the pulp of Cola was the highest among all the cultivars (particularly the flavanol content) except for Northen Spy; Cola Gelato had low polyphenol levels in both peel and pulp.

Table 8 - Polyphenol content in pulp (mg/kg) of the apple varieties.

Polyphenol	Cola[a]	Cola Gelato[a]	Golden Delicious[a]	Golden Delicious[b]	Empire[b]	McIntosh[b]	Cortland[b]	Mutsu[b]	Red Delicious[b]	Northern Spy[b]	Ida Red[b]
Total hydroxycinnamics	139	109	93	164.6	162.4	235.4	132.2	141.6	136.7	328	243
Total flavanols	434	241	128	220.2	-	236.4	353.5	152.1	366.2	582.9	230.4
Total flavonols	--	-	6.4	-	-	-	-	3.7	-	-	
Total dihydrochalcones	12	13	57	25.4	15	16.4	12.1	19.3	27.9	22.6	16
Total polyphenols	585	363	278	416.6	158.6	428.1	536.5	329	445.8	755.2	413.1

[a] This work.

[b] TSAO *et al.*, (2003).

CONCLUSIONS

The aromatic value of the Cola Gelato apples was noticeably higher than that of the other varieties examined. The major compounds responsible for the aromatic impact in the peel were hexyl ethanoate, ethyl hexanoate and α-farnesene, while hexyl ethanoate, hexanal and (*E*)-2-hexenal characterized the pulp odour. The polyphenol composition was relevant from a qualitative point of view, because the dominant metabolites are 3,4-dihydroxyphenolic in structure, which is very effective against oxidant species. Cola apples are rich in flavanols and 5-caffeoylquinic acid in both peel and pulp, but have fewer quercetin glycosides in the peel, when compared with Golden Delicious.

The results of this study provide experimental support to the popular reputation of Cola and Cola Gelato being fresh aromatic apples that have health benefits; Cola has more polyphenols, while Cola Gelato has more aromatic compounds.

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PAPER

RELATIONSHIP BETWEEN GRAPE PHENOLIC MATURITY AND RED WINE PHENOLIC COMPOSITION

RELAZIONE TRA LA MATURITÀ FENOLICA DELLE UVE E LA COMPOSIZIONE FENOLICA DEI VINI ROSSI

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ABSTRACT

Phenolic maturity of red Piedmont grape varieties Nebbiolo and Barbera was monitored during the grape harvest in 16 vineyards in 2000 and 2001. The study used the Glories' method which was modified to avoid some critical parts of the original protocol, mainly regarding the extraction solution used at pH 1. Experimental winemaking processes were performed on a part of the grapes from the vineyards being monitored. The analytical data revealed a correlation between the an-

RIASSUNTO

Nelle annate 2000 e 2001 è stato condotto un monitoraggio della maturità fenolica di uve rosse piemontesi Nebbiolo e Barbera, che ha riguardato 16 vigneti. Il lavoro ha permesso di mettere a punto modifiche al metodo per la valutazione della maturità fenolica proposto da Glories per ovviare ad alcune criticità dello stesso, segnatamente per quanto riguarda l'estrazione a pH 1. Parte delle uve dei vigneti sottoposti a monitoraggio sono state vinificate sperimentalmente. Lo studio dei dati analitici dei vini otte-

- Key words: Barbera, Nebbiolo, phenolic maturity, polyphenols, previsional indexes, wine -

thocyanins and flavonoid indexes of grapes and color indexes of wines. The cell maturity index (EA%) is representative of how quickly anthocyanins can be extracted. Moreover, a correlation was found between the seed maturity index (Mp%) and the content of low molecular weight flavanols in the wine. In 2002-2004 this correlation was studied on Nebbiolo, Barbera and Dolcetto grape varieties on an industrial scale. Winemaking carried out using different systems of maceration confirmed the experimental results.

nuti ha evidenziato correlazioni positive tra gli indici degli antociani e dei flavonoidi delle uve e quelli del colore del vino. L'indice EA% si è dimostrato rappresentativo della velocità di cessione della materia colorante. Inoltre è stato evidenziato un collegamento tra l'indice di maturità dei vinaccioli (Mp%) e il contenuto di flavanoli a bassa massa molecolare del vino. Negli anni 2002-2004 le correlazioni rilevate sono state nuovamente valutate su Nebbiolo, Barbera e Dolcetto in vinificazioni condotte su scala industriale con sistemi diversi di macerazione confermando i risultati già ottenuti su scala ridotta.

INTRODUCTION

Phenolic compounds, extractable from grape skins and seeds, have a notable influence on the sensorial properties of red wines, especially their chromatic characteristics, astringency and bitterness (ARNOLD *et al.*, 1980; ROBICHAUD and NOBLE, 1990). The phenolic compounds, together with the aroma precursors are the main factors that affect wine quality. Consequently they have been studied extensively in grapes and wine (AMRANI-JOUTEI *et al.*, 1994; MOUTOUNET *et al.*, 1996; CHEYNIER, 2000; ATASANOVA *et al.*, 2002).

The evaluation of the sugar content and acid profile alone do not fully express the real oenological potential of grapes. Knowing the polyphenolic characteristics of the grapes allows the maceration and winemaking process to be planned so as to allow winemakers to fully exploit the potentiality that the grape reaches in the vineyard (SAINT-CRIQ *et al.*, 1998ab; GONZALEZ-NEVES *et al.*, 2004).

Many studies have been conducted to define the best method to evaluate polyphenolic compounds in grapes (AUBERT and POUX, 1968; RIBEREAU-GAYON, 1971; MARGHERI *et al.*, 1985; BOURZEIX *et al.*, 1986; GUNATA *et al.*, 1987). GLORIES and AUGUSTINE (1993) used the term "grape phenolic maturity" to indicate the concentration of phenolic compounds in grapes, and the ease with which they are released. This definition encompasses the anthocyanin concentration in the skin, their degree of extractability, the flavanol concentration in the seeds and skin and their degree of polymerization. The method proposed by GLORIES consists of extracting the phenolic compounds from the whole berries liquidized under two different conditions, determining the concentration and subsequently comparing the data. Moreover, the authors indicate that the partial break-up of the seeds allows tannins to be partially extracted, and that skin tannins are extracted in proportion to the anthocyanins.

The first stage of the procedure attempts to extract nearly all of the phenolic content using a very low pH (~1) which favours the complete degradation of the

cell membrane (GLORIES and SAUCIER, 2000). The second stage repeats the extraction under normal maceration conditions using a buffer (pH 3.2) which does not cause any further degradation of the cell membrane other than that normally reached during ripening. The smaller the difference in the parameters between pH 1 and pH 3.2, the greater the level of phenolic maturation.

Many compounds are involved in the evolution of the maturation of the grape, so the definition of phenolic maturity cannot be represented by a few parameters and some confusion can arise when the data are interpreted (VENENCIE *et al.*, 1998).

Numerous studies were carried out in the 1990s to evaluate the phenolic potential of grapes using the method proposed by Glories working with whole berries (BARCELO, 1997; SAINT-CRIQ *et al.*, 1998bc; CELOTTI *et al.*, 2000a; GONZÁLES-NEVES *et al.*, 2004; 2007). Other studies were aimed at modifying the nature of the solvent and the working model of extraction while trying to be faithful to the original Glories method, working with skins and seeds separately (RIOU and ASSELIN, 1996; VENENCIE *et al.*, 1997; VENENCIE *et al.*, 1998; PEYRON, 1998; DI STEFANO *et al.*, 2000; MATTIVI *et al.*, 2002a) or with whole berries (LAMADON, 1995; CAYALA *et al.*, 2002; CRESPY, 2002; ROMERO-CASCALES *et al.*, 2005; MATTIVI, 2006).

Other methods for assessing the phenolic quality of grapes which differ drastically from the Glories method, such as the use of chromatic parameters (CELOTTI *et al.*, 2000b; 2007) or grape sensorial analysis, have also been developed (ROUSSEAU and DELTEIL, 2000; MARTINEZ, 2002).

The use of whole berries to evaluate phenolic maturity has been criticized (DI STEFANO *et al.*, 2000), but there have also been studies that have supported the technological validity of the Glories method (ROMERO-CASCALES *et al.*, 2005; GONZÁLES-NEVES *et al.*, 2004, GONZÁLES-NEVES *et al.*, 2007).

In this study, autochthonous grapes from the Piedmont region were used to study the relationship between the level of grape phenolic maturity and the characteristics of the various wines obtained at the experimental and industrial levels.

MATERIALS AND METHODS

Grape maturity

During the 2000-2001 vintage, the technological and phenolic maturity of the grapes were monitored, in eight vineyards of Barbera grapes and eight of Nebbiolo. The vineyards are located in the Langhe area (Cuneo province, Piedmont, northwestern Italy, where Barbera d'Alba DOC and Barbaresco DOCG wines are produced) and in the Alto Monferrato area (Asti province, Piedmont, where Barbera d'Asti DOC is produced).

A representative sampling of the grapes was made during harvesting. Three samples (ca. 500 berries) from all parts of the vine as well as from throughout the vineyard were gathered. Technological parameters and the anthocyanin profile of the grapes were determined on half of the berries from each sample. The remaining berries were used to determine the phenolic maturity parameters.

The technological maturity index: density (soluble solids), total acidity and pH according to official methods (EEC, 1990), was determined. Moreover the varietal anthocyanin profile was analysed by HPLC. Sample preparation was carried out as described by DI STEFANO and CRAVERO (1991): the berry skin extract was applied to a 300 mg SEP-PAK C18 cartridge (Waters Corporation, Milford, MA, USA) and eluted with methanol. The cartridge was preconditioned with methanol (2 mL) and H_2SO_4 (0.005 M; 2 mL)

before use. The chromatograph was a P100 equipped with a AS3000 auto-sampler (Spectra Physics Analytical, Inc, San Jose, CA, USA) and a 20 mL Rheodyne sample loop. A LiChroCART analytical column (25 cm x 0.4 cm i.d.) from Merck (Darmstadt, Germany) packed with LiChrosphere 100 RP-18 5-μm particles by Alltech (Deerfield, IL, USA) and a Spectra Focus Diode Array Detector (Spectra Physics Analytical, Inc, San Jose, CA, USA) operating at 520 nm was used.

The following conditions were used: solvent A=10% formic acid in water. Solvent B=10% formic acid with 50% methyl alcohol in water. These solvents were filtered through a 0.20 μm filter. The solvent flow rate was 1mL/min. The solvent program used was 72% A to 55% A over 15 min; to 30% A over 20 min; to 10% A over 10 min; to 1% A over 5 min; to 72% A over 3 min; an equilibrium time of 10 min was used (ROLLE and GUIDONI, 2007; ZEPPA *et al.*, 2001). Data treatment was carried out using the ChromQuest™ chromatography data system (ThermoQuest, Inc, San Jose, CA, USA).

The identification of the free form anthocyanins, in the berry skin extract was performed by comparison with external standards (delphinidin-3-O-glucoside chloride, malvidin-3-O-glucoside chloride, peonidin-3-O-glucoside chloride, petunidin chloride, cyanidin chloride; Extrasynthèse, Genay, France); the acylated forms of anthocyanins were identified by comparing the retention time of each chromatographic peak with available data in the literature (DI STEFANO *et al.* 1995). The percentages of individual anthocyanins were determined by comparing the area of the individual peak with the total peak area (HEBRERO *et al.*, 1988; LETAIEF *et al.*, 2007).

The Glories' protocol used to determine phenolic maturity is described by SAINT-CRIQ *et al.* (1998c). This protocol was modified to simplify the handling process and minimise the effect of the buffering capacity of the juice to improve the extraction yield:

- the pH 1 extractant solution was prepared immediately before use by mixing equal volumes of the following solutions: 1) HCl 1.0 M (to stabilise the pH value in extraction solution near 1 unit); 2) $K_2S_2O_5$ 2.0 g/L (to improve the cell membrane permeability according to AMRANI-JOUTEI and GLORIES, 1994, AMRANI-JOUTEI and GLORIES 1995a);

- the extracts after the maceration period were separated from the solid parts using a centrifuge at 3,500 rpm for 5 min.

The following parameters were determined in pH 1 and 3.2 solutions: phenolic richness (expressed as Absorbance at 280 nm, A280) according to RIBEREAU-GAYON (1970) and total anthocyanins (A1 and A3.2), total flavonoids (TF1 and TF3.2) and non-anthocyanin flavonoids (NAF1 and NAF3.2) as reported in the wine analysis. The analytical data were in reference to berry mass.

The indexes of phenolic maturity calculated are those defined by GLORIES and AUGUSTINE (1993): potential anthocyanins (A1), extractible anthocyanins (A3.2), cell maturity index (EA%) and seed maturity index (Mp%). The latter index was determined, according to the indications of the authors, by taking into consideration the medium ratio (TAR) between the total polyphenols (expressed as the absorbance at 280 nm) and the total anthocyanins of the skin (expressed as g/L), equal to the value 40.

The EA% and Mp% indexes were calculated as follows:

$$EA\% = \frac{A1 - A3.2}{A1} \times 100 \qquad Mp\% = \frac{A280 - ((A1/1000) \times TAR)}{A280} \times 100$$

Preliminary assays showed that the TAR value of 40 was too low for the Nebbiolo grapes and that the value of 70 was more correct. To evaluate the mean of Nebbiolo TAR, the skins of 50 grams of berries were liquidized with 2V mL of pH 3.2 extractant solution (where V is the volume corresponding to the must from 50 grams of berries as described to SAINT-CRIQ *et al.*, 1998) and the protocol of grape phenolic maturity was applied. For twenty Nebbiolo grape samples a mean TAR of 70±5.1 was obtained. The variability was in agreement with Glories' data (GLORIES, 2001). Therefore this value was used in the calculations for the Nebbiolo grapes.

Experimental winemaking

The grapes that were used to monitor the maturity came from six vineyards and three for each cultivar, were fermented. Winemaking was carried out using an experimental protocol. It was repeated three times per vineyard.

The winemaking was done in stainless steel tanks using about 500 kg of grapes for each trial. Fifty mg/L of SO_2 (as potassium metabisulfite) and 150 mg/L of ammonium sulphate were added to the must obtained from crushing and destemming. The must was inoculated with 200 mg/L of dry yeasts BRL97 Lalvin (Lallemand, Grenaa, Denmark). The pomace floating cap was punched down twice a day during maceration. Skin contact was continued for 120 h, after which draining off was carried out.

Industrial winemaking

Industrial scale (ca. 10,000 kg grapes) winemaking was carried out in 2002, 2003 and 2004 to evaluate the provisional index efficacy of phenolic maturity assessed on a reduced scale in the previous harvests (2000, and 2001). Nebbiolo, Barbera and Dolcetto grapes were harvested from vineyards in the provinces of Cuneo and Asti.

Various fermenters with different functions were used: horizontal rotating types with vanes, model VMO 100 (Velo spa, Altivole, TV, Italy) and a punching-down device (Tosto spa, Chieti Scalo, CH, Italy) during the first two years. In 2004 a rotating tank was used exclusively. Winemaking was carried out using the following vinification protocol. During skin contact rotating fermentors and punching-down devices were put into action three times a day for five min each time. For all the vinifications, the temperature of the must in fermentation was controlled so that it did not exceed 30°C (Nebbiolo), 29°C (Barbera) and 28°C (Dolcetto).

Wine analysis

The first racking off (15 days after the draining off) wines were analysed using the following parameters: alcoholic strength, total acidity, pH and total dry matter (EEC, 1990). Wine acids were determined by HPLC (SCHNEIDER *et al.*, 1987). Phenolic compound indexes were determined as described by DI STEFANO *et al.* (1989): total phenols (TP), total flavonoids (TF), non-anthocyanin flavonoids (NAF) and flavanols reactive to vanillin (flavanols vanillin assay, FVA) all expressed as (+)-catechin (mg/L), proanthocyanidins (PR) expressed as cyanidin chloride (mg/L). Total anthocyanins (AT) and monomeric anthocyanins (AM) were expressed as malvidin-3-glucoside chloride (mg/L) (DI STEFANO *et al.*, 1991). The relative standard deviation of phenolic indexes based on repeated analyses (n=10) of seven red wines were: 1.39% (TP), 0.93% (TF), 2.80% (FVA), 1.14% (AT), 3.90% (AM). Anthocyanins was determined by HPLC as described above in grape maturity.

Chromatic properties were determined: colour intensity (CI), tone (CT) and yellow (A420), red (A520) and blue (A620) components according to GLORIES (1984). The CIELAB index values

were determined with reference to illuminant C (PIRACCI, 1994): clarity (L*), red-green component (a*), yellow-blue component (b*), chroma (C*) and hue (H*). All absorbance measurements were made using a UV-1601PC spectrophotometer (Shimazdu Scientific Instruments Inc., Columbia, MD, USA) and chromatic properties were carried out using a glass cuvette (2 mm optical path).

The kinetics of extraction of the anthocyanin (AT) and flavonoid compounds (TF) was also monitored during the 2001 winemaking.

Statistical analysis

The data were analysed using STATISTICA for Windows Release 6.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Table 1 shows the combined descriptive experimental parameters of the technological and phenolic maturity determined at harvest time. Analogous parameters are shown in Table 2 for the industrial trial grapes (2002-2004).

The grapes reached a good level of technological maturation as shown by the sugar content (Brix) data. The total acidity was lower in the 2000 vintage due to intense respiration favoured by the higher average summer temperatures. The pH values are in accordance with those described previously; the 2001 values are lower due to notable rains. The absolute values of the total anthocyanin and flavonoid indexes at harvest obtained under various extraction conditions (pH 1: A1, TF1; pH 3.2: A3.2, TF3.2), show a notable disparity in the anthocyanin potential (A1) between Nebbiolo and Barbera confirming data of other authors (CRAVERO and DI STEFANO, 1992; MATTIVI *et al.*, 2002b). In particular, the A1 values for the Nebbiolo cultivar varied between the minimum value of 332 (in 2001) and 574 mg/kg (in 2000). The variability between the minimum and maximum for different vineyards in the same year were ca. 180 mg/

Table 1 - Grape maturity indexes of Barbera and Nebbiolo at harvest in 2000 and 2001 (mean±standard deviation). Data from eight vineyards.

		Barbera		Nebbiolo	
Harvest-period	beginning end	2000 21 Sept. 2 Oct.	2001 24 Sept. 28 Sept.	2000 25 Sept. 9 Oct.	2001 2 Oct. 3 Oct.
Berry weight[a] (g)		262.5±23.5	268.0±29.5	188.6±15.4	178.2±19.4
Soluble solids (Brix)		24.1±1.5	23.7±1.5	25.2±0.9	23.6±1.0
Total acidity (g/kg)		8.5±1.2	9.6±2.0	7.1±0.4	7.0±0.6
pH		3.17±0.07	2.99±0.05	3.26±0.08	3.04±0.08
Anthocyanins pH 1 - A1 (mg/kg)		983±175	1131±197	494±51	428±60
Anthocyanins pH 3.2 - A3.2 (mg/kg)		538±104	658±76	318±23	366±53
Flavonoids pH 1 - FT1(mg/kg)		2768±348	2588±369	2582±146	2406±339
Flavonoids pH 3.2 - FT3.2 (mg/kg)		1304±224	1777±200	2130±150	2287±324
Absorbance at 280 nm - A280		44.8±4.9	72.3±4.6	62.0±11.7	64.9±6.2
EA%		50.3±3.9	41.2±4.3	35.3±3.4	14.3±5.6
Mp%[b]		52.0±7.5	60.5±3.0	58.9±8.9	56.8±4.6

[a]Expressed as weight of 100 berries; [b]Mp% data were calculated using a tannins/anthocyanins ratio (TAR) equal to 40 for Barbera grapes and 70 for Nebbiolo grapes.

Table 2 - Grape maturity indexes at harvest in 2002, 2003 and 2004 (mean±standard deviation).

	Year	2002	2003	2004
Number of	Barbera	2	4	7
Vineyards	Dolcetto	2	2	2
	Nebbiolo	1	3	1
Berry weight[a] (g)	Barbera	250.1±14.1	198.0±14.4	218.4±16.1
	Dolcetto	192.8±4.2	169.5±21.9	155.0±24.0
	Nebbiolo	110.3	188.3±3.2	156-1
Soluble solids (Brix)	Barbera	20.2±0.3	25.3±0.4	24.7±0.8
	Dolcetto	19.3±0.4	23.9±0.6	22.2±3.0
	Nebbiolo	23.0	24.6±0.4	24.3
Total acidity (g/kg)	Barbera	13.0±0.1	6.8±0.6	10.4±1.3
	Dolcetto	8.2±0.1	4.3±0.7	6.5±0.4
	Nebbiolo	10.3	5.0±0.2	5.6
pH	Barbera	2.92±0.05	3.16±0.07	3.01±0.05
	Dolcetto	3.19±0.02	3.52±0.25	3.08±0.04
	Nebbiolo	3.04	3.32±0.02	3.10
Anthocyanins pH 1 - A1 (mg/kg)	Barbera	1027±148	1062±100	1171±119
	Dolcetto	958±62	801±39	1107±32
	Nebbiolo	757	500±27	696
Anthocyanins pH 3.2 - A3.2 (mg/kg)	Barbera	572±71	523±64	607±38
	Dolcetto	576±16	487±25	667±33
	Nebbiolo	466	314±1	470
Flavonoids pH 1 - FT1 (mg/kg)	Barbera	2828±416	3151±290	3052±136
	Dolcetto	3723±301	3814±307	3500±419
	Nebbiolo	4284	3511±272	3526
Flavonoids pH 3.2 - FT3.2 (mg/kg)	Barbera	1621±235	1934±123	1651±57
	Dolcetto	2565±309	2789±344	2226±385
	Nebbiolo	3345	2745±229	2922
Absorbance at 280 nm - A280	Barbera	64.3±1.6	69.6±8.2	61.2±4.4
	Dolcetto	68.0±3.4	76.9±4.3	59.5±9.2
	Nebbiolo	71-1	67.9±5.0	70.8
EA%	Barbera	44.0±1.4	50.4±4.9	47.9±4.2
	Dolcetto	39.5±2.1	39.0±0.1	39.7±0.9
	Nebbiolo	38.1	36.1±2.6	32.4
Mp%	Barbera	61.5±3.5	70.4±2.8	55.9±3.1
	Dolcetto	62.5±0.7	71.8±0.3	51-7±4.4
	Nebbiolo	49.0	64.7±2.5	48.7

kg. In 2001, the values were lower with a minimum value of 332 mg/kg.

The FT1 values in both cultivars were high. The ratio between TF3.2 and TF1, which represents an assessment of the extractability fraction of the flavonoids, is characteristic of the cultivar. This ratio is on average >80% for Nebbiolo and <60% for Barbera.

The EA% values for cultivar Barbera were higher than Nebbiolo in both vintages (Δ=15-16). The Barbera grape values are due to a more rigid cell wall structure according to ROMERO-CASCALES *et al.* (2005).

The EA% values from the 2001 vintage were lower than those of 2000 because of a greater cell wall fragility that facilitated anthocyanin extraction (AMRANI-JOUTEI and GLORIES, 1994; 1995a).

The contribution wine tannins (Mp%) from the seeds in Barbera grapes was

Table 3A - Anthocyanin pattern of grapes produced in 2001 vintages (±standard deviation); n=3.

	N1	N2	N3	B1	B2	B3
Delphynidin-3-glucoside (%)	6.2±0.3	6.8±0.2	6.4±0.3	11.8±1.1	12.0±1.4	14.3±1.3
Cyanidin-3-glucoside (%)	16.0±1.3	18.9±1.9	16.4±1.4	10.3±1.0	11.4±1.2	9.4±1.0
Petunidin-3-glucoisde (%)	5.4±0.5	6.2±0.5	5.1±0.6	11.2±1.1	11.1±1.0	13.0±1.3
Peonidin-3-glucoside (%)	42.6±0.9	43.6±0.7	44.0±0.7	24.9±2.0	27.5±2.2	21.7±2.4
Malvidin-3-glucoside (%)	20.1±1.9	16.4±1.7	18.9±2.0	36.4±2.1	33.0±1-9	36.9±2.2
Σ acetylglucoside anthocyanins (%)	3.6±0.4	3.1±0.4	3.8±0.3	1.3±0.1	1.4±0.1	1.1±0.2
Σ cinnamoylglucoside anthocyanins (%)	6.2±0.5	5.1±0.6	5.4±0.6	4.1±0.1	3.6±0.2	3.6±0.2

[a]Abbreviation: B (Barbera), N (Nebbiolo).

higher in 2001 – a clear sign of incomplete maturation. In fact, according to AMRANI-JOUTEI and GLORIES (1994), DE FREITAS *et al.* (2000) and GLORIES (2001), the proanthocyanidin extraction decreases to the seed maturation level. The Mp% values in Nebbiolo show a limited variability between the vintages.

The anthocyanin profile was determined on the six grape stocks used in winemaking (Table 3A). The percentages of anthocyanidins examined were similar to those reported in the literature (CRAVERO and DI STEFANO, 1992; GUIDONI *et al.*, 1997).

Fig. 1 shows that anthocyanin extraction during maceration reached its highest value after three days for trial B2 (Barbera grapes); the EA% value was lower (41.0). Trial B1 yielded the anthocyanins more slowly, the EA% value was 45.6. The differences were less in the Nebbiolo grapes because of the low anthocyanin content values as well as the cell maturity index EA (9-20%).

The situation described above is also valid for the total flavonoid extraction (Fig. 2), where the slope of the extraction curve for Barbera was higher, with a decreasing EA% value. After the third day of maceration, the formation of ethanol tended to influence the extraction kinetics, making the trends more uniform. The cell maturity index (EA%) values are therefore a measure of the facili-

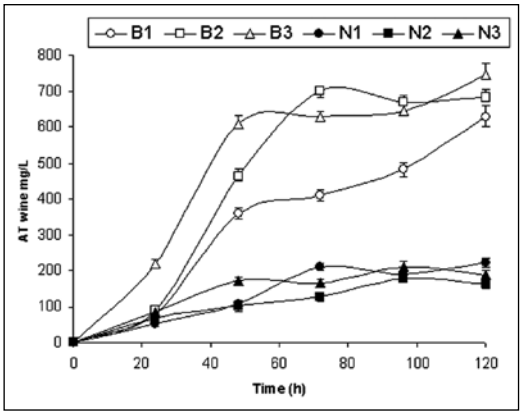

Fig. 1 - Evolution of the mean total anthocyanin (AT) contents during skin contact (2001); data as malvidin-3-glucoside chloride equivalent, mg/L. Grape: Barbera (B) and Nebbiolo (N).

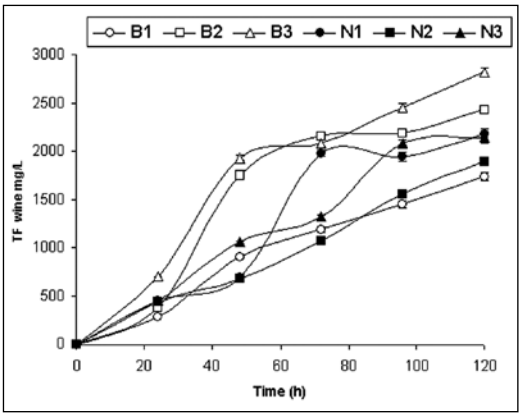

Fig. 2 - Evolution of the mean total flavonoid (TF) contents during skin contact (2001); data as (+)-catechin equivalent, mg/L. Grape: Barbera (B) and Nebbiolo (N).

Table 3B - Anthocyanin pattern of wines produced in 2000 and 2001 vintages (±standard deviation); n=3.

Sample[a]	N1		N2		N3		B1		B2		B3
year	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001	2001
Delphynidin-3-glucoside (%)	0.2±0.1	0.2±0.1	0.2±0.1	0.6±0.2	4.5±0.7	2.0±0.5	0.3±0.2	9.3±0.6	0.3±0.1	1.0±0.6	4.0±0.6
Cyanidin-3-glucoside (%)	0.1±0.1	0.9±0.4	0.2±0.1	1.3±0.2	4.5±0.6	1.8±0.3	0.3±0.1	2.8±0.2	0.3±0.1	1.1±0.6	2.4±0.4
Petunidin-3-glucoisde (%)	1.9±0.4	3.7±0.4	1.0±0.2	3.2±0.3	10.8±0.7	3.6±0.4	4.2±0.5	13.3±0.6	2.4±0.4	9.0±0.5	11.2±0.7
Peonidin-3-glucoside (%)	25.2±2.5	30.3±1.2	22.2±0.9	27.4±0.9	10.6±1.3	7.4±0.9	4.9±0.9	6.1±0.9	4.4±0.7	4.0±0.7	4.9±0.7
Malvidin-3-glucoside (%)	63.5±2.3	58.4±1.8	69.8±2.0	59.4±1.1	53.5±1.3	57.8±1.8	68.8±2.5	50.4±1.2	67.8±1.7	61.4±1.2	55.2±1.5
Σ acetylglucoside anthocyanins (%)	8.7±1.1	5.8±0.6	6.7±1.0	6.9±0.5	5.7±0.6	24.9±0.9	21.0±1.0	12.6±0.4	24.7±1.2	19.0±0.7	16.8±1.3
Σ cinnamoylglucoside anthocyanins (%)	0.6±0.2	0.6±0.2	0.1±0.1	1.1±0.4	1.3±0.3	2.2±0.6	0.2±0.1	5.5±0.6	0.3±0.1	4.3±0.6	5.4±0.6

[a]Abbreviation: B (Barbera), N (Nebbiolo).

ty with which the polyphenols, especially of the anthocyanins, were extracted during the first phases of maceration. Decreasing values correspond to increased ease of extraction.

The wines obtained in the 2000 and 2001 vintages from grapes taken from vineyards in which the phenolic maturity was monitored are described by general analytical parameters (Table 4A) and chromatic parameters (Table 4B).

The percentage recovery of anthocyanin pigments, expressed as the ratio between the average index of total anthocyanins in the wine (AT) and the corresponding index evaluated in grapes (A1 and A3.2) in the 2000 vintage, shows that there was 50% recovery in Nebbiolo wines compared to A3.2, and only 35% of the potential value at pH 1. Barbera wines presented better results especially with regard to the A3.2 values (on average 80%). These results were repeated in the 2001 vintage.

The amount of anthocyanins in the wines is dependent on the grape variety. In fact, in Barbera, the grapevine has an anthocyanin profile made up mainly of molecules tri-substituted in the B-ring (CRAVERO and DI STEFANO, 1992, DI STEFANO *et al.*, 2002; GERBI *et al.*, 2004) and therefore more protected against oxidation. The decrease in concentration of these pigments appears remarkably inferior to that of the Nebbiolo variety.

The wine anthocyanin profile (Table 3B) shows a considerable drop in di-substituted anthocyanins compared to the grape, particularly those with a catechol type structure on the B-ring. Moreover, a prevalence of the malvidin-3-glucoside (Mv3G) emerges as well in the case of Nebbiolo, instead of peonidin-3-glucoside (Pe3G), as in the grapes. Generally, a ratio Pe3G/Mv3G >1 is found in Nebbiolo wines (CAGNASSO *et al.*, 2001), but the opposite has also been found (GERBI *et al.*, 2002). The remarkable loss of di-substituted anthocyanins, easily extractable since the first phases of maceration,

Table 4A - Physicochemical characteristics of wines in 2000 and 2001 vintages (± standard deviation); n=3.

Sample[a]:	N1		N2		N3		B1		B2		B3
Vintage:	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001	2001
Alcohol (% vol.)	14.39±0.25	13.83±0.10	13.16±0.19	12.47±0.32	13.18±0.08	13.02±0.19	11.85±0.11	13.66±0.22	13.99±0.10	14.06±0.08	15.53±0.24
Reducing Sugars (g/L)	3.1±1.0	1.5±0.8	1.7±0.4	1.4±0.2	1.4±0.4	1.4±0.4	1.8±0.5	2.9±0.5	2.9±0.6	2.2±0.2	4.5±0.7
Total Dry Matter (g/L)	32.6±1.5	nd[b]	31.0±1.4	nd	29.2±0.8	nd	28.9±0.7	nd	35.4±0.7	nd	nd
pH	3.39±0.02	3.57±0.02	3.34±0.04	3.37±0.02	3.25±0.02	3.38±0.03	3.20±0.07	3.23±0.003	3.30±0.07	3.19±0.02	3.28±0.03
Total Acidity (g/L as tartaric acid)	6.7±0.1	6.2±0.2	7.2±0.5	8.8±0.4	7.8±0.4	7.2±0.2	10.8±0.6	10.2±0.3	8.5±0.4	8.0±0.1	8.2±0.4
Volatile Acidity (g/L as acetic acid)	0.28±0.04	0.26±0.06	0.21±0.04	0.23±0.02	0.27±0.05	0.23±0.05	0.35±0.06	0.44±0.04	0.40±0.02	0.40±0.02	0.45±0.02
Tartaric Acid (g/L)	2.2±0.1	2.2±0.2	2.5±0.4	2.4±0.1	2.5±0.2	2.3±0.2	3.7±0.2	3.7±0.2	3.2±0.2	4.2±0.2	3.2±0.1
Malic Acid (g/L)	1.7±0.5	1.8±0.25	2.3±0.2	1.7±0.3	2.3±0.2	2.3±0.2	5.4±0.4	3.7±0.2	2.2±0.3	1.4±0.1	1.8±0.1
Citric Acid (g/L)	0.38±0.07	0.26±0.04	0.4±0.1	0.18±0.05	0.30±0.15	0.23±0.04	0.3±0.0	0.40±0.06	0.72±0.07	0.48±0.03	0.35±0.04
Lactic Acid (g/L)	0.62±0.13	1.3±0.2	0.89±0.09	1.8±0.2	1.0±0.1	1.9±0.1	1.0±0.3	1.2±0.2	1.0±0.1	0.90±0.04	1.1±0.2
Succinic Acid (g/L)	0.7±0.1	nd	0.9±0.2	nd	0.8±0.1	nd	0.6±0.3	nd	0.5±0.1	nd	nd
Total Anthocyanins (mg/L as malvidin-3-glucoside chloride)	184±13	195±14	200±18	231±12	149±13	168±14	454±23	707±30	519±22	547±25	663±32
Combined Anthocyanins (%)	36.4±2.9	43.5±0.9	43.0±1.2	35.0±1.0	31.5±0.9	43.5±0.4	19.2±0.5	31.1±0.4	22.7±0.6	25.8±0.4	36.6±0.8
Total polyphenols (mg/L as (+)-catechin)	1863±43	2750±84	2351±58	2838±53	1883±78	2750±62	1453±54	2329±50	1854±38	2217±40	2880±63
Total Flavonoids (mg/L as (+)-catechin)	1458±53	2245±78	1846±63	2484±47	1372±79	1994±75	1337±53	2192±51	1504±47	2126±35	2657±56
Flavanols Vanillin Assay- FVA (mg/L as (+)-catechin)	1067±68	1348±91	1360±51	1539±49	1080±50	1517±68	360±21	721±30	340±21	741±33	917±42
Proanthocyanidins - PR (mg/L as cyanidin chloride)	2791±155	4370±149	3349±102	4801±110	2697±106	3976±110	1110±84	2244±107	1360±86	2139±109	2953±112
FVA / PR	0.38±0.01	0.31±0.01	0.41±0.00	0.32±0.00	0.42±0.01	0.38±0.01	0.32±0.01	0.32±0.00	0.25±0.00	0.35±0.02	0.35±002

[a]Abbreviation: B (Barbera), N (Nebbiolo);

[b]nd: not detected.

Table 4B - Chromatic characteristics of wines in 2000 and 2001 vintages (± standard deviation); n=3.

Sample[a]:	N1		N2		N3		B1		B2		B3
Vintage:	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001	2001
Clarity – L*	24.7±0.8	27.3±1.0	25.3±0.7	22.0±0.7	33.0±0.9	27.1±0.8	14.2±0.7	5.3±0.6	10.8±0.6	7.6±0.5	2.8±0.3
Hue – H* (rad)	0.599±0.003	0.610±0.002	0.606±0.002	0.581±0.003	0.618±0.002	0.613±0.002	0.471±0.002	0.259±0.004	0.399±0.002	0.322±0.002	0.234±0.003
Chroma – C*	71.2±0.06	72.7±0.4	72.9±0.5	66.1±0.4	79.2±0.5	69.8±0.4	53.8±0.5	34.8±0.4	47.7±0.5	41.3±0.4	21.1±0.3
Colour Intensity – CI (1 mm)	0.731±0.011	0.6658±0.009	0.773±0.007	0.878±0.012	0.572±0.010	0.840±0.010	1.421±0.009	2.693±0.012	1.778±0.011	2.114±0.008	2.697±0.013
Colour Tone – CT	0.651±0.004	0.693±0.003	0.602±0.004	0.570±0.005	0.588±0.004	0.648±0.004	0.451±0.002	0.399±0.004	0.437±0.003	0.411±0-003	0.439±0.004
% Yellow (A420/CI)	35.5±0.4	37.1±0.3	34.3±0.2	32.9±0.5	34.2±0.3	36.1±0.5	28.2±0.2	26.1±0.4	28.0±0.2	26.7±0.2	27.6±0.3
% Red (A520/CI)	54.9±0.7	54.0±0.4	57.1±0.3	58.1±0.7	58.4±0.3	55.7±0.2	63.2±0.4	65.5±0.6	63.1±0.4	64.7±0.3	62.2±0.4
% Blue (A620/CI)	9.5±0.2	8.9±0.1	8.1±0.2	8.9±0.3	7.4±0.1	8.2±0.2	8.5±0.1	8.4±0.2	8.8±0.1	8.7±0.1	10.1±0.2

[a]Abbreviation: B (Barbera), N (Nebbiolo).

is probably due to the complex processes of combination, oxidation and insolubilization that characterise anthocyanin-like substances during the course of winemaking (CHEYNIER *et al.*, 1994; 1997; CHEYNIER, 2000).

The situation regarding flavonoids in wine (TF) differs between the two cultivars studied. For Nebbiolo, the percentage found in wine with respect to grape is relatively low (≈ 60-70%) for TF3.2 and only 50-60% for TF1 in the 2000 vintage. Instead, higher values were found in 2001 with an increase in both percentages recovered.

Wines from Barbera show a TF content in wine greater than the potential shown in grapes at pH 3.2 (TF3.2) in both vintages. When TF wine was compared to the TF1 of grapes, the recovery factor varied, on average, from 50% in 2000 to 70% in 2001. Barbera grapes have few skin extractable proanthocyanidins (CRAVERO and DI STEFANO, 1992). The apparent anomaly of recovery percentage of TF3.2 can be explained on the basis of the high extractability of the seed proanthocyanidins during winemaking that depends, above all, on the ethanol concentration of the product. The aqueous solution (especially the pH 3.2 extraction) allows tannins with a low molecular mass only to be easily extracted.

A good correlation between wine and grape composition was recorded (Table 5). Total anthocyanins (AT) and chromatic parameters of the wine were highly correlated with the grape indexes (A1 and A3.2). The correlation associated with anthocyanin pigments highlights a secondary role of the cell maturity index (EA%) in determining the overall anthocyanin extraction. In fact, it seems that technology and the time of maceration produce a levelling action. Nevertheless, EA% does not appear superfluous because it provides information about how quickly anthocyanins can be extracted during the skin contact phase (Fig. 2).

The EA% parameter is useful for pro-

gramming the winemaking process in particular for grape varieties rich in easily degradable anthocyanins (Nebbiolo, Sangiovese, Pinot noir, Freisa).

The correlations found between the colour indexes of wine (Glories' and CIELAB parameters) with the same A1 and A3.2 indexes is significant for the colour of the future wine. They are characterised by correlation coefficients >0.93 and, in both cases, they are better than those carried out with the A3.2 index (Table 5).

The proanthocyanidin concentrations in red wines are represented by the PR and FVA indexes. These parameters were well correlated with the non-anthocyanin flavonoids (NAF1 and NAF3.2) and total flavonoids (TF1) in grapes. In particular, NAF3.2 seems better for assessing the flavanols that are reactive to vanillin (low-molecular proanthocyanidins). Considering that the seeds usually contain principally low-molecular weight flavanols (PRIEUR *et al.*, 1994; SOUQUET *et al.*, 1996) compared to the skin, the NAF3.2 value will tend to increase when the seed is less mature.

The linear regression equations of the best correlation, found when all data were considered, are shown (Table 6). The high R^2 value confirms that the grape indexes are useful for predicting the composition of the future wine by expressing the quantitative potential of the grape.

Industrial scale winemaking was carried out during the 2002, 2003 and 2004 vintages. Climatic conditions were very different during the three years; 2002 had a rainy pre-harvest period, 2003 had high temperatures for the entire ripening period along with widespread drought. 2004 was generally favourable with intermediate conditions between vintages. The correlation coefficients recorded in the industrial winemaking are shown in Table 7. The data refer to the single years; substantial improvements in the correlation coefficients in all three years were only noted in a few cases.

There was a good correlation between the total anthocyanins (AT) and colour parameters of the industrial wines with the A1 and A3.2 indexes of the grapes. These data seem to be in agreement with the ex-

Table 5 - Correlation coefficient between grape indexes and wine colour components in experimental winemaking (2000 and 2001).

Grape wine	A1	A3.2	A280	FT1	FNA1	FT3.2	FNA3.2
TP	-0.191	-0.040	0.650*	-0.125	0.202	0.703*	0.543
FT	0.129	0.263	0.645*	0.136	-0.059	0.560	0.301
FVA	-0.735**	-0.663*	0.549	-0.407	0.767**	0.798**	0.906***
PR	-0.643*	-0.533	0.574	-0.438	0.613*	0.762**	0.818**
AT	0.973***	0.978***	-0.074	0.695*	-0.874***	-0.380	-0.742**
L*	-0.946***	-0.964***	0.115	-0.668*	0.854***	0.347	0.711*
a*	-0.934***	-0.960***	-0.020	-0.735**	0.782**	0.207	0.605*
b*	-0.959***	-0.973***	0.078	-0.698*	0.849***	0.341	0.711*
H*	-0.949***	-0.969***	0.032	-0.716*	0.819**	0.268	0.654*
C*	-0.939***	-0.970***	0.045	-0.685*	0.829**	0.250	0.642*
TC	-0.909***	-0.877***	0.105	-0.666*	0.808**	0.470	0.762**
IC	0.946***	0.968***	-0.011	0.717**	-0.814**	-0.239	-0.633*
A420 (yellow)	0.935***	0.964***	0.001	0.710*	-0.804**	-0.212	-0.610*
A520 (red)	0.947***	0.966***	-0.018	0.715*	-0.818**	-0.254	-0.643*
A620 (blue)	0.934***	0.962***	0.007	0.729*	-0.788**	-0.199	-0.600

* p>0.95; ** p>0.99; *** p>0.999.

Table 6 - Regression coefficient (y=a+bx) between grape indexes and wine phenols and colour components in experimental winemaking (confidence interval for p=0.95).

Y wine	X grape	a	b	R^2	F
AT	**A1**	-28.94±79.12	0.5049±0.090	0.946	159.5***
AT	**A3.2**	-199.8±97.5	1.193±0.194	0.956	193.3***
IC	**A1**	-0.1045±0.4097	0.001887±0.000468	0.902	83.1***
IC	**A3.2**	-0.7553±0.4432	0.004495±0.000882	0.936	132.9***
TC	**A1**	0.7209±0-0719	-0.000241±0.000082	0.826	42.8***
TC	**A3.2**	0.7907±0.1120	-0.000540±0.000223	0.769	30.0***
C*	**A1**	91.14±10.11	-0.04316±0.01156	0.888	71.3***
C*	**A3.2**	106.54±9.80	-0.1039±0.0195	0.942	145.1***
H*	**A1**	0.7571±0.0778	-0.000350±0.000089	0.899	80.1***
H*	**A3.2**	0.8830±0.0816	-0.000850±0.000162	0.939	139.0***
L*	**A1**	36-66±5.33	-0.02367±0.00610	0.895	77.0***
L*	**A3.2**	45.04±5.91	-0.05670±0.0118	0.929	118.7***
A420	**A1**	0.0631±0.1092	0.000437±0.000125	0.875	62.8***
A420	**A3.2**	-0.0976±0.1108	0.00106±0.00022	0.929	118-3***
A520	**A1**	-01242±0.2782	0.001249±0.000318	0.898	78-9***
A520	**A3.2**	-0.5682±0.3019	0.002996±0.000601	0.934	127.2***
A620	**A1**	-0.01523±0.0440	0.000175±0.000050	0.873	61.9***
A620	**A3.2**	-0.07931±0.04564	0.000424±0.000091	0.925	111.5***
FVA	**FNA3.2**	29.3±358.1	0.7724±0.2722	0.821	41.2***

* p>0.95; ** p>0.99; *** p>0.999.

perimental wines described. The correlation coefficients are lower but still significant and are better if compared with A1. The regression equations (Table 8) have different values for the *a* and *b* coefficients for all three years; the values are less than those obtained in the experimental trials (Table 6). In 2003, a year with high temperatures during the ripening period, the AT values in wine were higher than those (AT3.2) in the grapes. The variability is mostly due to the different states of cell wall fragility which favoured easier extractions in 2002 and 2004 (AMRANI-JOUTEI and GLORIES, 1994; AMRANI-JOUTEI and GLORIES 1995ab). The lower A3.2 values in the 2003 harvest seems to have been due to lower efficiency of aque-

Table 7 - Correlation coefficient between grape indexes and wine phenols and colour composition in industrial winemaking (2002-2004).

Grape wine	A1	A3.2	A280	FT1	FT3.2
FT	-0.557**	-0.516**	0.626**	0.645***	0.708***
AT	0.930***	0.850***	-0.271	-0.338	-0.673***
TC	-0.705***	-0.750***	0.155	0.249	0.434*
IC	0.904***	0.786***	-0.310	-0.420*	-0.727***
A420 (yellow)	0.853***	0.723***	-0.300	-0.380	-0.685***
A520 (red)	0.934***	0.821***	-0.322	-0.449*	-0.756***
A620 (blue)	0.716***	0.624**	-0.226	-0.300	-0.556**

* p>0.95; ** p>0.99; *** p>0.999.

ous pH 3.2 extracting solution with low cell wall fragility.

The comparison of the TF indexes (Table 8) is good when the three years are grouped together, while the data for 2003 alone are not significant. This appears to have been due to cell walls that were less brittle which prevented the release of tannic flavonoids enclosed in the more complex cellular structures of anthocyanins (AMRANI-JOUTEI *et al.*, 1994; AMRANI-JOUTEI and GLORIES, 1994;1995a,b). In fact, the very high temperatures in July and August were excessively stressful to the vines in 2003, slowing down the enzymatic degradation of the polysaccharide structures.

In 2004 a non-linear relationship between TF wine and TF3.2 grape indexes was recorded (Fig. 3); this seems to indicate a lower estimate of the potential flavonoids in grapes (to medium-low concentrations). In fact, this is actually due to the low extraction efficiency of the aqueous buffer (pH 3.2) for the Barbera grape samples and to the more rigid cell wall structure.

In a less favourable vintage, the extraction of tannic substances is more standardized in industrial winemaking. This effect, caused by maceration conditions, tends to annul the grape variety differences.

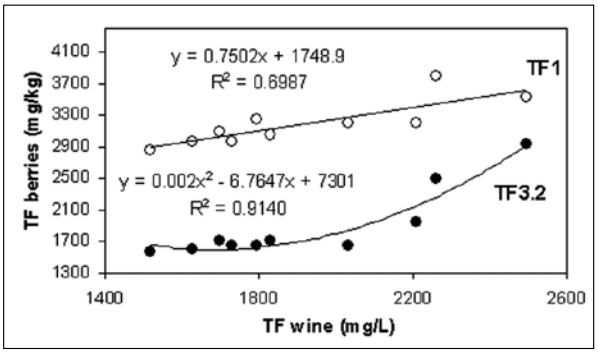

Fig. 3 - Regression between total flavonoids (TF) of the wine (industrial scale) and corresponding grape indexes to pH1 (TF1) and pH 3.2 (TF3.2) in 2004 (n=10).

CONCLUSIONS

The various parameters evaluated, including flavonoid indexes, have shown a clear connection with the phenolic composition indexes of wines. The phenolic parameters of the grapes considered can function as good prediction indexes of the future wine and are therefore of special technological interest. The EA% index measures the ease with which anthocyanins can be extracted in the first phase of skin contact.

It has been shown at the experimental level, and later verified in industrial winemaking, that the modality and time

Table 8 - Regression coefficient (y=a+bx) between grape indexes and wine phenols and colour components in industrial winemaking (confidence interval for p=0.95).

Y wine	X grape	a	b	R^2	F
AT	**A1**	-160.5±157.1	0.8163±0.1571	0.853	116.1***
AT	**A3.2**	-280.3±253.4	1.699±0.465	0.723	57.3***
IC	**A1**	-0.7101±0.6974	0.003104±0.000697	0.810	85.2***
IC	**A3.2**	-1.022±1.205	0.006274±0.002231	0.630	34.0***
TC	**A1**	0.8944±0.1480	-0.000385±0.000148	0.593	29.1***
TC	**A3.2**	1.0220±0.1622	-0.000947±0.00300	0.681	42.7***
A420	**A1**	-0.0872±0.2279	0.000777±0.000228	0.714	50.0***
A420	**A3.2**	-0.1343±0.3677	0.001513±0.000681	0.515	21.2***
A520	**A1**	-0.5769±0.3629	0.002001±0-000363	0.867	130.8***
A520	**A3.2**	-0.8224±0.6724	0.004129±0.001245	0.703	47.3***

* p>0.95; ** p>0.99; *** p>0.999.

of maceration can make the yield of the extraction process more uniform even for each variety studied. It is also true that different, carefully selected extraction modalities can highlight the differences between different grapes thus affecting the final characteristics of the wine. When designing a wine it is indispensable to choose the best-suited extraction conditions for the desired results.

The phenolic maturity indexes described here play an important role in the timing and modality during the maceration process so as to highlight the inherent potential of the grape.

The simplicity of the model showed that the correlation was strongly influenced by vintage. To interpret the data, a specific vintage reference must be defined. Therefore, new studies are indispensable if this limitation is to be overcome.

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PAPER

EFFECT OF LOW-DOSE GAMMA IRRADIATION ON THE QUALITY OF SWEET CHERRY DURING STORAGE

EFFETTO DI BASSE DOSI DI RADIAZIONI GAMMA SULLA QUALITÀ DI CILIEGIE DOLCI DURANTE LO STOCCAGGIO

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ABSTRACT

The efficacy of postharvest gamma irradiation treatment on sweet cherry cv. "0900 Ziraat" was tested under controlled atmosphere. Cherries were stored at six different atmosphere combinations for up to 60 days after being exposed to gamma irradiation. Weight loss values were higher in cherries stored under normal atmosphere compared to controlled atmosphere. Initial firmness was 14.21 and 15.97 N in non-irradiated and irradiated fruits, respectively. While the final values were 2.94

RIASSUNTO

È stata valutata l'efficacia di radiazioni gamma su ciliegie dolci della cultivar "0900 Ziraat" raccolte e conservate in atmosfera controllata. Le ciliegie, dopo essere state sottoposte a radiazioni gamma, erano conservate a 6 diverse combinazioni di atmosfera controllata fino a 60 giorni. I valori della perdita di peso erano più alti per le ciliegie conservate in atmosfera normale rispetto a quelle conservate in atmosfera controllata. La consistenza iniziale era 14,21 e 15,97 N nei frutti non irradia-

- Key words: controlled atmosphere, fungal decay, irradiation, *Prunus avium*, quality, storage -

N in normal atmosphere and 5.49 N in normal atmosphere+gamma irradiation. The highest acidity and ascorbic acid values were recorded in the fruit stored under controlled atmosphere (20:5, 25:5)+gamma irradiation. Irradiation reduced spoilage from 25.06 to 5.00%. The fungi most frequently isolated from sweet cherries were *Botrytis cinerea*, *Penicillium expansum*, *Monilinia fructicola*, *Alternaria alternata* and *Rhizopus stolonifer*. Better fruit quality was obtained when controlled atmosphere was combined with gamma irradiation compared with either normal atmosphere or controlled atmosphere. Sweet cherry cv. "0900 Ziraat" can be stored successfully for more than 60 days under controlled atmosphere (20:5)+gamma irradiation and partially controlled atmosphere (25:5)+gamma irradiation conditions.

ti ed irradiati rispettivamente; mentre i valori finali erano 2,94 N per i frutti conservati in atmosfera normale e 5,49 N nei frutti in atmosfera normale sottoposti a radiazioni gamma. Il più alto valore di acidità e di acido ascorbico veniva registrato nei frutti conservati in atmosfera controllata (20:5, 25:5) e sottoposti a radiazioni gamma. L'irradiazione riduceva lo sviluppo microbico dal 25,06 al 5,00%. I funghi più frequentemente isolati dalle ciliegie dolci erano il *Botrytis cinerea*, il *Penicillium expansum*, la *Monilinia fructicola*, l'*Alternaria alternata* e il *Rhizopus stolonifer*. La migliore qualità del frutto si otteneva quando l'atmosfera controllata era combinata al trattamento con radiazioni gamma sia se comparata con l'atmosfera normale o con l'atmosfera controllata. Le ciliegie dolci, cultivar "0900 Ziraat", possono essere conservate inalterate per più di 60 giorni in atmosfera controllata (20:5) dopo irraggiamento con raggi gamma ed in atmosfera parzialmente controllata (25:5) dopo irraggiamento con raggi gamma.

INTRODUCTION

Postharvest losses of sweet cherry fruit due to fungal decay often occur in spite of postharvest application of fungicides and other control measures. Postharvest fungicides are quite effective in controlling most fruit storage rots and are commercially used to minimize decay. However, some fungicides have lost their effectiveness because resistant strains have emerged (HOLMES and ECKERT, 1999). With increasing concern about the use of chemicals in food and the environment, there is renewed interest in nonchemical approaches to postharvest disease control (MATTHEIS and ROBERTS, 1993; EL-GHAOUTH *et al.*, 1995). Moreover, consumers are looking for fruit that is free of chemical residues. Consequently, alternative control strategies, such as antagonists, natural compounds and physical treatments have been investigated.

Gamma irradiation (GI) is a physical treatment that can be used to control postharvest diseases. GI has been used for disinfestation and to control decay and extend storage and shelf life of fresh fruits and vegetables. Besides exhibiting fungicidal effects, these treatments can also induce resistance in fruit. The choice of a suitable dose of irradiation

is particularly important to determine the conditions for disinfestation by irradiation of stored foods (WILSON *et al.*, 1994). Irradiation inhibits ripening and senescence, prolongs shelf life and reduces spoilage in many fruits. DRAKE and NEVEN (1997) reported that gamma rays reduce storage rot and delay ripening in sweet cherry fruit. Interest in the use of irradiation has increased in recent years due to its effectiveness for disinfestation and enhanced food safety. Moreover, the overall quality of irradiated cherries is better than methyl bromide-treated cherries (NEVEN and DRAKE, 2000).

For fruit having high export value such as sweet cherry, it is important to improve storage conditions, prolong storage time and develope new pre- and postharvest treatments. Sweet cherries can usually be stored for 1 to 4 weeks at 1-0°C and 85-90% relative humidity (RH). The storage time can be extended if the cherries are kept under modified atmosphere packaging (MAP) and controlled atmosphere (CA) conditions combined with postharvest treatments (THOMPSON, 2001; TIAN *et al.*, 2001; JIANG *et al.*, 2002; PADILLA-ZAKOUR *et al.*, 2004; TIAN *et al.*, 2004).

In this study, sweet cherry cv. "0900 Ziraat", which is widely grown in Turkey and of high export value, was used. The combined effect of GI and different atmosphere concentrations on the quality characteristics and growth of pathogenic fungi in fruit stored in 0.03:21 (CO_2%:O_2%) (normal atmosphere: NA, control), 5:5, 10:5, 15:5, 20:5 and 25:5 were investigated.

MATERIALS AND METHODS

Sweet cherry cv. "0900 Ziraat" (*Prunus avium* L.) was used in studies conducted in 2004 and 2005. Trees were located in the Commercial Agricultural Cooperative Orchard at Keles, Bursa, Turkey. The cherries were hand-picked at commercial maturity and were immediately transported to the laboratory. Cherries without wounds and/or rots were sorted for uniformity and maturity. A total of 150 kg of sweet cherries cv. "0900 Ziraat" were used in each year of the experiments. In the study, a total of 150 kg of sweet cherries were used, 75 kg for each treatment. The initial analyses were carried out after the sweet cherries were harvested; the fruit was divided into four groups: 1) fruit stored under NA conditions (control, 0.03:21 (CO_2%:O_2%) at 0°C) without irradiation, 2) fruit stored under CA (5:5 (CO_2%:O_2%), 10:5, 15:5, 20:5 and 25:5 at 0°C) without irradiation, 3) fruit stored under NA after being treated with post harvest irradiation and 4) fruit stored under CA after being treated with post harvest irradiation.

Following harvest, cherries were transported to an irradiation facility and exposed to GI at a dose of 0.30 kGy at 23°C. Irradiation was carried out in a Gamma Beam 650 (Nordion International Inc, Kanata, Ont., Canada) facility located at the Ali Osman Sonmez Nuclear Medicine Center in Bursa, Turkey. The Gamma Beam facility contains approximately 14,000 curies of cobalt-60. All samples were irradiated in a single geometric configuration at a dose rate of approximately 240 Gy/h and calibrated at the preferred geometry. The actual dose distribution was determined using alanine dosimeters (Bruker Instruments Inc., Billerica, MA). The ratio of maximum to minimum dose received was 1.4. Details of irradiation treatment were described earlier by FAN *et al.* (2001).

After irradiation, cherries were precooled with cold air (room cooling) in the Cold Storage Unit and Postharvest Physiology Laboratory, Uludag University, Bursa, Turkey. The cherries were placed in cardboard boxes (30x40x13 cm) with a 5 kg capacity and stored in

cabinets in a cold room at 0±0.5°C and 90±5% RH for 60 days. Shelf-life was determined by keeping the cherries under ambient conditions (20±2°C and 60±5% RH) for 2 days after removing them from storage.

Sweet cherries packaged in cardboard boxes with 5 kg capacity were placed in cold CA cabinets of 120 L volume, in which the internal atmosphere combinations could be controlled. Sweet cherries were stored under different atmosphere combinations (0.03:21 (CO_2%:O_2%), 5:5, 10:5, 15:5, 20:5 and 25:5). The atmospheric combinations in the CA cabinets were controlled by using a "BBG Goerz Metrowatt" recorder (Servomex Group Limited, Sussex, UK), "Servomex 1410" CO_2 analyzer (Servomex Group Limited, Sussex, UK), and "Servomex 1420" O_2 analyzer (Servomex Group Limited, Sussex, UK). If there were atmospheric changes, the atmosphere combinations were maintained through full automatic gas addition and/or CO_2 scrubbers (activated charcoal). Changes in the CO_2 and O_2 rates in each plastic container were maintained at 0.1-0.2%.

Weight loss during storage was determined by weighing samples on a Sartorius precision balance (0.01 g precision) (Sartorius Co., Göttingen, Germany) before storage and at different sampling times during storage. Fruit firmness and stem abscission force measurements were read as N using penetrometer probes (Sundoo SH-50, Wenzhou Sundoo Instruments Co., Ltd., Zhejiang, China). Monthly samples were taken from each treatment with two replicates; there were 30 cherries in each replicate and the means are reported. Measurements were taken as a single reading of each cherry sampled. Titratable acidity was determined by titration with 0.1 N NaOH to pH 8.1 and is expressed as percent of malic acid (BERNALTE *et al.*, 2003). The ascorbic acid content in the cherries was determined by subjecting of the samples to extraction with oxalic acid (0.4%) and then reading and calculating the absorbance at 520 nm in the spectrophotometer (Shimadzu UV-120-01) (Shimadzu Co., Duisburg, Germany) (KILIC *et al.*, 1991). The anthocyanins of the samples taken from the fruit pericarp were extracted in methanol:1% HCl and the absorbance values were recorded at 530 nm. The results were reported as the optical absorbance value of 1 g of sample in 100 mL methanol-HCl (SHULMAN and LAVEE, 1971). The number of rotten cherries was determined and compared to the total number of cherries and expressed as percentage. The fungi from diseased fruit were isolated according to general procedures and identified according to morphological structures. The pathogenicity of the fungi isolated from the fruits was determined according to EL-GHAUTH *et al.* (1995). Pathogenicity studies were carried out in an incubator at 25°C with three replicates of 30 cherries per replicate. The diameters of the lesions that formed on the cherries were measured 48 h after inoculation. These replicates of thirty noninoculated cherries each were kept in NA and CA at 0±0.5°C and 90±5% RH and served as the controls; the diameters of the lesions that formed on the fruit were measured. The percentages of isolated fungi were calculated by dividing the total number of isolated fungi by the total number of isolated material (fungi-infested and noninfested material).

The experimental design was a Randomized Plot Factorial Experimental Design with two replicates, consisting of 5 kg of fruit (one box) per replicate. Each replicate consisted of three samples of 350 g each that were analyzed separately and the results were combined to obtain the mean/replication. The results were analyzed using ANOVA and the means were compared using the LSD test ($P<0.05$).

RESULTS AND DISCUSSION

Weight loss

Weight loss increased (20.52% without GI, 17.32% with GI at the end of cold storage) during NA storage (Table 1). However, under CA conditions cherry maturation was delayed and weight loss reduced compared to those stored without CA. A higher moisture content in the CA minimized weight loss in the fruit. The cherries stored at a low temperature had less weight loss. The atmosphere surrounding the fruit provided a good barrier to moisture transfer. The lowest weight loss after cold storage+shelf life (2.83%) was observed in the CA (20:5)+GI treatment in which fungal growth was retarded or even suppressed (Table 2). Weight loss and fruit rot proceeded slowly due to less water loss in these treatments. At the end of storage, weight loss values under CA (5:5, 10:5, 15:5, 20:5, 25:5) and CA+GI were 8.23%, 7.87%, 7.82%, 6.52%, 6.80% and 6.98%, 6.37%, 6.18%, 2.83%, 3.64%, respectively. The differences between weight losses of fruit sealed in different atmospheres and those with GI treatment indicated that weight losses are related to the treatment. So the positive effects of storing the cherries in sealed plastic cold chambers may be due to a combination of the effects on the CO_2 and O_2 contents in the fruit and the maintenance of high moisture content. WANG and VESTRHEIM (2003), ERIS *et al.* (2004) and QIN *et al.* (2004) also reported weight loss in untreated sweet cherry fruit. In another study, peaches treated with pulsated ultraviolet light had a greater water loss than the untreated control after15 days, but not after 30 days of cold storage (CRISOSTO *et al.*, 1998).

Fruit firmness and stem abscission force

The firmness of the sweet cherries decreased in all treatments. The lowest firmness value (2.94 N) was obtained from cherries kept in NA (Table 1), while those kept in higher CO_2 concentrations, 20-25%, were firmer. Stem abscission force declined with the storage time. A marked decline was recorded on day 60 of storage in NA (Table 1); the situation was similar at the end of the shelf-life. The lowest stem abscission force value was obtained from cherries kept in NA. Cherries with less weight loss and higher firmness values had higher stem abscission force values. CA (20:5)+GI and CA (25:5)+GI gave good results (9.02 and 8.13 N) compared to other treatments. The stem abscission force values decreased with the ripening of the fruit. Previous studies have shown that sweet cherries may respond favorably to non-ambient gas levels. Sweet cherry firmness and color, for instance, improved with storage in 20-25% CO_2 or 0.5-2% O_2 (THOMPSON, 2001; WANG and VESTRHEIM, 2003). Cherries stored in MAP retained their firmness more than the control cherries stored in air (SERRANO *et al,.* 2005; HARB *et al.,* 2006). CA delayed the loss of fruit firmness (TIAN *et al.,* 2004). KAPPEL *et al.* (2002) reported that sweet cherries stored in NA became softer, whereas MA-stored sweet cherries tended to have a similar firmness or were even firmer than at harvest. CHAPON and BONY (1990) found that retention of sweet cherry firmness was best in 15% CO_2+5% O_2, but no increase in firmness during storage was detected. In addition to these studies, irradiation has been used with apricots, cherries, and peaches as a quarantine treatment at 300 Gy or less with little change in quality. Some loss of firmness and texture occurs when irradiation is used, but this quality loss is minimal. If an irradiation dose above 300 Gy is used, quality loss in firmness, color and internal breakdown occurs (DRAKE and NEVEN, 1998). When "Hedelfingen" and "Lapins" sweet cherries (*Prunus avium*) were stored in air or in two types of modified

Table 1 - Weight loss, firmness, stem abscission force and acidity of sweet cherry cv. "0900 Ziraat" under NA and CA.

Stor. time (days)[a]	Treat. (CO_2%: O_2%)	Weight loss (%)		Firmness (N)		Stem abscission force (N)		Acidity (%)	
		WGI[d]	GI	WGI	GI	WGI	GI	WGI	GI
0	NA[c]	0.00	0.00	14.21 a	15.97 a	8.62 a	10.39 b	0.77 a	0.79 a
LSD		-		1.16		0.18		0.03	
30	NA	12.55 a[e]	9.26 b	5.10 b	8.13 ab	5.49 a	7.45 a	0.67 ab	0.70 ab
	5:5	1.93 c	0.55 c	8.62 ab	8.72 ab	7.06 a	12.15 a	0.68 ab	0.73 ab
	10:5	2.36 c	0.64 c	9.02 ab	9.31 ab	7.64 a	11.17 a	0.65 a	0.72 ab
	15:5	1.77 c	0.55 c	8.33 ab	9.21 ab	7.74 a	12.64 a	0.69 ab	0.76 ab
	20:5	1.48 c	0.17 c	10.78 a	11.86 ab	7.45 a	12.64 a	0.72 ab	0.78 a
	25:5	1.54 c	0.27 c	10.00 a	10.09 a	8.04 a	12.25 a	0.68 ab	0.73 ab
LSD		2.86		0.47		0.74		0.11	
60	NA	20.52 a	17.32 a	3.82 c	8.37 abc	2.74 e	5.78 cd	0.37 f	0.47 e
	5:5	4.57 b	3.15 b	6.47 abc	8.53 ab	5.59 cd	8.62 b	0.53 de	0.64 ab
	10:5	4.70 b	3.35 b	5.78 bc	8.04 ab	4.21 de	8.53 b	0.52 de	0.63abc
	15:5	4.26 b	2.59 b	5.88 bc	8.62 ab	5.29 d	9.11 ab	0.55 cde	0.67 ab
	20:5	3.94 b	1.60 b	7.15 ab	9.80 a	8.43 b	11.07 a	0.59 bcd	0.69 a
	25:5	3.99 b	2.00 b	7.15 ab	8.04 ab	7.55 bc	9.70 ab	0.60 bcd	0.67 ab
LSD		4.79		0.30		0.23		0.09	
62[b]	NA	22.95 a	18.97 a	2.94 d	5.49 bc	1.86 d	4.70 c	0.25 f	0.40 de
	5:5	8.23 b	6.98 bc	4.80 cd	6.37 abc	4.02 cd	5.98 bc	0.34 e	0.34 e
	10:5	7.87 bc	6.37 bc	5.59 bc	6.76 abc	5.68 bc	6.47 bc	0.39 e	0.40 de
	15:5	7.82 bc	6.18 bc	5.00 cd	7.15 abc	4.80 c	6.57 bc	0.37 e	0.39 e
	20:5	6.52 bc	2.83 c	6.17 bc	8.82 a	7.64 ab	9.02 a	0.52 bc	0.62 a
	25:5	6.80 bc	3.64 bc	6.47 abc	7.55 ab	6.37 bc	8.13 ab	0.47 cd	0.56 ab
LSD		5.14		0.26		0.25		0.07	

[a] Storage conditions were 0±0.5°C and 90±5% RH.
[b] Shelf life conditions were 20±2°C and 60±5% RH for 2 days.
[c] Normal atmosphere (0.03:21 CO_2%:O_2%).
[d] Without gamma irradiation.
[e] Means within a column and between WGI and GI at each storage time of observation followed by different letters differ significantly at P<0.05.
The data in the table are the average of the experimental values from two years.

atmosphere (MA) bags (LifeSpan 204 and 208) at 3°C (37.4°F) and 90% relative humidity for 4 weeks, the control cherries had significant shriveling and browning of stems while MA-stored cherries had healthy green stems after 4 weeks (PADILLA-ZAKOUR *et al.*, 2004).

Titratable acidity

There were slight differences in titratable acidity of cherries among the treatments. Titratable acidity of sweet cherries decreased during storage (Table 1); the decrease was greater in the sweet cherries stored under NA conditions, than those stored under CA. The lowest titratable acidity values were found in CA-stored (5:5) cherries (0.34%), while the highest value (0.62%) was obtained with the CA (20:5)+GI treated cherries. The reduction in titratable acidity was the result of the acids being involved in physiological processes such as respiration. This is another important factor with regard to the treatments, es-

pecially in terms of slowing the loss of acidity, which led to a decrease in fruit quality. Using these methods, changes in fruit quality during storage could be kept within certain limits, since sugar accumulation was stable, and loss of titratable acidity was retarded in the fruit stored under the CA+GI conditions. Results from previous studies were ambiguous regarding the effects of CA on acid retention. While, CHAPON and BONY (1990) did not find any effects of storage atmosphere on acid retention in cherries, MATTHEIS *et al.* (1997) found that the highest titratable acidity concentrations occurred in fruit stored in the highest CO_2 concentrations. In addition, it has been reported that the titratable acidity was reduced by irradiation regardless of dose in "Rainier" cherries, but no change in titratable acidity was evident for irradiated "Bing" cherries (DRAKE and NEVEN, 2006).

Ascorbic acid

Ascorbic acid content decreased in cherries stored at either ambient temperature or cold temperature. Similar results were reported by TIAN *et al.* (2001) and JIANG *et al.* (2002). The ascorbic acid contents of sweet cherries varied according to the treatment. Ascorbic acid decrease was usually greater in the sweet cherries stored under NA conditions, than in those stored under CA. Similar to the results in this study, ascorbic acid loss was greatest in the sweet cherry stored at high temperatures under NA (JIANG *et al.*, 2002; TIAN *et al.*, 2004). In this study, the ascorbic acid loss was the highest (16.55 mg/100 g) in cherries stored in high CO_2 and GI (Table 2). TIAN *et al.* (2004) found that sweet cherries stored in 10% CO_2:5% O_2 had higher ascorbic acid content than those stored in other treatments. High CO_2 treatment retarded the changes in ascorbic acid. Moreover, YAMAN and BAYINDIRLI (2002) reported that semperfresh coatings were effective in reducing the ascorbic acid loss during storage due to the low oxygen permeability of sucrose polyester coating which reduced enzyme activity and prevented oxidation of ascorbic acid.

Total anthocyanin

Anthocyanins are responsible for the red color in cherries (GARDINER *et al.*, 1993). Increases were observed in total anthocyanin values of sweet cherries at different rates over storage time. There were differences among NA and CA groups. During storage, the anthocyanin content increased by 42 and 34% in NA and NA+GI, respectively. The lowest total anthocyanin values (33.49 mg/100 g) recorded at the end of storage occurred in 20% CO_2+5% O_2 with GI (Table 2). The anthocyanin values were in the range of 30-40 mg per 100 g of fruit. Increase in anthocyanins during storage has been reported by several authors (KALT *et al.*, 1999; WANG and STRETCH, 2001). According to GAO and MAZZA (1995), fruit from NA can be considered "dark red cherries" as they have an anthocyanin content higher than 82 mg per 100 g fresh weight.

Disease incidence
and fungal isolation

The highest percentage of rotten fruit was recorded at the end of storage plus shelf life from the fruit stored under NA (NA: 25.06%; NA+GI: 17.60%) conditions. The decay rate was lower in fruit stored in CA+GI compared to CA at the end of storage. In these treatments, 20:5 atmosphere combinations gave the lowest decay rate, and the fruit had a better overall appearance at the end of shelf life (Table 2). GI has been used to control decay, disinfest and extend the storage and shelf life of fresh fruit and vegetables (DRAKE and NEVEN, 1998). In the present study, the most frequently isolated fungal pathogens were *B. cinerea*

Table 2 - Ascorbic acid, total anthocyanin and percentage of fruit decay in sweet cherry cv. "0900 Ziraat" under NA and CA.

Stor. time (days)[a]	Treat. (CO_2%:O_2%)	Ascorbic acid (mg/100 g)		Total anthocyanin (mg/100 g)		Fruit decay (%)	
		WGI[d]	GI	WGI	GI	WGI	GI
0	NA[c]	24.47 a[e]	25.49 a	38.26 a	33.13 a	0.00	0.00
LSD		3.95		6.51		-	
30	NA	12.72 bc	11.31 c	58.43 a	47.25 b	4.48 a	1.41 b
	5:5	16.32 abc	14.39 abc	40.85 bcd	32.89 c-f	1.59 b	0.00 b
	10:5	16.63 abc	16.97 abc	41.08 bc	32.25 def	1.13 b	0.00 b
	15:5	18.55 ab	18.97 ab	40.59 bcd	31.50 ef	0.88 b	0.00 b
	20:5	20.20 a	19.20 ab	39.11 b-f	30.39 f	0.00 b	0.00 b
	25:5	19.82 a	19.25 ab	39.83 b-e	31.61 ef	0.00 b	0.00 b
LSD		6.56		8.77		1.62	
60	NA	10.13 c	10.62 c	58.85 a	48.74 b	16.82 a	11.69ab
	5:5	12.64 bc	13.14 abc	43.88 bc	34.02 de	7.16 bc	5.95 c
	10:5	13.58 abc	13.78 abc	43.58 bc	34.60 de	6.68 bc	5.51 c
	15:5	14.52 abc	14.86 abc	41.05 bcd	32.29 e	5.16 c	3.84 c
	20:5	17.72 ab	18.36 a	39.56 cde	30.99 e	4.40 c	2.90 c
	25:5	16.99 ab	17.57 ab	42.22 bcd	34.34 de	5.41 c	3.79 c
LSD		5.27		8.73		5.44	
62[b]	NA	7.30 d	9.90 cd	64.96 a	49.96 b	25.06 a	17.60 b
	5:5	10.82 bcd	11.88 bc	45.14 b	34.33 de	10.79 cd	7.69 c-f
	10:5	10.56 bcd	13.01 abc	44.45 bc	36.06 cde	10.96 c	8.17 c-f
	15:5	12.36 abc	13.91 abc	42.89 bcd	34.44 de	9.67 cde	7.34 cf
	20:5	14.69 ab	16.55 a	41.62 b-e	33.49 e	7.22 def	5.00 f
	25:5	13.42 abc	14.76 ab	43.36 bcd	34.85 de	7.96 c-f	6.21 ef
LSD		4.22		9.05		3.78	

[a] Storage conditions were 0±0.5°C and 90±5% RH.
[b] Shelf life conditions were 20±2°C and 60±5% RH for 2 days.
[c] Normal atmosphere (0.03:21 CO_2%:O_2%).
[d] Without gamma irradiation.
[e] Means within a column and between WGI and GI at each storage time of observation followed by different letters differ significantly at P<0.05.
The data in the table are the average of the experimental values from two years.

Pers.:Fr., *P. expansum* Link., *M. fructicola* (G.Wint.), *A. alternata* (Fr.:Fr.) Keissl. and *R. stolonifer* (Erhenb.:Fr.). The isolation ratios of these pathogens under NA (0:21), NA (0:21)+GI, CA (20:5) and CA (20:5)+GI conditions were 87.75, 69.63, 84.50 and 95.00% for *B. cinerea*, 9.38, 21.88, 7.25 and 5.75% for *P. expansum*, 2.62, 12.50, 5.00 and 0.50% for *M. fructicola*, 1.00, 9.00, 3.25 and 0.00% for *A. alternata*, 0.00, 0.00, 0.00 and 0.00% for *R. stolonifer*, respectively (Fig. 1). The GI treatment was effective against fungal diseases. *A. alternate* and *R. stolonifer* did not grow under NA and CA conditions at 0°C and 90% RH. GI was more effective than the WGI, but not as effective as desired in all the other treatments. This might be the result of a higher artificial inoculum density compared with a natural inoculum density. CA (20:5)+GI treatment was the most effective against the fungi in "0900 Ziraat" but they were not 100% effective in either atmosphere con-

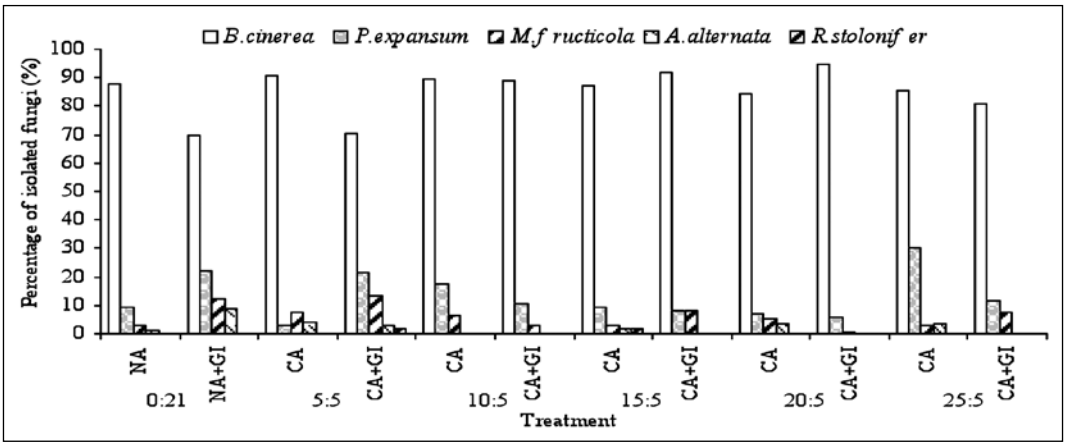

Fig. 1 - Percentage of fungi isolated from sweet cherry cv. "0900 Ziraat" after 60+2 days of storage+shelf life (The data in the figure are average of the experimental values from two years).

dition. The most important fungal pathogens that cause postharvest decay in sweet cherry were *B. cinerea*, *P. expansum*, *M. fructicola*, *A. alternata* and *R. stolonifer* (TIAN *et al.*, 2001; QIN *et al.*, 2004; YAO and TIAN, 2005). The application of additives can improve the biocontrol of brown rot in sweet cherry under various storage conditions. Disease control is enhanced directly because the additives inhibit pathogen growth, and indirectly because the additives have little influence on the growth of antagonistic yeasts (QIN *et al.*, 2006).

CONCLUSION

Sweet cherry cv. "0900 Ziraat" can be stored for 1-3 weeks under NA conditions, while this period can be increased by using different treatments under CA conditions. Changes in the quality of CA fruits can be kept within determined ranges by also using GI. Postharvest diseases were reduced under high CO_2 and low O_2 with GI treatment during storage. Spoilage and maturity were accelerated in NA. Irradiation and CA can be used as a disinfectant to preserve the quality of "0900 Ziraat" sweet cherry. In conclusion, the sweet cherry cv. "0900 Ziraat" can be successfully stored for more than 60 days using CA (20:5)+GI.

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PAPER

SOME QUALITY PROPERTIES OF THREE SWEET CHERRY (*PRUNUS AVIUM* L.) CULTIVARS DURING SHELF LIFE

ALCUNI PARAMETRI DI QUALITÀ DI TRE CULTIVARS DI CILIEGIE DOLCI (*PRUNUS AVIUM* L.) DURANTE LA CONSERVAZIONE

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ABSTRACT

Some quality properties (stem rupture force, firmness, tensile strength and penetration depth, total soluble solids and percentage of decayed fruit) of three cherry cultivars ("Van", "Bing" and "0900 Ziraat") during shelf life were investigated. The cherries were kept at 20±1°C and 60±5% relative humidity for 7 days and quality changes in the fruit were determined at one day intervals. Stem rupture force and firmness varied among cultivars, "Van" being the best, followed by "Bing" and

RIASSUNTO

Sono stati studiati alcuni parametri di qualità (resistenza al distacco del picciolo, consistenza, resistenza alla tensione e profondità di penetrazione, solidi solubili totali e percentuale di frutti deteriorati) di tre cultivars di ciliegie ("Van", "Bing" e "0900 Ziraat") durante la conservazione. Le ciliegie venivano conservate a 20±1°C ad una umidità relativa di 60±5% per 7 giorni e i cambiamenti nei parametri di qualità del frutto venivano determinati ad intervalli di un giorno. La resistenza al di-

- Key words: decay ratio, firmness, shelf life, stems rupture force, sweet cherries, tensile strength, total soluble solids -

"0900 Ziraat" cultivars. The highest TSS content of the fruit was obtained from "0900 Ziraat" (14.62%); "Van" and "Bing" were 13.00% and 12.39%, respectively. At the end of the study, the lowest TSS content was obtained from "Bing" and the highest from "0900 Ziraat". The highest initial tensile strength was 1.024 MPa for "Van" at harvest. However, this cultivar had the lowest tensile strength (0.171 MPa) on the 7th day. The "0900 Ziraat" cv. had the lowest percentage of decayed fruit (8.38%) and had better quality results than the other cultivars under shelf life conditions.

stacco del picciolo e la consistenza variavano fra le diverse cultivars, la migliore risultava essere la "Van", seguita dalla "Bing" e dalla "0900 Ziraat". La cultivar "0900 Ziraat" presentava il contenuto più alto di solidi solubili totali (SST) con il 14,62%, la "Van" e la "Bing" presentavano un contenuto di SST del 13,00 e del 12,39%, rispettivamente. Al termine di questo studio la cultivar "Bing" presentava il contenuto minore di SST mentre la cultivar "0900 Ziraat" ne presentava il maggiore. La più alta resistenza alla tensione iniziale era di 1,024 MPa per la cultivar "Van" al momento della raccolta. Tuttavia, questa cultivar, presentava la più bassa resistenza alla tensione (0,171 MPa) al 7° giorno. La cultivar "0900 Ziraat" presentava la più bassa percentuale di frutti deteriorati (8,38%) e una qualità migliore rispetto alle altre cultivars durante la conservazione.

INTRODUCTION

Sweet cherries are an important crop for fresh consumption in Turkey, and in the past decades production has expanded rapidly to 250,000 tonnes (SIS, 2003; FAO, 2003). While a considerable part of the cherry production is exported, the expansion of fresh cherry exports is hampered by its relatively short storage and shelf life (PAULL, 1999).

The following sweet cherry cultivars are cultivated in Turkey: Noir De Guben, Stella, "Van", "Bing", "Bigarreau", "Edirne", "Turfanda", "Karabodur", "0900 Ziraat", "Karagevrek" and "Lambert". The most common sweet cherry exported from Turkey is the "0900 Ziraat", also known as Uluborlu Napolyon. Information regarding the physical, mechanical and chemical properties of sweet cherry fruit is needed for designing harvesting equipment and post-harvest technology such as transporting, storing, cleaning, separating, sorting, sizing, packaging and processing it into different foods (VURSAVUŞ *et al.,* 2006).

Fruit firmness is an important quality attribute that is directly related to enhancing storage and shelf life potential. It also influences susceptibility to decay and mechanical damage (BARRET and GONZALEZ, 1994). Fruit stem rupture force and total soluble solids (TSS) can be used to determine the best time to harvest the fruit. Many studies have been conducted on the physico-chemical, physico-mechanical, storage, and nutritional properties of fruit, such as sweet cherry (ESTI *et al.*, 2002; MUSKOVICS *et al.*, 2006; VURSAVUŞ *et al.*, 2006; BERNALTE *et al.*, 2003; MITCH-

AM *et al.*, 1998) and cornelian cherry (DEMIR and KALYONCU, 2003; GULERYUZ *et al.*, 1998).

Sweet cherry is prized by consumers because it is an early season fruit of excellent quality. The main quality indices are skin colour, which is related to fruit ripening and is affected by the anthocyanin concentration (SERRANO *et al.*, 2005), and the total soluble solids total/acidity ratio (TSS/TA) at harvest. Both parameters, together with the absence of stem browning, greatly influence consumer acceptance (CRISOSTO *et al.*, 2003). TSS ranges from 11° to 25° brix depending on the cultivar and depends mainly on the glucose and fructose content and less on the presence of sucrose and sorbitol. Sweet cherry fruit deteriorates rapidly after harvest and, in some cases, does not reach consumers at optimal quality after transport and marketing. The main aspects of sweet cherry deterioration are weight loss, colour changes, softening, surface pitting, stem browning and loss of acidity, while the variation in TSS is limited (BERNALTE *et al.*, 2003; IPPOLITO *et al.*, 2005).

For fruit having high export value such as sweet cherry, it is crucial to prolong shelf life. No detailed study concerning the shelf life of sweet cherry exists in the literature. The aim of this study, therefore, was to evaluate the physical properties of three high export value sweet cherry cultivars, which are grown widely in Turkey. The measurements were made at various times of shelf life.

MATERIALS AND METHODS

Fruit

Three sweet cherry cultivars ("Van", "Bing" and "0900 Ziraat") grown in the South-Marmara Region of Turkey were used for all the experiments. Samples of "Van", "Bing" and "0900 Ziraat" were carefully hand-picked in 2007 from an orchard located in Inegol, Dipsizgol village, Bursa, Turkey. After harvest, the fruit was immediately placed in thermal bags, cooled and transported to the postharvest physiology laboratory. The fruit, without wounds or decay, was sorted according to size and maturity. The cherries were then placed in cardboard boxes (30x40x13 cm) having a 5 kg capacity. A total of 15 kg of sweet cherries were used, 5 kg per cultivar. Parameters such as stem rupture force, firmness, tensile strength and penetration depth, TSS and percentage of decayed fruit were measured daily throughout the shelf life period. Experiments were carried out at a room temperature of 20±1°C and 60±5% relative humidity. All of the tests were performed at the Biological Material Laboratory of the Agricultural Machinery and Postharvest Physiology Laboratory of Department of Horticulture of Uludag University.

Stem rupture force and firmness

The stem rupture force and firmness properties of the cherries were estimated by performing compressive stress-strain experiments by means of a biological material test device. The device was equipped with a 50 N capacity load cell (Sundoo 50 SH Digital Push Pull Gauge, Wenshou, China). Hooked tips were mounted on the measuring device to measure the stem rupture force of the cherries. The fruit was placed on the hook and the stem was pulled vertically by hand. The force values as the stem broke off from the fruit were recorded. The accuracy of N was 0.01. Samples were taken daily from each cultivar. Three replicates of 10 fruit per replicate were used and the means are reported.

A 9 mm diameter conical probe that was mounted on the top of the penetrometer was used to measure firmness in each fruit and the accuracy of the firmness values was 0.01 as N. Firm-

ness, the mechanical resistance of the skin, was determined by puncturing the cheek area of 30 cherries from each cultivar. Other authors have used similar devices to measure firmness in cherries (BROWN, 1988; MITCHAM *et al.*, 1998). The crosshead speed was 10 mm/min and the same replicates were used as those described above.

Total soluble solids

Sweet cherry samples were passed through a blender in the laboratory and the TSS ratio of the fruit juice was determined as a percentage using a NOW refractometer (0–32%) (Tech-Jam International Inc., Tokyo, Japan). Thirty cherries were used in each sample.

Tensile strength and penetration depth

Tensile load for firmness is the force required to push a probe into a product to a depth that causes irreversible crushing. It is an indicator of the mechanical strength of the cherry that can withstand damage during mechanical harvesting and postharvest handling and during shelf life. The maximum force of the penetrator was determined by measuring the penetration depth (*h*). The apex angle of the conical penetrator was 55° (Fig. 1). The penetration depth was measured with a digital calliper (Mitutoyo, CD-15CP model, London, England) mounted on the test device. The tensile strength value of the cherries was calculated according to the following equation (FRACZEK *et al.*, 2005):

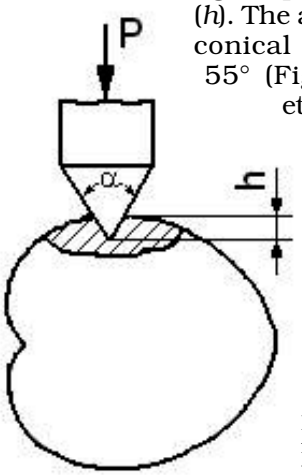

Fig. 1 - Firmness measuring scheme for sweet cherry.

$$\sigma_t = P \frac{\sin(\alpha/2)}{\pi h_p^2 \tan^2(\alpha/2)} \quad (1)$$

Where σ_t is the tensile strength (MPa); P is the maximum force loading the penetrator (N); h_p is the penetration depth (mm) and α is the apex angle of the penetrator (degree).

Percentage of decayed fruit

After harvest the cherries were kept for 7 days under shelf life conditions to determine the percentage of decayed fruit. Fruit decay caused by physiological and fungal diseases is reported as the number of decayed fruit per day. The ratio of decayed fruit was calculated as a percentage based on the total number of fruit. In addition, the cumulative decayed ratios were determined for each day during the shelf life period.

Statistical analysis

Statistical analysis was carried out according to BAHNASAWY *et al.* (2004). The results were analyzed using ANOVA and the means were compared using the LSD test ($P<0.05$). Means and standard deviations for the data were calculated.

RESULTS AND DISCUSSION

Stem rupture force

The results of the stem rupture force for the cherry cultivars at different times during shelf life are reported in Table 1. It can be seen that the stem rupture force decreased linearly as shelf life increased.

Table 1 - Stem rupture force, firmness and TSS changes of sweet cherry cvs. "Van", "Bing" and "0900 Ziraat" during shelf life.

Cultivar	Shelf life (days)							
	0	1	2	3	4	5	6	7
	Stem rupture force (N)							
"Van"	12.80 (1.37)[a]	11.37 (1.02)[a]	10.21 (0.68)[a]	9.24 (0.88)[a]	8.08 (0.84)[a]	6.71(1.38)[a]	4.71 (1.78)[a]	2.82 (0.86)
"Bing"	10.79 (0.93)[b]	9.62 (0.78)[b]	8.55 (0.78)[b]	7.54 (0.78)[b]	6.52 (0.72)[b]	5.38 (0.88)[b]	4.04 (1.15)[ab]	2.67 (0.82)
"0900 Ziraat"	11.32 (1.42)[b]	9.80 (1.14)[b]	8.27 (0.79)[b]	7.09 (0.97)[b]	5.81 (1.02)[c]	4.56 (0.73)[c]	3.53 (0.64)[b]	2.48 (0.76)
Level of significance	*	*	*	*	*	*	*	ns
	Firmness (N)							
"Van"	7.78 (0.53)	7.19 (0.42)	6.79 (0.32)[a]	6.36 (0.25)[a]	6.02 (0.26)[a]	5.69 (0.29)[a]	5.26 (0.39)[a]	4.76 (0.50)[a]
"Bing"	7.68 (0.72)	6.98 (0.43)	6.47 (0.26)[b]	6.17 (0.20)[b]	5.94 (0.12)[a]	5.67 (0.22)[a]	5.25 (0.36)[a]	4.71 (0.46)[b]
"0900 Ziraat"	7.42 (0.48)	6.91 (0.36)	6.36 (0.37)[b]	5.95 (0.24)[c]	5.47 (0.35)[b]	5.03 (0.31)[b]	4.56 (0.33)[b]	4.15 (0.32)[c]
Level of significance	ns	ns	*	*	*	*	*	*
	Total soluble solids (%)							
"Van"	13.00 (0.85)[b]	13.64 (0.37)[b]	13.93 (0.22)[b]	14.24 (0.22)[b]	14.69 (0.56)[b]	15.13 (0.57)[b]	15.47 (0.45)[b]	15.64 (0.19)[b]
"Bing"	12.39 (1.08)[b]	13.20 (0.42)[b]	13.71 (0.46)[b]	14.09 (0.39)[b]	14.42 (0.19)[b]	14.71 (0.39)[b]	15.07 (0.37)[b]	15.24 (0.19)[c]
"0900 Ziraat"	14.62 (1.07)[a]	15.47 (0.71)[a]	16.09 (0.41)[a]	16.53 (0.46)[a]	17.07 (0.56)[a]	17.69 (0.79)[a]	18.36 (0.60)[a]	18.62 (0.38)[a]
Level of significance	*	*	*	*	*	*	*	*

The values in parentheses are the standard deviations.
All data are the means of three replications with 10 fruit.
[a-c] letters indicate the statistical difference in columns.
* significance level at $p<0.05$. ns: not significant.

When the stem rupture forces were examined in relation to the different sweet cherry cultivars, there were significant differences among the cultivars at Days 0, 1, 2, 3, 4, 5 and 6 ($P<0.05$). There were no significant differences among cultivars on Day 7 ($P>0.05$). The initial stem rupture force values were 12.80, 10.79 and 11.32 N in "Van", "Bing" and "0900 Ziraat", respectively. There was a marked decline on Day 5 of shelf life. At the end of Day 7, cv. "Van" had the highest stem rupture force (2.82 N) compared to the other two cultivars: "Bing" was 2.67 N and "0900 Ziraat" was 2.48 N. Notable decreases in the stem rupture force occurred towards the end of shelf life in parallel to maturity.

Stem water loss occurred during shelf life in all cultivars. Temperature and humidity are two factors that have been implicated in stem browning in sweet cherries (DEWEY, 1951; SIEGELMAN, 1952). After harvest, heat from the sun and from respiration contributes to heat loading in cherries (YOUNG and KUPFERMAN, 1994). Keeping the cherries at lower temperatures immediately after harvest helps maintain fruit firmness and greener stems and reduces decay (DRAKE *et al.*, 1988; YOUNG and KUPFERMAN, 1994). Maintaining the relative humidity in this range helps maintain green stem colour. Water evaporates much more quickly from cherry stems than from the fruit (SESKE, 1996) and desiccation is generally related to stem browning (DRAKE *et al.*, 1988). It is clear that fruit deterioration and loss of stem quality can occur rapidly and therefore im-

proved strategies to moderate both temperature and humidity immediately after harvest need to be developed.

Firmness

The variations of the firmness with shelf life of the cultivars are summarized in Table 1. As the shelf life increased from 0 to 7 days, the firmness values decreased. There were no statistically significant differences between the cherry cultivars on harvest day and on the first day of shelf ($P>0.05$). The firmness values on the first day were 7.78 N for "Van", 7.68 N for "Bing", and 7.42 N for "0900 Ziraat". The "Van" cultivar had the highest fruit firmness value for all days. On Day 7, the fruit firmness had decreased 38.8, 38.7 and 44.0% for "Van", "Bing" and "0900 Ziraat", respectively. Again the "Van" cultivar had the highest firmness value with 4.76 N on Day 7. "Bing" and "0900 Ziraat" had firmness values of 4.71 N and 4.15 N, respectively.

No significant differences were found between the cultivars in the resistance to penetration. In experiments with two cherry cultivars ("Sciazza" and "Ferrovia") ESTI *et al.* (2002) reported that force values on the starting day decreased by the end of the study. VURSAVUŞ *et al.* (2006) measured the force values of "Van", "Noir De Guben" and "0900 Ziraat" cultivars at a temperature of 25°C the day after harvest (13.67 N, 13.34 N and 15.04 N, respectively). In another study, BERNALTE *et al.* (2003) found that the initial 3 N puncture load for the "Van" cherry cultivar decreased to 2.5 N after Day 17 of storage.

Total soluble solids

The TSS of the sweet cherry cultivars for different shelf life periods are presented in Table 1. Between Day 0 and Day 6, the total soluble solids contents of the "0900 Ziraat" cultivar was statistically significant ($P<0.05$) from the "Van" and "Bing" cultivars, but the variation between "Van" and "Bing" cultivars was not significant. The three cultivars were significantly different on Day 7. The TSS value increased as a consequence of water loss. The TSS ratio, which is generally related to sugar-acid metabolism and mineral content, shows different changes depending on the temperature, atmospheric conditions and stage of maturity of the fruit. Low temperature, in particular, limits this change under storage conditions. Changes in the carbohydrate content are limited due to the suppression of respiration. VURSAVUŞ *et al.* (2006) determined the TSS contents in "Van", "Noir De Guben" and "0900 Ziraat" cultivars: 14.2, 14.0 and 14.4%, respectively. Other studies on the changes in TSS in different sweet cherry cultivars during storage using MAP and shelf life support the results obtained in this study (HEVIA *et al.*, 1998; ERIS *et al.*, 2004).

Tensile strength
and penetration depth

Using the firmness values of three cherry cultivars through out the shelf life, the depth of penetration in the fruit using conic tip, and Equation 1, the tensile strengths were then calculated and, are reported in Table 2. The tensile values of "Van", "Bing" and "0900 Ziraat" cultivars on the starting day were 1.024 MPa, 0.986 MPa and 0.879 MPa, respectively. On the 5th day of shelf life, the tensile strength of "Bing" was higher (0.401 MPa) than the other cultivars. At the 7th day, the tensile strengths of "Van", "Bing" and "0900 Ziraat" cultivars were 0.171 MPa, 0.226 MPa and 0.185 MPa, respectively. In the last three days, the peel resistance of "Bing" cultivar was higher than the other two cultivars. The differences between tensile strength values in relation to shelf days of the cultivars based on the LSD test were not significant ($P>0.05$).

Table 2 - Depth of penetration and tensile strength changes of sweet cherry cvs. "Van", "Bing" and "0900 Ziraat" during shelf life.

Shelf life (days)	Depth of penetration (mm)			Tensile strength (MPa)		
	"Van"	"Bing"	"0900 Ziraat"	"Van"	"Bing"	"0900 Ziraat"
0	2.45 (0.18)	2.32 (0.15)	2.35 (0.11)	1.024 (0.178)	0.986 (0.141)	0.879 (0.100)
1	2.58 (0.19)	2.56 (0.17)	2.47 (0.12)	0.807 (0.114)	0.751 (0.109)	0.727 (0.079)
2	2.72 (0.20)	2.75 (0.18)	2.52 (0.16)	0.670 (0.090)	0.595 (0.083)	0.652 (0.072)
3	2.91 (0.21)	2.89 (0.19)	2.65 (0.17)	0.552 (0.075)	0.518 (0.073)	0.556 (0.055)
4	3.07 (0.23)	3.00 (0.20)	2.90 (0.20)	0.469 (0.064)	0.462 (0.065)	0.452 (0.055)
5	3.43 (0.24)	3.15 (0.21)	3.42 (0.24)	0.356 (0.052)	0.401 (0.056)	0.311 (0.042)
6	3.60 (0.26)	3.40 (0.22)	3.77 (0.26)	0.301 (0.043)	0.317 (0.046)	0.230 (0.032)
7	4.55 (0.31)	3.79 (0.24)	4.10 (0.32)	0.171 (0.028)	0.226 (0.032)	0.185 (0.026)

The values in parentheses are the standard deviations.

A plot of the experimentally determined values of force applied to break the peel resistance of the cultivars against penetration depth (Fig. 2) shows that the force decreased as the penetration depth increased during the shelf life period. The relationship between the force applied to break peel resistance of the fruit and penetration depth can be expressed as:

$$P = 16.087 h_p^{-0.9043} \quad (2)$$

with an R^2 value of 0.985.

Percentage of decayed fruit

The percentage of decayed fruit for the sweet cherry cultivars is given in Table 3. The percentage of decayed fruit of the cultivars "Van" and "Bing" between Day 3 and Day 5 was statistically significant ($P>0.05$) from cultivar "0900 Ziraat". On days 6 and 7 all cultivars were statistically significant. On the 6th and 7th days, the percentage of decayed fruit was highly significant in the "Bing" cultivar. On Day 7, "0900 Ziraat" had

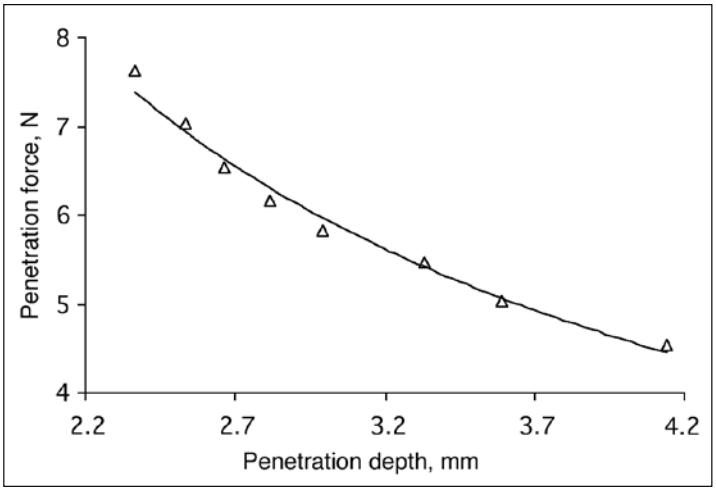

Fig. 2 - Relationship between penetration force and penetration depth of cultivars.

Table 3 - Fruit decay (%) of sweet cherry cvs. "Van", "Bing" and "0900 Ziraat" during shelf life.

Shelf life (days)	Cultivar			Significance level
	"Van"	"Bing"	"0900 Ziraat"	
0	0.00	0.00	0.00	ns
1	0.00	0.00	0.00	ns
2	0.40 (0.10)	0.36 (0.07)	0.08 (0.03)	ns
3	2.11 (0.48)[a]	1.90 (0.21)[a]	0.20 (0.04)[b]	*
4	7.92 (1.24)[a]	6.60 (0.78)[a]	2.65 (0.32)[b]	*
5	13.68 (2.40)[a]	12.88 (1.11)[a]	5.45 (0.59)[b]	*
6	15.37 (0.92)[b]	17.66 (0.76)[a]	6.38 (0.64)[c]	*
7	17.04 (1.35)[b]	22.37 (2.86)[a]	8.38 (0.58)[c]	*

The values in parentheses are the standard deviations.
All data are the means of three replications.
[a-c] letters indicate the statistical difference in rows.
* significance level at $p<0.05$. ns: not significant.

a 8.38% decay, followed by the "Van" and "Bing" cultivars, which had means of 17.04 and 22.37%, respectively. According to the data in Table 3, the shelf life for cvs. "Van" and "Bing" is 3-4 days, while the shelf life for "0900 Ziraat" is 5-6 days. BERNALTE *et al.* (2003) reported a decay percentage of around 3 and 7% at the end of storage (14 days at 1°C and 3 days at 7°C) and shelf life (4 days at 20°-22°C) in "Van", respectively. ERIS *et al.* (2004) reported that the highest percentage of decayed fruit during storage (30 days for "Bing", 60 days for "0900 Ziraat") was recorded at the end of storage plus shelf-life (2 days) from the sweet cherries stored under ambient conditions. Furthermore, in the study, the decayed ratio was lower in the fruit of cv. "0900 Ziraat" compared to cv. "Bing". In another study, after 21 days of storage at 1°C, TOIVONEN *et al.* (2006) reported decay percentages of "Bing", "Cristalina", "Lapins", "Samba", "Sandra Rose", "Sonato", "Staccato" and "Symphony" cherry cultivars of 3.2, 7.2, 4.0, 1.6, 4.8, 5.6, 2.4 and 17.6%, respectively.

CONCLUSIONS

The results of the investigations of various quality properties of sweet cherries show the following:

(1) In terms of stem rupture force and firmness, the best results were obtained from the "Van" cultivar.

(2) The highest TSS value was recorded for "0900 Ziraat" (14.62%). Values for "Van" and "Bing" were 13.00 and 12.39%, respectively. At the end of the study, the lowest TSS content was recorded for "Bing" and the highest for "0900 Ziraat".

(3) The highest tensile strength was obtained for "Van" (1.024 MPa) at harvest. This cultivar had the lowest tensile strength (0.171 MPa) on the 7th day.

(3) On the 7th day of shelf life, "0900 Ziraat" had the lowest decay percentage (8.38%); "Van" and "Bing" followed with 17.04 and 22.37%, respectively.

The "0900 Ziraat" cultivar had the lowest percentage of decay under shelf life conditions and the best results in terms of the quality parameters examined. In the future, new parameters will

be studied for longer shelf life. The effects of these parameters on fruit quality will be ascertained.

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PAPER

DETERMINING THE BEST PRODUCT QUALITY SET FOR PACKED YOGURT IN TURKEY

DETERMINAZIONE DEL GRUPPO DI FATTORI CHE IDENTIFICA IL MIGLIOR DI PRODOTTO DI QUALITÀ PER LO YOGURT CONFEZIONATO IN TURCHIA

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ABSTRACT

The factors affecting consumer demand for packed yogurt were explored, and the best product quality set was determined. A conjoint analysis in the SPSS statistical program was done with the data from 385 household surveys. The three most important factors affecting packed yogurt demand were brand, price and product content. On the other hand, the best product quality set was the Cizmelioglu private label, no-fat yogurt, sold in the supermarket at €0.97/kg with promotion. Results in-

RIASSUNTO

Sono stati considerati i fattori che influenzano la richiesta del consumatore per lo yogurt confezionato ed è stato determinato il gruppo di essi che identifica il migliore prodotto di qualità. È stata effettuata un'analisi congiunta nel SPSS con i dati dei sondaggi su 385 famiglie. I tre più importanti fattori che influenzavano la domanda di yogurt confezionato erano la marca, il prezzo ed il contenuto del prodotto. All'interno di questo gruppo di fattori, il migliore prodotto di qualità è risulta-

- Key words: brand, conjoint analysis, consumer, purchase intention, yogurt -

dicate that consumers prefer a private label of low priced no-fat yogurts.

to essere il marchio privato "Cizmelioglu", yogurt privo di grassi, venduto nel supermercato in promozione a €0,97/kg. I risultati indicano che i consumatori preferiscono yogurt magri di marchio privato, a basso costo.

INTRODUCTION

The total annual world production of milk is about 618 million tonnes. Of this, 148 million tonnes (24%) are produced by the EU25. While Turkey produces about 10 million tonnes of milk yearly, the average productions of Germany, the Netherlands, Italy and Spain are 27, 10.5, 9.9 and 5.8 million tonnes, respectively. Only 20% of the milk produced in Turkey is processed in modern dairy factories, compared to 97-98% in the developed countries (AERI, 2006). This means 80% of the milk produced in Turkey is unregistered[1] (TEKINAY, 2006). This problem obviously affects the whole dairy sector.

Dairy products play an important role in the human diet. The annual average consumption of dairy products per capita, i.e. cheese, milk, butter and yogurt, in Turkey is 8.5 kg, 30 L, 1.3 kg and 34 kg, respectively, compared to 14 kg, 85 L, 4.7 kg and 20 kg in the developed countries (TAN and OZTURK, 2002; KARAGOZLU, 2004). These data clearly show that the over all dairy product consumption in Turkey is much less than that in developed countries, except for yogurt.

Turkey has the highest per capita consumption of yogurt in the world (AYBEK, 2006). That is mainly because yogurt is a traditional food, and the Turkish people have particular tastes and preferences. However, due to the problems in production and marketing, 90% of the yogurt consumed is home-made[2] and only 10% of the total yogurt consumption is packed yogurt (FIRAT, 2002; UNSAL, 2007).

Due to health concerns, food safety and quality control, urbanization, technological developments, regulations to comply with the European Union (EU) standards etc., processed and labelled yogurt consumption in Turkey has been increasing steadily (VURAL, 2001; YURTTUT, 2001; TOPCU, 2006). In the past only generic and national brand yogurts were sold on the market as packed yogurts. After the introduction of giant supermarkets (e.g., Migros and BIM), private label (store brand)[3] yogurts can now be found on the store shelves, as well. The market share and growth rate of private label yogurts are 19 and 34%, respectively (RETAILING INSTITUTE, 2005).

With the steady increase in the demand for private label products, the retailers must determine what attributes their products should have. Increas-

[1] Of this, about 40% is consumed domestically, and another 40% is processed in small, unregistered dairy farms.

[2] People make yogurt themselves using milk they produce on farms or milk they buy from unregistered milk venders in the streets.

[3] Generic packed yogurts are produced by smaller scale factories, and are sold with no brand name. National brand packed yogurts are produced directly by manufacturers, and sold nationwide in the stores under their own name. Private label yogurts are produced by manufacturers for retailers, and sold only in the retailers' own stores under their own store name. Therefore, the words "private label" and "store brand" are used interchangeably.

ing competition between the national and private brands forces both parties to pay more attention to the attributes of their rivals' product. In addition, they can benefit from acquiring information about consumer heterogeneity to position their products and set prices according to consumer preferences (APELBAUM, 2000). Thus, an analysis of consumer responsiveness to different product attributes with an emphasis on the choice of private labels over national brands is needed.

Consumers, on the other hand, have access to much more information and a greater choice in today's competitive and global market (LAROCHE and TOFFOLI, 1999). They have limited processing capacity and hence use only a part of the information available when choosing a brand. When evaluating brand attributes, consumers limit themselves to 3-5 items of information in order to reduce the complexity of selection (LAROCHE *et al.*, 2001). For example, for any brand choice, price and quality play an important role since they are often central to consumers' judgment and decisions (KALYANARAM and WINER, 1997). For most brands, consumers believe that price and quality are correlated and their preferences are affected by external variables such as income, family size, social status, profession, etc. (KAMAKURA and RUSSELL, 1989; ALLENBY and ROSSI, 1999). Influenced by all these factors, a consumer defines the purchasing problem, gathers necessary information, evaluates alternatives and makes a purchasing decision (MESÍAS *et al.*, 2003; TOPCU, 2004).

The effects of product attributes on consumer attitudes towards product evaluation have been widely studied (ROWAN, 2000; WANSINK *et al.*, 2001; DELIZA *et al.*, 2003). One of the greatest difficulties in this type of research is to quantify the effect that each product attribute has on the consumer's decision to purchase. Conjoint analysis is a useful tool for investigating the effect of these attributes. It is a market research tool for developing effective product design. Using conjoint analysis, researchers can determine product attributes that are important to the customers, the consumer idea of most desirable level of product attributes and the market preference for different brands (SPSS CONJOINT 15.0, 2006).

Conjoint analysis was used in this study to explore the factors affecting consumer demand for packed yogurt and then, to determine the best product quality set which maximizes the total utility for the customer. With the increasing demand for packed yogurt, and the growing competition between manufacturers and retailers in Turkey, it is interest to determine variations in consumer responsiveness to different yogurt attributes.

MATERIALS AND METHODS

Materials and determination of sample size

The data were obtained from a survey conducted from March to June in 2006. To minimize sample bias and represent the population correctly, the Erzurum city centre[4] was divided into four districts. Based on the population and subdistricts within each district, weighted sample size and survey distributions were determined proportionally for each district (Table 1).

The following formula was used to determine the sample size for each district, (YILDIZ *et al.*, 2006):

$$n = \frac{Z^2 * p * (1 - p)}{c^2} = 385$$

[4] Erzurum (39°57' N latitude and 41°15' E longitude) is located on the Silk Road in the north-east region of Turkey (Fig. 1). Erzurum is one of the biggest cities in the region with a total population of 962,000; the city center population is 402,000.

Table 1 - Sample size and distribution in the districts.

Districts	Number of households	The ratio of the number of households in districts (%)	Sample size
Yenisehir	30,022	37	142
Yakutiye	26,099	32	122
Kazimkarabekir	17,976	23	89
Dadaskent	6,562	8	32
Total	**80,659**	**100**	**385**

Source: (DEMM, 2007).

Where:

n = sample size;

Z = Z value, (used 1.96 for 95% confidence level);

p = percentage picking a given choice, (0.5 used for sample size needed);

c = confidence interval, (used 0.05 = ±5).

A simple random sampling method was used to select consumer households. A face-to-face survey method was used with 385 adults. The survey was conducted with adults (with or without children) either at their residence or at shopping centres.

Generation of orthogonal design and questionnaire

Conjoint analysis is a multivariate technique based on the assumption that purchasing behaviour reflects a choice,

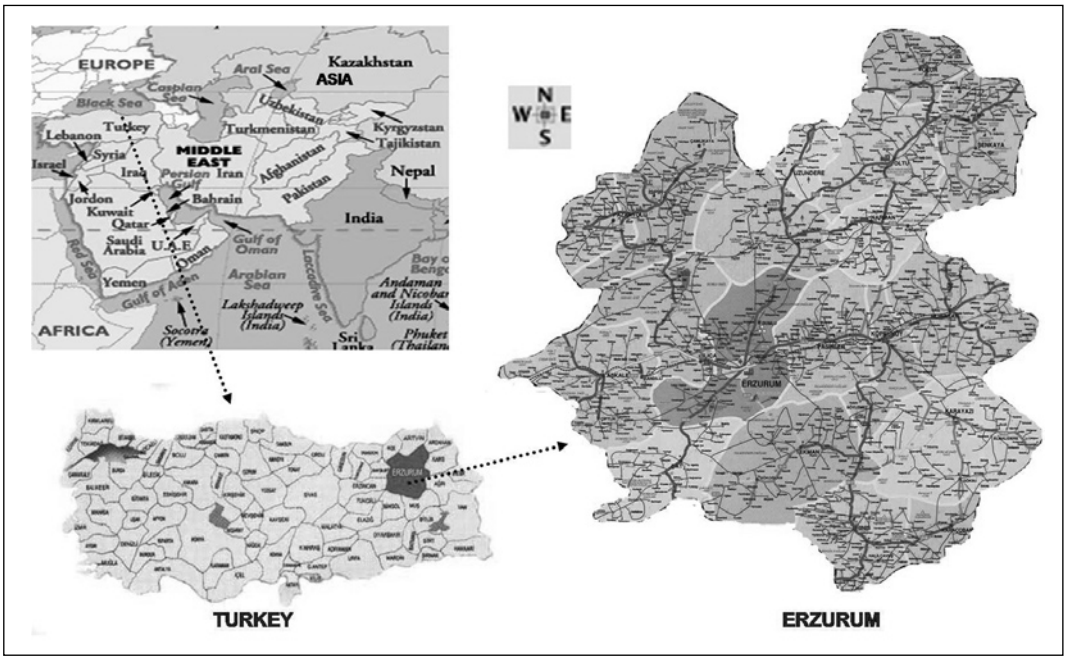

Fig. 1 - Maps of Turkey and Erzurum.

Table 2 - Factors and their levels for packed yogurt used in conjoint analysis.

Factors	Factor levels			
Brands*	P (Pinar)	D (Danone)	M (Migros)	C (Cizmelioglu)
Price (€/kg)**	1.47	1.44	1.32	0.97
Selling point	Supermarket (SM)		Market (MM)	
Content	Whole yogurt (WY)	Low-fat yogurt (LY)	No-fat yogurt (NY)	
Promotion	YES		NO	

* Where, P and D are national brands, and M and C are private labels (store brands). Here, private label "C" is produced locally.

** The product prices were converted from the New Turkish Lira (NTL) to Euro (€) using the exchange rate on October 15, 2007. The conversion rate used was 1.70 NTL/€.

within a product category, among products which possess a set of differentiating attributes. This technique has been used widely in many marketing studies including dairy products such as cheese (SOUZA MONTEIRO and LUCAS, 2001), yogurt (SABA and ROSATI, 2002; LUCKOW *et al.*, 2005; O'CONNOR *et al.*, 2006; HADDAD *et al.*, 2007), and pasteurized milk (DOLEKOGLU, 2003; AKPINAR, 2004; VALEEVA *et al.*, 2005).

To determine the factors influencing consumer purchasing decisions for packed yogurt, a pre-market study was done in March, 2006 to find out the most popular packed yogurt brands (private and national), their prices and availability in the stores[5]. After obtaining these data, based on the factors and factor levels listed in Table 2, the plan which consists of product profiles (i.e., combinations of factor levels) to be rated by the participants were generated using the orthogonal design procedure in the SPSS statistical program.

With 5 factors and a total of 15 factor levels, there are 192 potential product profiles, which is quite unmanageable to deal with[6]. In order to avoid this problem, it was necessary to generate a representative subset known as an orthogonal design, which is the typical starting point of a conjoint analysis. After generating an orthogonal design, the number of product profiles was reduced to 18 cases, 2 of which are holdouts[7] (Table 3).

The design of the survey forms was based on these 18 product profiles. SPSS Conjoint uses the "full-profile" approach, where participants rank, order, or score a set of profiles, according to preference (SPSS CONJOINT 15.0, 2006). In this study, the participants were asked to rank the 18 profiles from the most to the least preferred.

Statistical method

The data file was created using the preference rankings of those profiles collected from the participants. Before an-

[5] Store refers to both small closed markets and larger markets (supermarket) in the city centre.

[6] Five factors affecting consumer preference for packed yogurt are brand, price, selling point, content and promotion. There are a total of 15 factor levels, four factor levels for brand; four for price; two for selling point; three for content; and two for promotion. The combinations of these 15 factor levels give a total of 192 potential product profiles (i.e. 4x4x2x3x2 = 192).

[7] Holdout cases are judged by the subjects but are not used by the conjoint analysis to estimate utilities. They are used to check the validity of the estimated utilities.

Table 3 - Combinations for packed yogurt used in conjoint analysis.

Card number	Brand	Selling point	Price (€/kg)	Content	Promotion
1	C	MM	1.44	WY	Yes
2	P	SM	1.32	NY	Yes
3	M	MM	1.44	NY	No
4	D	MM	0.97	WY	Yes
5	C	MM	1.32	LY	Yes
6	M	MM	1.32	WY	No
7	D	SM	1.32	WY	No
8	D	SM	1.44	LY	No
9	P	MM	0.97	LY	No
10	C	SM	1.47	WY	No
11	M	SM	0.97	WY	Yes
12	P	SM	1.44	WY	Yes
13	M	SM	1.47	LY	Yes
14	D	MM	1.47	NY	Yes
15	C	SM	0.97	NY	Yes
16	P	MM	1.47	WY	No
17[a]	M	SM	1.47	WY	Yes
18[a]	M	SM	1.44	LY	No

[a] Holdout.

alysing the data with the conjoint procedure, the "*factors*" subcommand must be described. The model can be specified by describing the expected relationship between factors and rankings via "*factors*" subcommand (SPSS CONJOINT 15.0, 2006). The discrete model indicates that factor levels are categorical and no assumption is made about the relationship between the factor and the ranks. On the other hand, the linear model indicates an expected linear relationship between the factor and the ranks. The expected direction of the linear relationship can be specified with the keywords "*more and less*". Less linear indicates that lower levels of a factor are expected to be preferred, while more linear indicates that higher levels of a factor are expected to be preferred. Specifying more or less will not affect estimates of utilities (HAIR *et al.*, 1998; SPSS CONJOINT 15.0, 2006).

According to the characteristics of the factors, discrete, less linear and more linear models were used in this study. Brand, selling point and yogurt content were modelled as discrete because there is *no priori* knowledge about the influence of yogurt attributes on purchase intent. Price and promotion[8], however, were modelled as less linear and more linear, respectively. Price was assumed to follow a less linear model since it, typically, shows an inverse relationship with purchase intent. Promotion, on the other hand, was assumed to follow more linear relationships in that consumers are expected to exhibit more positive attitudes toward product promotions (HADDAD *et al.*, 2007).

The conjoint analysis of the data generates a utility score, called a part-worth,

[8] Promotion involves disseminating information about a product, product line, brand, or company. In this study, we use the term promotion especially for sales promotion, in particular, consumer sales promotion. There are a number of consumer sales promotion techniques such as price deal (a temporary reduction in the price), price-pack deal (the packaging offers a consumer a certain percentage more of the product for the same price), product deal (with the purchase of a product, offering the second of the same product or different product for free or at a cheaper price), coupons and rebates.

for each factor level. These utility scores, analogous to regression coefficients, provide a quantitative measure of the preference for each factor level, with larger values corresponding to greater preference. Part-worths are expressed in a common unit, which allows them to be added together to give the total utility, or overall preference, for any combination of factor levels (NESS, 2000; TOPCU, 2006). For example, the total utility (TU) of a packed yogurt with brand "C", selling point "SM", price "€0.97/kg", content "NY" and promotion "YES" (i.e., card number 15) is:

$$TU = \text{constant} + U(C) + U(SM) + + U(€0.97) + U(NY) + U(YES)$$
$$TU = 6.22 + 0.70 + 0.01 + 3.25 + + 0.41 + 0.27 = 10.86$$

RESULTS AND DISCUSSION

Table 4 shows the correlations between the utility scores (part-worth) and relative importance for each factor level. Pearson's R and Kendall's tau statistics imply that there is a significant correlation between the observed and estimated preferences (NESS, 2000; SPSS CONJOINT 15.0, 2006; TOPCU, 2006). This means the model fits the observed data well.

Higher utility values (part-worth) indicate greater preference. The most and the least preferred brands in Erzurum are the private label "C" (Cizmelioglu) and the national brand "P" (Pinar), respectively. The reasons that consumers prefer the private labels over national brand could be related to knowing the origin. Consumers may have an incentive to support locally produced products to help local or regional economy grow. Moreover, they may trust local products more since the origin is known. Finally, this locally produced product may cause consumers to have a perception of higher quality and/or lower market price due to less transportation (shorter distance to ship), input and labour costs.

The supermarket "SM" as a selling

Table 4 - Conjoint analysis results.

Factors	Factor levels	Part-worth	Relative importance (%)	Standard error
Brand	P	-0.60		1.14
	D	0.15	**33.24**	1.14
	M	-0.25		1.14
	C	**0.70**		1.14
Selling point	**SM**	**0.01**	11.01	0.66
	MM	-0.01		0.66
Sale price (€/kg)	**0.97**	**3.25**		2.36
	1.32	2.44	**22.45**	1.77
	1.44	1.67		1.18
	1.47	0.81		0.59
Content	WY	-0.38		0.88
	LY	-0.03	**20.88**	1.03
	NY	**0.41**		1.03
Promotion	**Yes**	**0.27**	12.42	2.67
	No	-0.13		1.32
Constant	6.22		2.56	
	Correlations among observed and estimated preferences			
		Value		Significance level
Pearson's R		**0.80**		**0.00**
Kendall's tau		**0.60**		**0.00**

Table 5 - Total utilities of the product profiles in orthogonal design.

Card no	Brand	Selling point	Price (€/kg)	Content	Promotion	Total utility	Ranking
1	C	MM	1.44	WY	Yes	8.47	7
2	P	SM	1.32	NY	Yes	8.75	5
3	M	MM	1.44	NY	No	7.91	9
4	D	MM	0.97	WY	Yes	9.50	3
5	**C**	**MM**	**1.32**	**LY**	**Yes**	**9.59**	**2**
6	M	MM	1.32	WY	No	7.88	11
7	D	SM	1.32	WY	No	8.31	8
8	D	SM	1.44	LY	No	7.89	10
9	P	MM	0.97	LY	No	8.70	6
10	C	SM	1.47	WY	No	7.23	14
11	M	SM	0.97	WY	Yes	9.12	4
12	P	SM	1.44	WY	Yes	7.19	15
13	M	SM	1.47	LY	Yes	7.03	16
14	D	MM	1.47	NY	Yes	7.85	12
15	**C**	**SM**	**0.97**	**NY**	**Yes**	**10.86**	**1**
16	***P***	***MM***	***1.47***	***WY***	***No***	***5.91***	***18***
17	***M***	***SM***	***1.47***	***WY***	***Yes***	***6.68***	***17***
18	M	SM	1.44	LY	No	7.49	13

point has a higher utility than smaller markets although the difference between them was minor. It could be said that consumers prefer to purchase their yogurts from bigger stores, perhaps because bigger markets have more product varieties, better quality and price, better customer services, etc. (Table 4). As for "sale price", there was an inverse relationship between price and utility, as expected. Consumers prefered yogurts with the price of €0.97 more than the yogurts with the price of €1.47 per kg since lower prices lead to higher utility.

"No-fat" yogurt content, on the other hand, gave consumers the highest utility. This could be due to growing health concerns. Increasing knowledge and consciousness about the detrimental and adverse effects of fat[9] could induce consumers to demand more products with lower fat content. Presence of "promotion" corresponded to a higher utility, as expected. This means that consumers preferred more packed yogurts with consumer sales promotion (Table 4). The aim of promotions is Promotions aim to increase consumer demand for a product. In general, the products with promotions were more attractive to consumers in terms of their price, quality, physical appearance, etc.

Table 4 also shows the relative importance of each factor. The results indicate that "brand" had the most influence on overall preference with 33.24% relative importance, followed by "price" with 22.45% and "content" with 20.88%. "Selling point", played the least important role in determining overall preference. Promotion and selling point were not as significant as brand, price and content; the ranges of the first two factors were not as large as the ranges of the latter three factors.

Table 5 shows the total utilities obtained from the part-worths in Table 4, and the ranking of the 18 product profiles in orthogonal design. The results indicate that the product profile "C, SM, €0.97/kg, NY and YES" gave the maxi-

[9] Saturated fat found mostly in animal and dairy products has higher levels of total and low-density lipoprotein cholesterol, and increase the risk of coronary heart disease (PUGLIESE, 2006).

Table 6 - Product profiles maximizing and minimizing consumers' total utilities.

Card #: 15		Card #: 16	
Brand	: C	Brand	: P
Selling point	: Supermarket (SM)	Selling point	: Market (MM)
Sale price	: €0.97/kg	Sale price	: €1.47/kg
Content	: No-fat (NY)	Content	: Whole (WY)
Promotion	: Yes	Promotion	: No
a) Maximum utility		b) Minimum utility	

mum total utility (10.86). On the other hand, the product profile "P, MM, €1.47/kg, WY and NO" gave the minimum total utility (5.91) (Table 6). Since these combinations have all the factor levels with the highest and lowest part-worths in Table 4, there is no way to have total utility values higher or lower than these two profiles.

CONCLUSIONS

This paper estimates the consumer preferences for packed yogurt attributes in Erzurum, Turkey where yogurt is a traditional food and *the per capita* consumption is the highest in the world. Conjoint Analysis was used to investigate the relative importance of five factors and the utility scores of 15 factor levels of packed yogurts in the marketplace. As expected, brand and price influenced consumers' preference the most, while promotion and selling point had the least influence.

The results also show that consumers prefer yogurts with a private label, lower price, promotion and no-fat content. Consumers were more likely to purchase whose origin is known (i.e., a private labelled local product). In addition, price played a central role in consumer purchasing decisions. Given the product attributes, assigning the right price is crucial for marketers. Product content greatly affected consumer preferences. Nutritional information and the health conditions of consumers are expected to strongly influence food choices due to the perceived detrimental long-term health effects of dietary fats. These findings may be beneficial to marketers, manufacturers and retailers who are (re) designing a product or developing new marketing tactics and strategies.

Although this study has some scientific merit for the academic and food manufacturing communities, there are some limitations. The results of this study have a limited generalizability since the data were collected in only one city. The survey should now be conducted nationwide; more data will give more objective results about population preferences. Moreover, in future studies, this model could be expanded to incorporate more factors and factor levels into the model, and the population could be divided based on demographic and socio-economic characteristics. Further study is needed to understand what is driving the preference for private label yogurt over the national brand. This information could be invaluable to manufacturers who could advertise those qualities and thereby build equity within the community.

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SHORT COMMUNICATION

PRODUCTION OF BIOGENIC AMINES BY SOME *ENTEROBACTERIACEAE* STRAINS ISOLATED FROM DAIRY PRODUCTS

PRODUZIONE DI AMINE BIOGENE DA PARTE DI CEPPI DI *ENTEROBATTERIACEAE* ISOLATI DA PRODOTTI LATTIERO-CASEARI

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ABSTRACT

Eighty-two strains of *Enterobacteriaceae* isolated from cheese were examined to evaluate their capacity to produce four biogenic amines at 37°C *in vitro*. All of the strains produced at least one biogenic amine (almost all putrescine). In a second step, the formation of histamine by one strain of *Klebsiella oxytoca*, two strains of *Citrobacter freundii*, one strain of *Proteus vulgaris*, one strain of *Hafnia alvei* and two strains of *Morganella morganii* were evaluated at: 4°, 10°, 15° and 22°C. Six

RIASSUNTO

In questo lavoro sono stati esaminati a 37°C *in vitro* per la produzione di amine biogene 82 ceppi di *Enterobatteriaceae* isolati da formaggi. Tutti i ceppi producevano almeno un'amina biogena (soprattutto putrescina). In un secondo momento è stata studiata la capacità di produrre la sola istamina da parte di un ceppo di *Klebsiella oxytoca*, due ceppi di *Citrobacter freundii*, un ceppo di *Hafnia alvei* e due ceppi di *Morganella morganii* a: 4°, 10°, 15° e 22°C.

- Kew words: biogenic amines, cheese, *Enterobacteriaceae*, histamine -

strains out of seven produced more histamine at 22°C than at 37°C. Putrescine and cadaverine appear to be hygienic quality indicators. Histamine production should be evaluated at a minimum of two different temperatures.

Sei ceppi su sette producevano più istamina a 22° che a 37°C. Putrescina e cadaverina sembrano essere utili indicatori di igiene nei formaggi; suggeriamo di valutare la produzione di istamina ad almeno due differenti temperature.

INTRODUCTION

Biogenic amines are a group of naturally-occurring amines derived from the enzymatic decarboxylation of free amino acids. Some of them play an important role in cell metabolism while others are formed during the ripening of cheese and meat products or in other fermented food. Their presence in food is sometimes regarded as a sign of spoilage or a cause of poisoning (SHALABY, 1996, ÖNAL, 2007).

Although their toxic level has still not been clearly established, a value less than 200 mg/kg of tyramine + histidine + 2-phenylethylamine is considered as an index of acceptable hygienic quality for meat products (SUZZI and GARDINI, 2003), while the sum of histamine + putrescine + cadaverine + tyramine should not exceed 900 mg/kg for dairy products (VALSAMAKI *et al.*, 2000). The maximum range of histamine and tyramine in foods should be 50-80 mg/kg and 100-800 mg/kg, respectively (ÖNAL, 2007).

Several bacterial species have decarboxylase activity; among these *Photobacterium* spp, *Morganella* spp, *Lactobacillus* spp, *Carnobacterium* spp ed *Enterobatteriaceae* are frequently associated with the presence of biogenic amines in many food items (SHALABY, 1996; VALSAMAKI *et al.*, 2000; SANTOS *et al.*, 2003; MARTUSCELLI *et al.*, 2005; AYMERICH *et al.*, 2006; ÖNAL, 2007). In cheese, the biogenic amine content is generally associated with the decarboxylase activity of lactobacilli, enterococci, micrococci and some strains of *Enterobacteriaceae* (GARDINI *et al.*, 2001; PEREIRA *et al.*, 2001; GIRAFFA, 2002; ÖNAL, 2007).

The progression of tyramine, histamine, putrescine and cadaverine contents has been investigated during the ripening of some artisanal Italian cheeses (PATTONO *et al.*, 2002; GENNARO *et al.*, 2003). The results showed a considerable increase of putrescine and cadaverine while the initially low levels of histamine and tyramine remained nearly the same throughout the ripening time.

The aim of the present work was to investigate the decarboxylase activity of some enterobacteria strains isolated from different dairy products.

MATERIAL AND METHODS

Microorganism

A total of 82 bacterial strains were isolated from different dairy products, mostly fresh cheese. They were biochemically identified by means of API 20E Biomerieux galleries (Marcy-L'Etoile, France).

Instruments

The putrescine, cadaverine, histamine and tyramine contents were determined by HPLC using a LaChrom

L7100 (Merck-Hitachi, Darmstadt, Germany) HPLC apparatus equipped with a L7000 pump, an L7400 UV detector set at UV wavelength 254 nm and a 50 μL loop. The HPLC column used was a Merck LiChrospher 100 RP-18,250x4 mm (5 μm) and a pre-column with the same stationary phase (Merck, Darmstadt, Germany).

Reagents

The reagents used for HPLC were of LC grade and the water was obtained using a MilliQ system (Millipore, Billerica, MA). Amines, amino acids and dansyl-chloride were purchased from Sigma (St. Louis, MO).

Qualitative determination of biogenic amines

The bacterial strains were grown following the method proposed by MARINO *et al.* (2000). This method uses two media to ascertain the decarboxylase capacity. After propagation at 37°C for 24 h in TSB (Tryptone Soy Broth), the strains were inoculated in Moeller Broth supplemented with 1% (w/v) L-lysine-hydrochloride, L-arginine-hydrochloride and L-ornithine-hydrochloride and inoculated on Niven medium. A positive reaction was recognizable by a color change to green in Moeller Broth and to violet in Niven medium.

Quantitative determination of biogenic amines

The quantitative determination entailed the inoculation of the positive strains (1% v/v) in a liquid medium for quantitative analysis. The tubes were incubated at 37°C for four days. The liquid medium was composed of the following (g/L): tryptone 1.0, NaCl 5.0, K_2HPO_4 2.5 pyridoxal-phosphate $6.67x10^{-3}$, L-arginine-hydrochloride 3.5, L-histidine-hydrochloride 3.5, L-lysine-hydrochloride 3.5, L-ornithine-hydrochloride 3.5 and L-tyrosine-hydrochloride 3.5 (final pH adjusted to 5.3 by means of HCl 1N).

The derivatization was performed as follows: 0.5 mL of filtered culture (pore size 0.22 μm) was added to 2.0 mL of a dansylchloride solution (10 mg/mL) and to 0.5 mL of a saturated solution of $NaHCO_3$ then incubated at 60° C for 20 min. After 20 min, 50 μL of 28 % ammoniun hydroxide was added. Thirty minutes later the reaction mixture was filtered (pore size 0.45 μm) and injected into the HPLC apparatus. Ultrapure H_2O and acetonitrile were the mobile phases. The composition was: 0-5 min H_2O/ACN 35:65, 5-20 min H_2O/ACN 25/75 at a flow rate of 0.8 mL/min. The samples were analyzed in duplicate.

The strains producing more than 20 ppm of histamine were tested after having been kept at 4°, 10°, 15° and 22°C for fours days. The analyses were performed in triplicate in the conditions described above.

RESULTS AND DISCUSSION

All of the strains were identified with API20E. The percentage of identification varied between 81.2 and 99.9%.

Regarding the qualitative determination, all of the bacteria tested showed a change in the color of the qualitative medium, indicating that all of the bacteria could produce at least one biogenic amine. For this reason, all of the strains were submitted to the quantification test. The amounts of biogenic amines produced by the strains examined are reported in Table 1. Putrescine was produced by 98.8% of the strains isolated, cadaverine by 92.6%, histamine by 79.3% and tyramine by 64.6%.

Given the laboratory conditions, putrescine was the amine that was produced in the largest quantity. In fact 32.9% of the strains formed more than 100 ppm and a strain of *Morganella mor-*

Table 1 - Production of biogenic amines (ppm) at 37°C.

	PUT mean (min-max)	CAD mean (min-max)	HIS mean (min-max)	TYR mean (min-max)
Proteus spp.[2]	289.3 (3.9-547.7)	8.9 (0.2-17.9)	1.1 (0-2.1)	0.8 (0.5-1.1)
Proteus vulgaris[3]	158.7 (3.9-467.4)	1.9 (0.5-5.0)	33.8 (0-98.9)	0.44 (0.1-1.0)
Proteus *mirabilis*[29]	283.2 (1.5-1171.4)	30.8 (0-209.2)	3.27 (0-14.4)	1.0 (0-10.3)
Providencia alcalifaciens[1]	4.33	2.97	0.92	n.d.
Escherichia coli[3]	75.7 (5.7-206.7)	299.7 (224.2-375.0)	0.9 (0-2.4)	0.2 (0-0.3)
Pseudomonas putrefaciens[1]	609.86	19.43	2.54	0.42
Klebsiella oxytoca[3]	3.8 (0.8-5.5)	108.26 (0.7-318.5)	86.9 (0-260.8)	5.1 (0-15.1)
Klebsiella pneumoniae subsp rhinosclerum[1]	4.69	n.d.	8.87	n.d.
Enterobacter sakazakii[1]	3.55	2.27	16.26	10.90
Enterobacter cloacae[2]	1.2 (1.0-1.4)	2.5 (4.7-5.4)	2.5 (0-5.0)	0.8 (0.2-1.4)
Enterobacter fergusonii[1]	0.91	0.81	n.d.	0.11
Enterobacter agglomerans[1]	0.35	4.94	2.54	n.d.
Hafnia alvei[15]	3.6 (0-17.7)	17.9 (0-200.9)	7.3 (0-17.6)	1.1 (0-10.6)
Morganella morganii[10]	162.5 (0.8-1263.0)	17.8 (0-200.9)	17.3 (0-17.6)	1.1 (0-9.7)
Erwinia spp.[3]	7.8 (0.9-20.5)	37.9 (1.2-110.1)	3.6 (0-8.4)	0.4 (0-1.1)
Citrobacter freundii[6]	247.6 (9.7-861.1)	33.8 (6.0-134.4)	85.3 (0.3-254.7)	0.3 (0-1.8)

Legend: PUT = Putrescine, CAD = cadaverine, HIS = histamine, TYR = tyramine, n.d. = not detected.
The number in parentheses after each strain indicates the number of strains tested.

ganii produced up to 1.263 ppm. Only eleven strains (13.4%) were able to form cadaverine in amounts greater than 100 ppm; all of the *Escherichia coli* strains were in this group. Histamine was produced by the majority of the strains; however only three strains (two *Citrobacter freundii* and one *Klebsiella oxytoca*) were able to form more than 100 ppm. The values ranged from 0 to 98.7 ppm for all the other strains with the latter value formed by *Proteus vulgaris*. Although *M. morganii* and *Hafnia alvei* are regarded as powerful amine producers, only three strains were considered for the subsequent tests.

The bacteria that were isolated from the different cheeses showed a low capacity to produce tyramine. No strains produced more than 20 ppm and only 4.9% gave more than 10 ppm.

Only seven strains produced more than 20 ppm of histamine; these were two strains of *C. freundii*, two strains of *M. morganii*, one strain of *K. oxytoca*, one strain of *P. vulgaris* and one strain of *H. alvei*. These were then tested to investigate the production of histamine at different temperatures (Table 2). The percentage of strains identificated with API20E varied between 91.3 and 99.9%.

There was no growth at 4°, and only three strains showed a limited growth at 10°C. At 15°C all of the strains except *H. alvei* produced histamine in amounts ranging from 82.6 to 379.5 ppm. At 22°C, three strains out of seven showed a growth greater than that at 37°C and five strains formed more than 300 ppm of histamine, i.e. the amount was greater than that formed at 37°C.

The association between the presence of some bacteria in food and the formation of biogenic amines has been investigated widely (MASSON *et al.*, 1996; SUZZI *et al.*, 2000). Regarding dairy products, attention has been focused mainly on lactic acid bacteria (LAB). In the present study, some *Enterobacteriaceae*

strains were considered. The results indicate a considerable variability in the amine-producing capability not only among genera, but also within the single species. This confirms what has been reported in literature (MASSON *et al.*, 1996; MARINO *et al.*, 2000; SUZZI *et al.*, 2000; BOVER-CID *et al.*, 2001b; DURLU-ÖZKAYA *et al.*, 2001; ÖZOĞUL, 2004).

Concerning the amount of biogenic amines produced, there were several differences among the species reported in the literature (MARINO *et al.*, 2000; DURLU-ÖZKAYA *et al.*, 2001). These differences can be attributed to different strains of the same species being investigated; laboratory conditions, different experimental methods, different composition of the medium, vitamins and coenzymes or oxygen tension are some other possible factors that are responsible for the differences (DURLU-ÖZKAYA *et al.*, 2001; PEREIRA *et al.*, 2001; SUZZI and GARDINI, 2003; FERNANDEZ *et al.*, 2007; ÖZGUL and ÖZGUL, 2007).

Most of the strains investigated proved to be powerful producers of cadaverine and putrescine. Similar findings were reported by AYMERICH *et al.* (2006).

The *Enterobacteriaceae* isolated in this study did not produce large amounts of histamine and tyramine, at least not when this culture test was used. These result suggests that the ability of cheese to produce these biogenic amines is probably ascribable to other genera such as LAB, enterococci and others (BOVER-CID *et al.*, 2001a; GARDINI *et al.*, 2001; PEREIRA *et al.*, 2001; SANTOS *et al.*, 2003; MARTUSCELLI *et al*, 2005; AYMERICH *et al.*, 2006).

The production of histamine by some strains was examined at different temperatures (Table 2). Between 10° and 22°C, the production increased as the temperature increased, as reported by VECIANA-NOGUES *et al.* (2004). At 10°C only *K. oxytoca* and *C. freundii* began to form histamine.

The peak of histamine production *in vitro* was reached at 22°C for 6 strains: only *H. alvei* produced less histamine at 22°C than at 37°C. For three of the six strains (*K. oxytoca*, *C. freundii* and *H. alvei*), the increase of production was coupled with an increased turbidity of the test medium. The same behaviour was observed by VECIANA-NOGUES *et al.* (2004) with one strain of *K. oxytoca* and one strain of *M. morganii* isolated from tuna fillets.

Regarding the other strains tested, the two *M. morganii* strains and that of *P. vulgaris* showed a completely different behaviour. Even if the optical density remained the same, the amount of amine formed decreased. This decoupling be-

Table 2 - Production of histamine at 4°, 10°, 15°, 22° and 37°C (mean values expressed in ppm). Number of bacteria in suspension were calculated by McFarland nephelometer standards. McFarland 1 standard corresponds to approximately $3x10^8$ bacteria/mL.

	4°C	Quantity	10°C	Quantity	15°C	Quantity	22°C	Quantity	37°C	Quantity
Species										
K. oxytoca	0	n.g.	27.67	<0.5	174.30	>1 <2	293.53	2	260.7	1
C. freundii[a]	0	n.g.	1.26	<0.5	202.82	1	336.71	2	254.7	0.5
C. freundii[b]	0	n.g.	3.36	<0.5	379.50	1	397.58	1	216.2	0.5
P. vulgaris	0	n.g.	0	n.g.	229.90	1	369.70	2	98.8	2
M. morganii[a]	0	n.g.	0	n.g.	82.67	1	300.32	1	89.9	1
M. morganii[b]	0	n.g.	0	n.g.	249.18	1	343.05	2	42.12	2
H. alvei	0	n.g.	0	n.g.	0	n.g.	3.41	1	25.2	2

Legend: n.g. = not grown.

tween optical density and biogenic amine formation has been observed by several other authors (DURLU-ÖZKAYA *et al.*, 2001; FERNÁNDEZ *et al.*, 2007). FERNÁNDEZ *et al.* (2007) suggest a different decarboxylating activity for a *Enterococcus durans* strain with a different expression of the decarboxylase and antiporter genes. A previous study showed that this behavior could be ascribable to a better decarboxylating activity of the examined strains at a lower temperature (SUZZI and GARDINI, 2003). MARTUSCELLI *et al.* (2005) explained this occurrence by supposing that at 37°C the monoaminoxidase (MAO) system would be more active, so rather than having a lower production of biogenic amines, there could be an acceleration of their inactivation carried out by monoaminoxidase. The results of this study report a 50% decrease in the activity of monoaminoxidases at 20°C, if the activity at 37°C is considered to be 100%.

CONCLUSIONS

Many factors are involved in the production of biogenic amines in food: temperature, bacteriological composition of the raw materials, type of starter cultures and contaminating microflora from the environment (ANSORENA *et al.*, 2001; GENNARO *et al.*, 2003 SANTOS *et al.*, 2003; VECIANA-NOGUES *et al.*, 2004; BALAMATSIA *et al.*, 2006). To study this phenomenon, it is not enough to consider only the bacterial species; single strains must be identified.

Biomolecular techniques and the study of protein and enzyme expression can be powerful tools for identifying the strains. However, this type of analysis is demanding and expensive. The decarboxylase-positive strains can be identified rapidly and reliably which is important for checking the indigenous population of fermented food and choosing the type of starter cultures.

The results of the present investigation show that the *Enterobacteriaceae* in dairy products produced large amounts of putrescine and cadaverine. For this reason it is advisable, as suggested for meat products (BOVER-CID *et al.*, 2001a; VINCI and ANTONELLI, 2002; COISSON *et al.*, 2004; BALAMATSIA *et al.*, 2006), to indicate both amines as markers of the microbial quality in cheeses (MARINO *et al.*, 2000; VALSAMAKI *et al.*, 2000; PINHO *et al.*, 2001).

It is important to check the hygiene of the milk and the cheese process in order to reduce the total number of *Enterobacteriaceae* as much as possible. A large production of cadaverine and putrescine could enhance the toxicity of other biogenic amines by saturating the detoxification systems.

Lastly, since all strains of *Enterobacteriaceae* do not have their maximum decarboxylating activity at 37°C, the production of histamine should also be investigated at temperatures less than 37°C; a temperature of 22°C is suggested.

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SHORT COMMUNICATION

PHYSICAL AND CHEMICAL CHARACTERISTICS OF FROZEN GROUND BEEF AND AGED BEEF MEAT FROM *BOS INDICUS* STEERS SUPPLEMENTED WITH α-TOCOPHEROL ACETATE

CARATTERISTICHE FISICO-CHIMICHE DI MACINATI DI MANZO CONGELATI E DI CARNE DI MANZO INVECCHIATA PROVENIENTE DA CASTRATI DELLA SPECIE *BOS INDICUS* ALIMENTATI CON DIETE ARRICCHITE CON α- TOCOFEROL-ACETATO

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ABSTRACT

The objective of this study was to evaluate the effects of diet supplementation with vitamin E on the physical and chemical characteristics of ground, frozen and stored or aged *Quadriceps femoris* (QF) and *Longissimus dorsi* (LD) muscles from Nellore steers fed high

RIASSUNTO

Lo scopo di questo lavoro era quello di valutare gli effetti di una dieta arricchita con vitamina E sulle caratteristiche fisiche e chimiche di campioni di muscolo di *Quadriceps femoris* (QF) e *Longissimus dorsi* (LD), macinati e congelati o invecchiati, prove-

- Key words: color, meat, Nellore, pH, stability -

concentrate diets. Muscles were obtained from 24 animals that were 30 months old with a mean live weight of 279 kg. Half of the animals received daily doses of 1,000 mg of α-tocopherol acetate (VIT E) per head per day that was added to 100 g of corn meal. The other half received 100 g of corn meal without the antioxidant. Twenty-four hours after slaughtering, QF samples from each animal were ground, frozen and stored for up to 6 months. In addition, 4 samples from the LD of each animal were vacuum packed individually and kept for 21 days. All samples were analyzed to determine the pH, color and water-holding-capacity. The VIT E supplementation improved only the water loss characteristics of frozen ground QF and did not have any positive effect on the physical-chemical characteristics of the aged LD.

nienti da castrati del distretto di Nellore alimentati con diete arricchite. Questi muscoli provenivano da 24 animali di 30 mesi con un peso vivo medio di 279 kg. La metà degli animali assumevano dosi giornaliere di 1.000 mg di tocoferol-acetato (VIT E) che veniva aggiunto a 100 g di farina di mais. L'altro meta degli animali assumevano 100 g di farina di mais senza antiossidante. Ventiquattro ore dopo la macellazione, 7 campioni di QF da ogni animale venivano macinati, congelati e stoccati fino a 6 mesi. Inoltre, per ogni animale 4 campioni del muscolo LD venivano confezionati sottovuoto individualmente e conservati fino a 21 giorni. Per tutti i campioni è stata effettuata la determinazione del pH, del colore, e della capacità di trattenere l'acqua. La VIT E influiva positivamente solo sulla capacità di perdere l'acqua dei QF macinati congelati, mentre non presentava alcun effetto positivo sulle caratteristiche fisico-chimiche dei campioni di LD invecchiati.

INTRODUCTION

Color and the lipid stability are some of the limiting factors that affect the quality and acceptability of meat and meat products. In red meat, such as beef, a bright red color is perceived by consumers to be indicative of freshness, while consumers discriminate against meat which has turned brown. The rate of discoloration of meat is related to the effectiveness of oxidative processes and enzymatic reducing processes in controlling metamyoglobin levels in beef (FAUSTMAN *et al.*, 1989). Therefore, muscle stability depends mainly on the balance between anti-oxidant agents, such as α-tocopherol and pro-oxidants, including the polyunsaturated fatty acids, and the free iron within the muscle (MITSUMOTO *et al.*, 1998).

In the case of refrigerated vacuum-packed meat and frozen ground beef, color changes may reduce quality product (DUFRASNE *et al.*, 2000). The discoloration of refrigerated meat and frozen ground beef is due of myoglobin alterations caused by lipid oxidation reactions. Therefore, meat flavor and shelf-life are compromised by lipid oxidation and surface discoloration. Some technologies, including the use of vitamin E in animal feed prior to slaughter have been tested (LYNCH *et al.*, 1999; DUFRASNE *et al.*, 2000) in an attempt to guarantee the quality of beef meat during storage. This

antioxidant may slow lipid oxidation, as well as myoglobin oxidation (MITSUMOTO *et al.,* 1998).

The objective of this study was to evaluate the effects of diet supplementation with vitamin E (synthetic α-tocopherol) on physical and chemical characteristics of frozen stored ground *Quadriceps femoris* (QF) or aged *Longissimus dorsi* (LD) muscles from Nellore steers.

MATERIALS AND METHODS

The muscles were obtained from 24 *Bos indicus* breed Nellore steers that were 30 months old and had a mean live weight of 279 kg. The steers, raised in confinement, were fed diets containing 85, 79 or 73% concentrate and sugarcane bagasse as roughage source. The concentrate was made up of corn grain, soybean meal, citrus pulp, urea, mineral premix, ammonium sulfate, potassium chloride and Rumensin. Half the animals received a daily dose of 1,000 mg α-tocopherol acetate added to 100 g of corn meal and the other half received 100 g of corn meal without any additives (CON).

The animals were slaughtered after 98 days of feeding, when the subcutaneous fat thickness, between the 12th and 13th ribs was 6 mm thick. Measurements were made with ultrasound equipment. Animals were slaughtered according to Humanitarian Slaughter Guidelines as required by Brazilian law. After slaughter, the carcasses were chilled at 0-1°C for 24h.

After chilling, a sample of *Quadriceps femoris* muscle (QF) was taken from the left side of each carcass and ground in an electric meat chopper. Seven subsamples of ground meat were then vacuum packed separately and frozen at -25°C for 0, 1, 2, 3, 4, 5 or 6 months. Subsequently, three samples of LD muscle (2.5 cm thick) were taken from each animal and vacuum packed individually and aged for 1, 7 or 21 days at 0-1°C. Another sample of LD muscle was taken on day 1 of aging for the Vitamin E (VIT E) and cholesterol (CHOL) analyses.

The QF and LD samples were also evaluated for pH, water-holding-capacity (WHC) and color. The pH was evaluated using a model HI8314 pH Meter (Hanna Instruments, Bedfordshire, UK) and color was determined in triplicate using a portable MiniScan XE Hunter Lab colorimeter (Reston, VA, USA), using the L*,a*,b* CIElab system scale. The WHC was calculated as follows: WHC=We/(Wb+We)x100 where Wb was the weight of the beef and We, the weight of the exudate.

VIT E and CHOL concentrations were also evaluated on the first day of aging in the LD samples of each animal. VIT E and CHOL analyses were performed using a high performance liquid chromatograph (HP series 050, Wilmington, DE, Usa), at 295 nm and 210 nm wavelengths, respectively, for a simultaneous detection procedure. CHEMSTATION-HP software was used (KATSANIDIS and ADDIS, 1999). Vitamins were differentiated by using a Microsorb column with an isocratic 1.3 mL/min flow system. VIT E and CHOL patterns were Sigma® T325 and Sigma® C-8667, respectively. For VIT E curve, concentrations were 0.51, 1.28, 2.57, 3.85 and 5.14 μg/mL and for CHOL, the concentrations were 0.07, 0.15, 0.3 and 0.4 μg/μL.

The QF analyses were completely randomized in a 3x2x6 factorial design (diets x level of α-tocopherol x time of freezing). For LD analyses a completely randomized design was used with a 3x2x4 factorial arrangement (diets x level of α-tocopherol x aging time), except for VIT E and CHOL where the factorial arrangement was 3x2 (diets x level of α-tocopherol).

Statistical analyses were conducted using the mixed procedure of the SAS® system (SAS/STAT, 1999). The fixed effects of diet, level of α-tocopherol, time of freezing and interactions on the pH, WHC and color analyses of QF samples

were evaluated. For the pH, WHC and color analyses on LD muscle the fixed effects of diet, level of α-tocopherol, period of aging and interactions were considered. VIT E and CHOL were evaluated considering the fixed effects of diet and level of α-tocopherol. The pH, WHC and color were analyzed as repeated measurements. Covariance structures were modeled and the best fitted (ARH(1)) was adopted. Effects of treatments were considered significant at $P<0.05$.

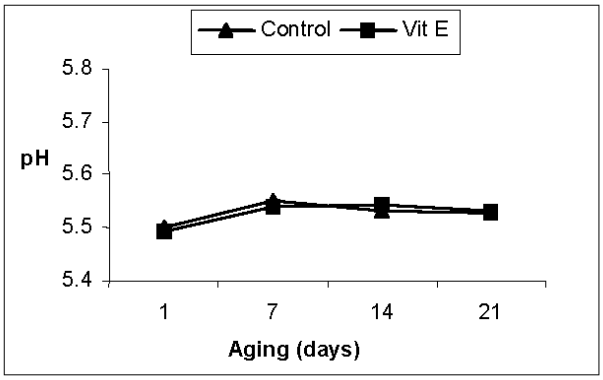

Fig. 1 - *Longissimus dorsi* muscle pH variation with aging.

RESULTS AND DISCUSSIONS

No significant differences were observed for the LD pH values over time between the VIT E and the control groups (Fig. 1). There was no significant difference in the WHC values between time and treatment (Fig. 2). However, MITSUMOTO *et al.* (1998) reported that vacuum-packed LD muscle, refrigerated at 1°C for 6 days, that had been supplemented with 5,000 mg of vitamin E, lost less water by exudation than the control samples. The membrane was maintained and the sarcoplasmatic components were retained which resulted in less exudation loss. Furthermore, JENSEN *et al.* (1997) did not find any significant differences in the WHC in the LD muscle of pork supplemented with 100, 200 and 700 mg of α-tocopheryl acetate; 48 h after slaughtering, the values were 3.7, 4.0 and 4.4, respectively.

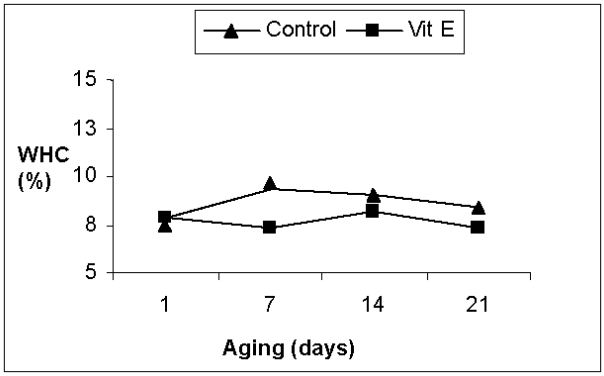

Fig. 2 - *Longissimus dorsi* muscle water bolding capacity (WHC) variation with aging.

For the LD muscle, the L* and a* color parameters had linear effects ($p<0.01$) (Figs. 3 and 4), and the b* parameter had a cubic effect ($p<0.01$) during aging, but the

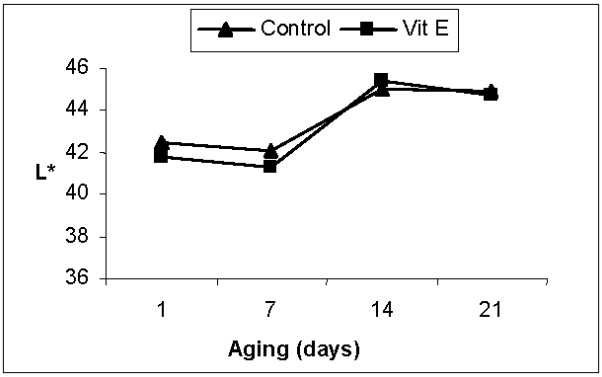

Fig. 3 - *Longissimus dorsi* muscle (L*) variation with aging.

muscles were not affected by the treatments (Fig. 5). A possible explanation for the high L* values could be the presence of liquid on the meat surfaces which resulted in higher L* values on the 21st day of aging. In general, a* did not vary with the time of aging, therefore there vitamin E supplementation did not have a protective effect on the membrane. However, the loss of color pigment did not occur in the times studied. The color of the meat was recovered after the packaging was removed due to myoglobin oxygenation (MITSUMOTO *et al.*, 1998). The discoloration seems to be related to the efficiency of the oxidative and enzymatic processes which decreased the metmyoglobin levels in the control meat systems.

When the results from the supplemented animals were correlated with the control group, no significant difference was observed between the treatments. However, the vitamin E concentration in the LD, at day 1 of aging, showed a significant interaction ($p<0.01$) between the treatments and concentrate levels, with mean values of 1.7 mg/kg and 4.7 mg/kg, for the control group and VIT E, respectively (Fig. 6). DUFRASNE *et al.* (2000) also reported a significant increase in the α-tocopherol concentration in the *Longissimus thoracis* muscle of beef, stored at 4°C for up to 14 days, with controlled lighting, with VIT E values of 1.9 mg/kg in the meat of the experimental animals and 0.9 mg/kg in the meat of the control group. These results indicated that the level of lipid oxidation was affected by a low α-tocopherol concentration in the aged meat for up to 14 days. However, no information was

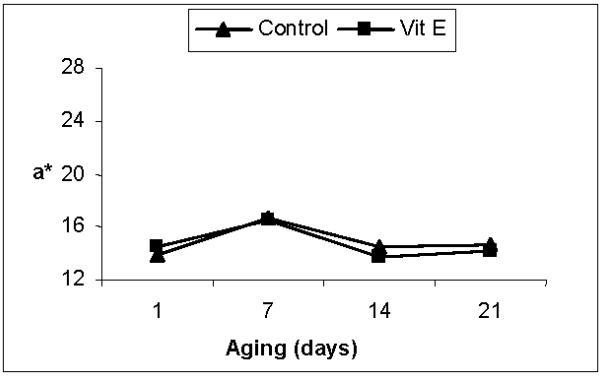

Fig. 4 - *Longissimus dorsi* muscle (a*) variation with aging.

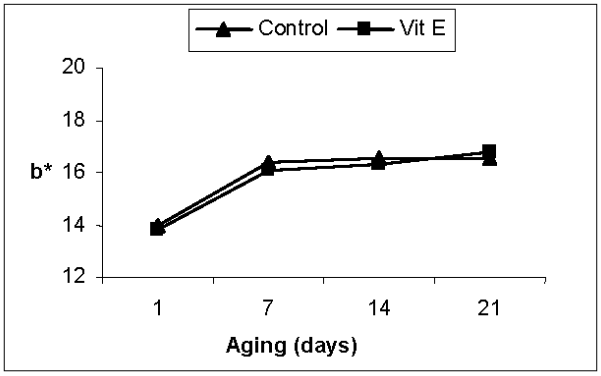

Fig. 5 - *Longissimus dorsi* muscle (b*) variation with aging.

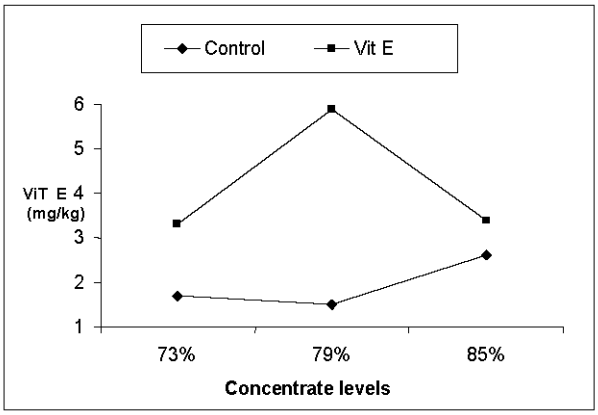

Fig. 6 - *Longissimus dorsi* vitamin E concentration as a function of concentrate levels.

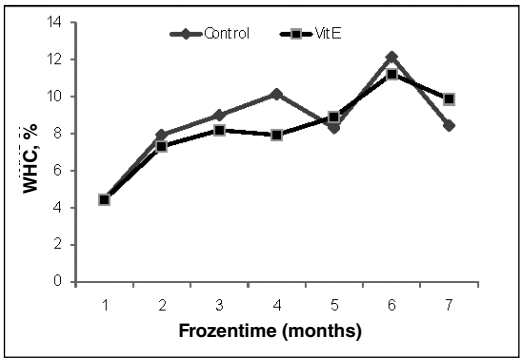

Fig. 7 - Water holding capacity of *Quadriceps femuris* muscle, during frozen storage for control and vitamin E supplemented groups.

found in the literature regarding vitamin E supplementation related to concentrate levels in bovine feed.

The cholesterol concentrations were not significantly different in the LD of VIT E supplemented steers and in the control group, 51.0 and 52.5 mg/100 g, respectively. The cholesterol values were linearly correlated ($p<0.01$) with frozen storage time, but were not affected by treatments.

Table 1 - Mean values of pH, L*, a* and b* in ground *Quadriceps femuris* muscle, during frozen storage for control and vitamin E supplemented groups.

Storage time	Physical-chemical characteristics			
Month	pH	L*	a*	b*
		Storage time		
0	5.69	44.60	16.22	16.09
1	5.70	44.17	15.13	16.09
2	5.67	44.36	15.35	16.22
3	5.70	41.12	16.69	16.74
4	5.69	41.99	14.96	16.02
5	5.73	40.45	16.07	16.39
6	5.70	41.66	14.85	15.92
Time effect[1]	Lin**	Cub**	Lin*	Quad**
		Treatment		
Control	5.68[a]	42.96[a]	15.69[a]	16.38[a]
VIT E	5.71[a]	42.28[a]	15.52[a]	16.03[a]

[1] Lin - linear; Quad - quadratic; Cub - cubic;
* $P<0.05$; ** $P<0.01$;
[a] Means with the same letter did not differ within the same characteristic at 5% by Student T test.

The WHC of frozen ground beef from vitamin E supplemented cattle was affected by the treatments ($p<0.01$); there was less water loss at 1, 2, 3 and 5 months (Fig. 7). This result suggests that the VIT E supplementation helped maintain cell membrane integrity and protected lipids against oxidation. It was observed a quadratic effect ($p<0.01$) with frozen storage time.

The pH had a linear effect ($p<0.01$) with frozen storage time, but was not affected by treatments (Table 1). The color characteristics were not affected by treatments. L* had a cubic effect ($p<0.01$), a* had a linear effect ($p<0.05$) and b* had a quadratic effect ($p<0.01$) with frozen storage time. LYNCH et al. (1999) reported that the vitamin E dietary supplementation in cattle increased the α-tocopherol levels in *Longissimus dorsi*, *Gluteus medius* and *Psoas major* muscles. They reported that at these levels the color and oxidative stability of fresh, frozen and vacuum-packaged beef cuts improved. They also concluded that differences in lipid stability in the muscles could explain the variability in meat color. In this way, the association of L* and a* seems to indicate color losses in the meat. The results of this study suggest that vitamin E did not protect the membranes in the ground beef, in contrast to what was expected and the VIT E supplementation only improved the water-holding-capacity (WHC) of QF frozen ground beef.

CONCLUSION

Despite the high vitamin E concentration in the supplemented feed, which increased the α-tocopherol acetate concentration in the LD muscle, no protective effect related to meat color was observed. This high level of α-tocopherol

acetate in the muscle did not influence the physical or chemical characteristics of the meat aged for 21 days or frozen for 6 months.

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SHORT COMMUNICATION

ESTABLISHING A HACCP SYSTEM IN A SMALL-SCALE TRADITIONAL MEAT PROCESSING ENTERPRISE

STABILIRE UN SISTEMA HACCP IN UNA PICCOLA IMPRESA DI LAVORAZIONE TRADIZIONALE DI CARNE

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ABSTRACT

The aim of the present work was to develop a HACCP program for a small-scale cured ham producing enterprise. The product is defined, the key-stages of production are described, and the prerequisite programs and supporting measures applied in the enterprise are given. Regarding hazard analysis, the HACCP team was formed, the potential hazards were identified, and after evaluation, the critical control points (CCPs) were determined. The CCPs of the processing steps are: reception and

RIASSUNTO

Lo scopo del presente lavoro era quello di implementare un sistema HACCP per una piccola azienda produttrice di prosciutto crudo. Il prodotto è stato definito, sono stati descritti gli stadi chiave della produzione e sono stati definiti i prerequisiti ed le misure di supporto applicate nell'azienda. Riguardo l'analisi del rischio, è stato formato il team dell'HACCP, individuati i potenziali rischi e, dopo un'attenta valutazione, sono stati determinati i punti critici di controllo (CCPs). I CCPs degli

- Key words: critical control points, cured ham, food safety management system, HACCP -

storage of raw materials, meat trimming, weighing of curing mixture, cooling, drying, resting and maturing, packaging-labeling and storage of the final product.

stadi del processo sono: la ricezione e lo stoccaggio delle materie prime, il taglio della carne, la pesatura del condimento, il raffreddamento, l'essiccamento, il riposo e la stagionatura e lo stoccaggio del prodotto finito.

INTRODUCTION

The HACCP (hazard analysis critical control point) system is a systematic approach for identifying, evaluating and controlling biological, chemical or physical hazards, at all stages of food production (CCFH, 2003; NACMCF, 1998). HACCP is widely accepted as the best method for assuring product safety and is internationally recognized as a tool for controlling food-borne safety hazards (KHANDKE and MAYES, 1998; KVENBERG *et al.*, 2000). In addition, HACCP reflects the uniqueness of a product and its production method; it can be described as a preventive, dependent, dynamic, interventive and interactive control system (JOUVE, 1998; UNTERMAN, 1999).

The food industry, nowadays, invests a considerable part of its resources to ensure the quality of their products, particularly regarding health and safety. This has become necessary because of the great economic losses which occur as a consequence of microbiological spoilage in food, as well as the appearance of food-borne diseases in consumers (BATA *et al.*, 2006; HENSON *et al.,* 1998). A guide has been published by ISO and ITC to help small businesses apply the HACCP in the framework of the ISO22000:2005 standard (ISO and ITC, 2007).

Since 1996 it has been obligatory to apply a HACCP system in the European Union (EU, 2004). Food enterprises are making efforts to comply with the new European legislation. The aim of the present work was to establish a HACCP program for a small-scale enterprise that produces cured ham. The key-stages of production are given, and the various types of hazards are identified. After hazard evaluation, the critical control points (CCPs) were determined.

MATERIALS AND METHODS

Production of cured ham

Cured ham is produced by a traditional fermented meat process in the mountainous village of Proussos, Euritania, Greece. It is a type of "prosciutto" that is produced according to the Parma "prosciutto" production program, but adapted to the local climatic conditions.

The ham is the rear thigh muscle of the pig which is cured with, semi-coarse salt, coating paste (a mixture of abdominal fat, salt and pepper), and a curing mixture (FSIS, 1995). Cured ham is generally made with a deboned thigh muscle, approximately 5 to 6 kg, produced by the action of dry salting and a mixture of other ingredients; it is aged for 9 to 12 months, under controlled temperature and humidity conditions.

Salt and curing ingredients were put on the surface of the meat after it had been trimmed; the ham was then placed in a tank at 1°-4°C and 80-90% RH for 1 week. The meat was massaged (500 revolutions/h). This procedure was re-

peated once. After curing, the meat was washed with water (60°-65°C) and dried at 12°-14°C and 65-75% RH for 1 week. After resting for 3 months at a dynamic temperature that fluctuated between 1° and 4°C every six hours and 65-75% RH the ham was placed in a maturation room at a temperature of 14°-15°C that increased to 18°C in the summer and 70-75% RH.

Cured ham produced in the Greek way has a pH of 5.6, and aw of 0.82. The whole thigh is vacuum-packed in a clean, food-grade plastic bag. It is also vacuum packed in quarters or slices, and is ready to eat; further heating is not necessary. The deboned packed product is stable and has a shelf-life of 1-2 months, at 4°C. The boned product has a shelf-life of up to 1 year. It must conform to the provisions of Community Regulation (EC) No 852/2004 for food hygiene (EU, 2004) and Regulation No 2073/2005 on microbiological criteria for foodstuffs (EU, 2005).

PREREQUISITES AND SUPPORTING PROGRAMS

Before establishing a HACCP system, the prerequisite programs applied by the meat processors has to be considered (SPERBER *et al.*, 1998; TOMPKIN *et al.*, 1999). These refer to the location of the enterprise, the construction of the buildings and the prevention and control of rodents and insects. Furthermore, care must be taken to insure that hygienic practices (cleaning and disinfection) are applied to the vehicles and other means of transport. Finally, the technological equipment must operate efficiently and the measurements obtained from instruments must be reliable especially in the cold and maturation rooms. In addition, the meat enterprise must apply support measures to check the water used, the overall hygienic conditions in storage rooms, the disinfecting of the preservation rooms, hygiene of the personnel and general conditions of the buildings.

ANALYSIS OF HAZARDS

Assembling the HACCP team

Hazard analysis includes identifying potential hazards to food safety and evaluating them; it is basic to all HACCP systems (MORTIMORE, 2001). The first step when developing a HACCP system is to set-up a HACCP team which is made up of only a few members in order to assure flexibility. In the present case, the HACCP team was made up of four members, i.e. the HACCP coordinator for the enterprise, the person responsible for production, receiving and storing of materials, and product distribution. The HACCP team members dealt with a number of functional disciplines, e.g. microbiology, regulatory affairs, packaging, logistics, food safety, etc., in order to be certain that a complete and accurate hazard analysis would be conducted.

Stages of production

The main steps for producing cured ham include: receiving raw materials, storing raw materials, trimming, weighing of curing mixture, curing, cooling, massaging, salting, 2nd cooling, 2nd massaging, washing, drying, resting, coating, maturing, deboning, removal of coating paste, packaging - labelling, cold storage of final product, and loading - distribution.

Identifying potential hazards - CCPs

Before dealing with hazard analysis and evaluation, it is important to identify the possible biological, chemical or physical hazards. Biological hazards in meat are mainly of microbiological origin (*Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus*

Table 1 - Plan for the CCPs, critical limits and corrective actions during cured ham production.

CCP No.	Processing stage	Critical limits	Corrective action
1.	Reception of raw material (meat)	No sign of microbial contamination during quality control	Recommendation to supplier, periodical microbiological analysis of raw materials
		Temperature of meat at reception ≤2°C Absence of antibiotics	Returning unsuitable raw material
	- Secondary materials	Absence of defective materials Absence of contaminants	Returning unsuitable materials
	- Packaging material	Absence of spoiled material	Returning unsuitable material
2.	Storing of raw materials	Meat temperature in cold storing ≤2°C. Curing salt and ingredients are stored at <18°C.	Reporting to maintenance engineer, adjust cooling, temperature correction
3.	Trimming	Maintenance of proper hygienic conditions of the equipment and utensils Processing room temperature ≤10°C Absence of wounded thighs during removal of pelvis bone Meat pH from 5.5 to 5.8	Adjust room temperature at ≤10°C Rejection of product
4.	Weighing of curing mixture	ascorbate ≤500 ppm, potassium nitrate <200 ppm	Control of recipe ingredients
5.	Cooling	Store at temperature ≤4°C and humidity ≤75%	Reporting to maintenance engineer, cooling adjustment, temperature correction
6.	Drying	Controlling temperature at ≤14°C for 1 week and humidity at ≤75% for 1 week	Correction of temperature and humidity
7.	Resting	Aw (target end: 0.85, Controlled fluctuation for temperature (1°-4°C) and humidity (80-90%), every 6h. pH:5.6 Cured ham should not be soft when pressing with thumb near bone.	Correction of temperature and humidity Prolong resting phase
8.	Maturing	Room temperature 15°C and ≤18°C in summer Room humidity 70-75% Keep in maturation room for 9-12 months aw:0.82	Correction of temperature and humidity
9.	Packaging - labeling	Absence of contaminated, moist and torn packaging material.	Rejection Investigate reasons for non-conformity
10.	Storage	Packed product at ≤4°C for 1-2 months Unpacked product at <12°C for 1 year	Correction of temperature Withdrawal of out-dated products

aureus, etc.). Chemical hazards in mature meat include mycotoxins, medicinal residues, pesticide residues, heavy metals, additives exceeding permitted dose, disinfectants or packaging materials. Physical hazards include glass, metals, stones, plastics, bones, insects, and contamination from personnel.

The CCPs identified in the present study, as well as the critical limits and corrective actions are reported in Table 1. The hazard evaluation at 20 different steps along the cured ham production line identified 10 CCPs. Most of the controls at the CCPs are focused on maintaining proper temperature and humidity levels. These extrinsic factors are vital for controlling microbial flora in the product. For CCP no. 4, concerning the amount of curing ingredients, the critical limits were determined according to current legislation (Food Code). With CCP no. 9, production codes on labels and the use of metal detectors are included to assure product traceability and recall and control physical hazards. The other critical limits included the ingredients in the curing mixture, especially nitrate and salt. The pH and aw values are the major multiple hurdles that develop during processing and correspond to the critical limits. The safety management of the product shall direct the production to the accomplishment of these safety hurdles (LEISTNER and RODEL, 1976).

Verification, validation and record-keeping activities of the applied system include periodical inspections of the production records and the results of microbiological and physico-chemical analyses in order to validate the effectiveness of CCPs. An on-site audit of the production line is also necessary. During the annual verification, suggestions are made to improve and update the system. Record keeping includes a written system, its procedures, the work instructions and the record-keeping forms. Data from the CCP monitoring, corrective actions and verification records are items for evaluating the effectiveness of the system. Records with amendments of the system are included in the documentation.

CONCLUSION

The CCPs in the production of cured ham at a small-scale processing plant were determined. A HACCP for the meat industry must begin at the farm because certain safety concerns cannot be eliminated during the slaughtering process, e.g., chemical residues and certain microbiological pathogens (e.g. *Salmonella*, *Campylobacter jejuni*) cannot be controlled without the longitudinally integrated safety assurance as incorporated in the EU General Food Law (EU, 2002; MOSSEL *et al.*, 1995).

Food safety hazards may be introduced or appear at any stage along the food chain "from farm to fork". The elimination of the "weak links" in the food chain is the objective of the new international standard ISO 22000:2005 "Food safety management systems – requirements for any organization in the food chain" (ISO, 2005). It should be noted, that the successful application of a HACCP program requires that the personnel involved be efficiently trained, and that the administration of the meat processing plant be committed to assuring food hygiene and safety for their enterprise.

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SHORT COMMUNICATION

ISOLATION AND IDENTIFICATION OF THERMOPHILIC *CAMPYLOBACTER* SPP. IN CATTLE CARCASSES FROM A NORTHERN ITALIAN SLAUGHTERHOUSE

RICERCA ED IDENTIFICAZIONE DI CAMPILOBATTERI TERMOFILI IN CARCASSE BOVINE PRESSO UN MACELLO DEL NORD ITALIA

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ABSTRACT

Rinse water samples were obtained from fifty cattle carcasses processed in a northern Italian slaughterhouse between January and April 2007. The incidence and prevalence of *Campylobacter* spp. in these samples were determined. Conventional cultural techniques, followed by a rapid latex agglutination test were used for testing. A polymerase chain reaction (PCR) followed by DNA sequencing was used to identify the thermophilic *Campylobacter*. *Campylobacter* spp. were detect-

RIASSUNTO

Sono stati indagati cinquanta campioni di acqua di lavaggio di carcasse bovine, prelevati presso un macello del Nord Italia nel periodo Gennaio-Aprile 2007, al fine di determinare l'incidenza di *Campylobacter* spp. e, qualora presente, definire la prevalenza delle specie coinvolte nella contaminazione delle carcasse bovine oggetto della ricerca. L'isolamento e l'identificazione di campilobatteri termofili sono stati effettuati impiegando le tecniche microbiologiche colturali classiche, affiancate dall'utiliz-

- Key words: cattle carcasses, PCR, rinse water samples, thermophilic *Campylobacter* -

ed in four (8%) samples using the cultural methods and in six (12%) samples using molecular techniques. *Campylobacter jejuni* was the only species detected in these samples. The results indicate a relatively low level of *Campylobacter* spp. contamination on the examined cattle carcasses.

zo di un test rapido di agglutinazione al lattice, e la metodica molecolare PCR seguita dal sequenziamento dei prodotti di amplificazione. *Campylobacter* spp. è stato riscontrato in quattro (8%) e in sei (12%) dei campioni indagati secondo il metodo microbiologico e molecolare, rispettivamente. L'identificazione di specie ha portato all'esclusivo riscontro di *Campylobacter jejuni*. I risultati evidenziano un basso livello di contaminazione da *Campylobacter* spp. delle carcasse bovine prese in esame.

INTRODUCTION

Thermophilic *Campylobacter* species are the bacteria that are most frequently isolated from diarrhoea in humans (SKIRROW, 1994). Among these, *Campylobacter jejuni* and *Campylobacter coli* are responsible for 85-95% and 5-10%, respectively, of human *Campylobacter* infections (VANDAMME, 2000).

Over the last ten years, cases of enteritis due to *Campylobacter* have increased markedly in most industrialised countries (LUZZI and PEZZOTTI, 2005). Campylobacteriosis is now the most frequently reported zoonotic disease in humans within the European Union, exceeding the number of cases of salmonellosis. In 2005 the number of reported *Campylobacter* cases in Europe increased 7.8% compared to the previous year. The incidence rate was 51.6 cases per 100,000 people, with a total of 197,363 recorded cases (ANONYMOUS, 2006).

The main source of foodborne *Campylobacter* infections is thought to be contaminated poultry meat because as much as 98% of fresh poultry meat samples may be positive for *Campylobacter* (MANFREDA, 2004). However, *Campylobacter* has also been isolated from a variety of other foods such as fresh bovine and pig meat, raw cow's milk, cheeses, fishery products, and fruit and vegetables, but the occurrence is less common than that reported in fresh chicken (LUZZI and PEZZOTTI, 2005).

In the EU in 2005, the proportion of samples of fresh beef found positive for *Campylobacter* at retail was generally low (0-2.1% in reports from Germany, Italy, the Netherlands and Spain) with consistently low proportions of positive samples reported for four consecutive years in Italy (1.1% in 2002, 0.6% in 2003, 0% in 2004 and 0.5% in 2005) where the most commonly found species was *C. jejuni* (ANONYMOUS, 2006).

The origin of *Campylobacter* on raw meat is thought to be due to contamination of carcass surfaces with faecal material during slaughtering (LUZZI and PEZZOTTI, 2005). Cattle are a natural reservoir for *Campylobacter* and these organisms are present in the faeces of healthy cattle (ACIK and CETINKAYA, 2005). The prevalence of *Campylobacter* in beef cattle at slaughter has ranged from 47 to 74%, with *C. jejuni* being the most commonly isolated species (LUZZI and PEZZOTTI, 2005).

Cattle are often carriers of *Campylobacter* and enteropathogenic campylobacters can be isolated from the carcass-

es of newly slaughtered animals (COMI and MAIFRENI, 1999), the slaughter process may be an important source of cross-contamination, that could lead to increased consumer risk. This fact underscores the importance of accurately determining the level of *Campylobacter* contamination on cattle carcasses at the slaughterhouse in order to evaluate the wholesomeness of beef. Few data are available regarding the presence of *Campylobacter* on bovine carcasses following slaughter in Italy.

The objectives of the current study were (1) to assess the incidence of campylobacters on cattle carcasses from a small northern Italian slaughterhouse and (2) to identify the campylobacters at the species level, particularly *C. jejuni* and *C. coli*.

MATERIALS AND METHODS

Experimental design and sample collection

Rinse water samples were collected from fifty carcasses of adult beef cattle slaughtered in a northern Italian slaughterhouse which was licensed to process a maximum of 2,000 animals per year using entirely non-mechanised, manual slaughtering procedures.

Samples were taken at the abattoir immediately after the animals were slaughtered but before the carcasses were taken to refrigerated storage. Sampling was as directed by the veterinarian supervising the slaughter. The neck region of each carcass was washed with sterile peptone water and 50 mL of the runoff was collected in a sterile resealable container. A total of fifty rinse samples were collected between January and April 2007.

The samples were placed in refrigerated containers (4°±2°C) and delivered the same day to the laboratory for analysis. All samples were analyzed for the presence of *Campylobacter* within 24 h following collection; microbiological and molecular analysis techniques were used.

Microbiological analyses

All samples were analyzed qualitatively for *Campylobacter* spp. Each 50 mL rinse sample was placed in a sterile screw-cap container and filled to the brim with 225 mL of Bolton Broth (Oxoid, Hampshire, UK) containing Bolton Broth Selective Supplement (Oxoid) and Laked Horse Blood (Oxoid). The broths were incubated for 4 h at 37°C, followed by 44 h at 42°C under microaerobic conditions created by placing the containers, with loosened screw caps, in 2.5 L jars. The microaerobic atmosphere was obtained by using commercial gas generating kits (CampyGen; Oxoid). After incubation, 100 μL samples were taken from each culture and streaked onto plates of *Campylobacter* blood-free selective agar base (CCDA; Oxoid) containing CCDA Selective Supplement (Oxoid). Inoculated plates were placed in a jar under microaerobic conditions generated by CampyGen sachets (Oxoid) at 42°C for 48 h.

Campylobacter suspect colonies were subjected to a rapid latex agglutination test (M46 Microscreen *Campylobacter*; Microgen Bioproducts, Surrey, UK) to confirm the identification of the enteropathogenic thermophilic campylobacters.

As specified by the manufacturer, several colonies with *Campylobacter*-like morphology were removed from each CCDA plate with an inoculating loop and mixed with 50 μL of sample diluent on the agglutination slide. A 50 μL volume of test latex reagent was then added and mixed. The reagent contained latex particles coated with rabbit immunoglobulins raised against enteropathogenic *Campylobacter* antigens. Bacterial suspensions were maintained in constant gentle movement for about two min until a sensitive and specific immunochemical reaction had taken place causing

the finely dispersed latex particles to agglutinate into aggregates that were easily visible to the naked eye. According to the manufacturer's instruction, the slides were assigned a score (+, ++, +++) according to the strength of the agglutination reaction observed.

PCR analyses

DNA was extracted from 1 mL of the 48 h enrichment broth cultures, using the Chelex 100 Resin (Bio-Rad Laboratories, CA, USA) extraction procedure as described by JOSEFSEN *et al.* (2004). PCR amplifications were carried out as described by DENIS *et al.* (1999). The initial PCR was carried out to amplify a gene that is specific to the *Campylobacter* genus; if *Campylobacter*-positive, a second PCR was carried out to identify the species in the samples. Three sets of primers for gene amplification were used: MD16S1 and MD16S2 for *Campylobacter* genus that amplify the 16S rRNA gene; MDmapA1 and MDmapA2 for *jejuni* species and COL3 and MDCOL2 for *coli* species that amplify *mapA* and *ceuE* genes, respectively. An internal control (IC) of amplification was used as described by DENIS *et al.* (2001). PCR reactions were performed in a total volume of 100 μL or 50 μL using the Taq PCR Core Kit (Quiagen, Hilden, Germany). The reaction mixture contained 5 μL or 2.5 μL of DNA template, 200 μM of each dNTP, 2.5 or 1.25 U of Taq DNA polymerase, 15 mM of $MgCl_2$, and 20 pmol of each primer. Amplification reactions were carried out using a Perkin Elmer 2400 thermocycler (Perkin Elmer-Applied Biosystems, Warrington, UK) with the following programme: one cycle of 10 min at 95°C, 35 cycles each consisting of 30 s at 95°C, 1 min 30 s at 59°C, 1 min at 72°C and a final extension step of 10 min at 72°C. Amplification generated an 857 bp DNA fragment corresponding to the genus *Campylobacter*, and 589 bp and 462 bp fragments corresponding to the species *jejuni* and *coli*, respectively. PCR products were checked for quality and quantity (refering to MassRuler DNA Ladder, Low Range; Fermentas International Inc, Ontario, Canada) by electrophoresis on 2% agarose gel stained with ethidium bromide. PCR products were then purified (QIAquick PCR Purification Kit; Qiagen) and sequenced (MWG-Biotech genomic service, Ebersberg, Germany). The sequences obtained were matched with nucleotide sequences in the EBI (European Bioinformatics Institute) database.

RESULTS

The results of the microbiological and molecular analyses are shown in Table 1. Plate colonies, grown from enrichment broths, that had a morphology consistent with that of *Campylobacter* were obtained from four (8%) of the fifty samples investigated. Positive latex agglutination tests confirmed the presence of *Campylobacter* spp. in each of the four samples, all of which displayed the maximum agglutination reaction (+++).

PCR and sequencing of amplified fragments showed the presence of *Campylobacter* spp. in six (12%) of the fifty samples investigated for the *Campylobacter* genus. Four of these six samples (samples 14, 26, 46, and 48) corresponded to the samples that were positive for *Campylobacter* spp. based on the microbiological analyses. PCR analyses and DNA sequencing to determine *Campylobacter* species revealed that *C. jejuni* was present in each of the six positive samples while *C. coli* was absent in all the samples.

DISCUSSION AND CONCLUSIONS

The present study assessed the prevalence of thermophilic *Campylobacter*

Table 1 - *Campylobacter* detection by microbiological and molecular analyses of rinse water samples from freshly slaughtered cattle carcasses.

Sample number	Microbiological analyses		Molecular analyses [b]		
	Growth on plate [a]	Latex agglutination test	Identification of *Campylobacter* genus	Identification species	
				C. jejuni	*C. coli*
10	no	N/A	+	+	-
11	no	N/A	+	+	-
14	yes	+	+	+	-
26	yes	+	+	+	-
46	yes	+	+	+	-
48	yes	+	+	+	-
Positive (%)	4/50 (8)	4/50 (8)	6/50 (12)	6/50 (12)	0/50 (0)

N/A = not applicable.
[a] Colonies with *Campylobacter*-like morphology grown on selective CCDA media following enrichment culturing.
[b] PCR followed by DNA sequencing.

contamination of beef carcasses in a small slaughterhouse. Molecular methods for bacterial identification were used to compare PCR with the conventional culture techniques for detecting *Campylobacter* spp.

The PCR assays indicated that 6 of the 50 (12%) samples were positive for *Campylobacter* spp., while only 4 of the 50 (8%) were positive for *Campylobacter* spp. using conventional cultural techniques. This finding suggests that campylobacters were present at higher levels in the original samples because the PCR technique detects target DNA in samples even when this organism cannot grow in culture. However, despite the greater sensitivity of the PCR method, only the microbiological results can indicate the presence of viable *Campylobacter* contamination, and therefore indicate the level of risk to the consumer.

The contamination frequency in cattle carcasses was 8%; this exceeded the 0-5% contamination values reported in other studies (BEACH *et al.*, 2002; KURSTEINER *et al.*, 1985; KWIATEK *et al.*, 2006; MADDEN *et al.*, 2001; MINIHAN *et al.*, 2004a; MINIHAN *et al.*, 2004b; PHILLIPS *et al.*, 2006). The higher value could be due to a greater risk of carcasses being exposed to faecal material or more opportunities for cross-contamination in a small abattoir where all operations are carried out manually.

However, an 8% contamination level is low compared to the incidence of *Campylobacter* reported in chicken (74.4%) (STERN *et al.*, 2007) and pig (62%) (MALAKAUSKAS *et al.*, 2006) carcasses at slaughter.

When *Campylobacter* contamination was found, only *C. jejuni* was present; this is in accord with previously published data that indicate that *C. jejuni* is the sole or most prominent *Campylobacter* contaminant of beef (ELMALI, 2004; PEZZOTTI *et al.*, 2003). The presence of *C. jejuni* on beef carcasses is important because approximately 90% of all human cases of campylobacteriosis are caused by *C. jejuni* (ROBINSON *et al.*, 2000).

In terms of consumer health, the risk of *Campylobacter* infection in humans through a bovine meat source should be considered low, because the percentage of samples that were positive for *Campylo-*

bacter in this study was low. In addition the bacteria cannot survive on the dry surface of beef carcasses during chilling (GRAU, 1988; LUZZI and PEZZOTTI, 2005).

In this study, thermophilic *Campylobacter* spp. were found on cattle carcasses in a small northern Italian slaughterhouse. A similar investigation on the presence of this pathogen on carcasses processed in large slaughterhouses is needed to have a more accurate assessment of the presence of *Campylobacter* on beef carcasses in northern Italy.

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