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Hygienic quality and freshness of shell eggs collected at the retail level in Italy

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Summary

Shell eggs sampled at the retail level in two large Italian cities were tested to assess their freshness, food safety, and the presence of veterinary drug residues.

Some samples were found to be irregular due to lack of compliance with freshness requirements or shell tainted by microcracks and foreign material; the most severe non-compliance was however due to the presence of veterinary drug residues, which exceeded either the maximum residue limits or were even prohibited

Keyzords

Eggs - Haugh Unit - Quality factors - Residues.

Introduction

Hen eggs are one of the most complete food of animal origin, due to the high biological value of its proteins and its phospholipidic component (phosphorilated lipids are fundamental elements of cell structure), and to their good energy supply (70 Kcal for one egg of 55 g) (14).

Two hundred and eighteen eggs pro capite were eaten in 2003 in Italy (14). This considerable consumption is due to:

i) low prices make them easily affordable for most consumers, regardless of their income;
ii) eggs' extreme versatility allow them to enter into the preparation of a wide range of foods to be consumed either raw or cooked;
iii) eggs can be stored for a long time even at room temperature, meanwhile maintaining acceptable characteristics (4-5 weeks) (4, 12).

Eggs, like every food of animal origin, can transmit a broad range of pathogens, drug residues or other chemicals commonly used for crop protection. Because of these hazards, eggs fall under official control, as far as the definition of qualitative and organoleptic standards, and food safety requirements are concerned (6, 7, 15). Legal provisions establish that shell eggs quality must be assessed by means of official inspection and control procedures; laboratory analysis have only a supplementary function, as far as detection of any xenobiotic substance is concerned.

In November 1999, 35 egg samples were collected in Rome and Milan supermarkets by technicians belonging to Altroconsumo (a consumers' association) and subsequently tested. The study

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aimed to verify the eggs' quality grade, freshness and some food safety requirements.

Materials and methods

Tested samples

Thirty-five egg samples were tested, of which 8 were classified as Extra grade and 27 as A grade, 21 were from Milan and the other 14 from Rome. Samples were randomly collected in as many supermarkets from large scale retail-trade. Every sample consisted of at least 2 boxes of 6 eggs belonging to the same lot of production, packaged into containers made of plastic or pressed pulp wood, with or without cardboard band.

In order to simulate storage conditions after purchase, samples were treated as following:

- thirty-three samples were stored at room temperature until one week before their preferable consumption, as reported on the label. Subsequently they were moved to a temperature ranging between 0° C ÷ +4° C until their best-before deadline, when tests started;
- two samples, kept in refrigerators also in the supermarket, were stored between 0°C ÷ +4°C till their best-before deadline, when tests started.

Every sample was tested to define their sanitary and health condition, and to detect possible residues of some veterinary drugs. Parameters and standards adopted, (Tables I and II), were either according to the requirements set in EU legislation (7) or in USA regulation (19, 20). In order to evaluate egg quality and freshness, for each parameter standards set in EU (7) and USA regulation (19, 20) were adopted and, whenever necessary, integrated with information from scientific literature (9, 11).

Parameters possibly affected by storage temperature (thickness and albumen pH, air cell depth) were compared to verify whether differences existed between results obtained in chilled samples (0° C ÷ +4° C) and in samples kept at room temperature.

Quality factors

One of the two units making up each sample (one unit = one package of 6 eggs) was randomly chosen and the eggs within were submitted to the following tests:

External inspection: integrity, cleanness condition, regularity and color of the shell (7, 19).

Candling: evaluation of shell integrity, yolk outline and position, viscosity of albumen (16, 19) and air cell depth [measured by a millimetre thin card according to Fagotti (9)].

Internal inspection: presence of foreign smells, yolk thickness, germinal disc, thickness and viscosity of albumen, meat and/or blood-spots (7, 19).

Table I
Tests performed on eggs

Tests	Method	Law references
Inspection	Visual inspection	Commission Regulatio (EEC) N. 1274/91
Tetracyclines	HPLC	Council Directive 96/23/EC
Quinolones	HPLC	Council Directive 96/23/EC
Sulphonamides	HPLC	Council Directive 96/23/EC
Nitrofurans	HPLC	Council Directive 96/23/EC
Coccidiostatics	HPLC	Council Directive 96/23/EC
Macrolids	HPLC	Council Directive 96/23/EC
pH	potentiometric	General

Table II
Standards adopted to assess eggs' quality and freshness

Standards adopted	Parameters of assessment	Ref
Clean shell	Shell without adherent material	19,20
	abnormal colourations or decolourations	19,20
Dirty shell	Shell with adherent material, abnormal colourations or decolourations	
Regular shell	Shell within typical aspect of the species whole and without little calcareous thickenings (concretions) with possible rough areas	19,20
Fracture of the shell	Shell and testacean membrane with break (albumen leaking or leakage of albumen)	7,11
Micro-fractures of the shell	Shell with cracks whose detection is exclusively by means of a direct light beam (candling)	-
Physiologic air chamber	Air chamber betweenshell and the external layer of albumen, normally located at the egg's blunt pole, with a maximum height of 6 mm	9,19,20
Content tainted by meat and blood stains	The egg's content (albumen and/or yolk) is tainted by blood residues, possibly without the typical red colour, and/or by reproductive organs tissue clusters; the stains' diameter does not exceed 3 mm and the stains are not due to the development of the germinal spot	19,20

Hydrogen-ion activity (pH) and albumen thickness were also measured, although EU legislation does not specifically provide for these requirements. The height of albumen, in Haugh Unit (HU), was measured by a micrometer Futura, (Aviomatic, Varese, Italy) having as reference the USA regulations standards: AA class (extra for the EU (CE)) >72 HU, A (A for the EU CE) 60 - 72 HU and B (B for the EU CE) < 60 HU (7, 20). Haugh Units were calculated on the basis of the average thickness of 10 eggs, randomly chosen from each sample. The Spearman non-parametric correlation coefficient was calculated between the number of HUs and air cell thickness to verify whether albumen thickness could possibly be used as a freshness parameter also in eggs stored at room temperature.

Chemical analysis: veterinary drugs residues were assessed by means of the following technique: for every sample at least 4 eggs were randomly chosen and albumen and yolk were pooled, homogenized in Stomacher® 400 (LAB SYSTEM, Norfolk, UK) and chilled in plastic food boxes at –18°C ± 3°C temperature till the day when tests started. Residue presence was assessed for substances, for which EEC (6) establishes either maximum residues limits (MRL) or absence (2, 3):

- tetracyclines: oxytetracycline, tetracycline;
- quinolones: enrofloxacin, flumequine and oxolinic acid;
- sulfonamides: sulfadiazine, sulfaquinoxaline, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfathiazole, sulfamethoxypyridazine;
- nitrofurans: nitrofurazone, nihydrazone, furazolidone, furaltadone;
- coccidiostatics: carbadox, olaquindox and nicarbazine;
- macrolides: tylosin and spiramycin.

Tests were carried out by means of solvent extraction and purification by solid-phase extraction (SPE) techniques, specific for each analyte, and further identification in HPLC, according to Regulation

93/256/EEC (8).

In particular:

Tetracyclines, extraction was performed by means of a MacIlvaine/EDTA buffer mixture 0.1 M, pH 4. Triethylammonium sulphate 0.5 M, pH 5 was added to a part of the extract, then purified on a SPE C_{18} cartridge. Interference substances were eliminated by washing the cartridge with water. Tetracyclines were eluted with oxalic acid 0.01 M in methanol. HPLC analysis was carried out using a Waters (Milford, USA) 600 MS pump on a C_{18} column (25 cm × 4 mm, 5 μm) with a mobile-phase consisting of acetonitrile/methanol/oxalic acid 0.01 M aqueous solution (20:75:5, v/v/v) and a diode array detector Waters PAD 996.

Quinolones, the extraction was carried out by means of a mixture of dichloromethane/acetone (1:1, v/v). Part of the extract was dried at 50°C ± 5°C, taken up in a phosphate buffer solution pH 7.8 and cleaned up on a SPE C_{18} cartridge. Interference substances were eluted by washing with hexane. Quinolones were eluted using a methanol/ammonia 0.1 N mixture (75:25, v/v). HPLC analysis was performed using a liquid chromatograph Star 9010 (Varian, Palo Alto, CA-USA), on a C_8 (25 cm × 4 mm, 5 μm) column with a gradient mobile-phase acetonitrile/oxalic acid 0.035 M and fluorimetric detection:

- enrofloxacin: 278 nm excitation wavelength, 445 nm emission wavelength
- flumequine and oxolinic acid: 250 nm excitation wavelength, 365 nm emission wavelength.

Sulfonamides, nitrofurans and coccidiostatics were extracted by means of dichloromethane/acetone (1:1,v/v) mixture. An aliquot of the extract was evaporated to dryness at 50°C ± 5°C, redissolved in a phosphate buffer solution pH 7.8 and purified on solid phase C_{18} – Al_2O_3 cartridges, connected in series. After washing with hexane, nitrofurans and coccidiostatics were eluted with methanol. Sulphonamides, retained on Al_2O_3 column, were eluted using a methanol/water solution (6:4, v/v). HPLC analysis was performed on a C_{18} (25 cm × 4 mm, 5 μm) column with a gradient mobile phase (acetonitrile/buffer acetate 0.01M pH4.6) and diode array detection, using a liquid chromatograph Waters 600 MS coupled with a Waters 996 PAD detector.

Macrolides were extracted by means of dichloromethane/acetone (1:1,v/v). Part of the extract was dried at 50°C ± 5°C, retrieved using a acetate buffer pH 5.5 and purified on a cyanopropyl SPE cartridge. Co-extracted substances were washed with water and methanol. Macrolides were eluted using methanol/diethylamine (99:1, v/v). HPLC analysis was performed on a C_{18} (15 cm × 4.6 mm, 5 μm) column with mobile-phase acetonitrile/monobasic sodium phosphate 005 M, pH 2.5 and diodes array detection, using a liquid chromatograph Waters 600 MS coupled with a Waters 996 PAD detector.

Data analysis

Results were aggregated in a Microsoft® Access 97 database. Main descriptive statistics, the non-parametric correlation coefficients (Spearman), χ^2 and Fisher exact probability were calculated using SPSS for Windows, version 9.0.1.

Probability distribution of anomalies detected in the sampled population was assessed by means of Bayesian analysis; Microsoft® Excel 97 was used for calculations.

Results

Esternal inspection (shell)

Integrity

Fifty-seven percent of samples contained no cracked eggs. The remaining 43% had 1 or 2 cracked eggs. Eggs were also candled to detect the presence of micro-fractures: only 31% of samples contained

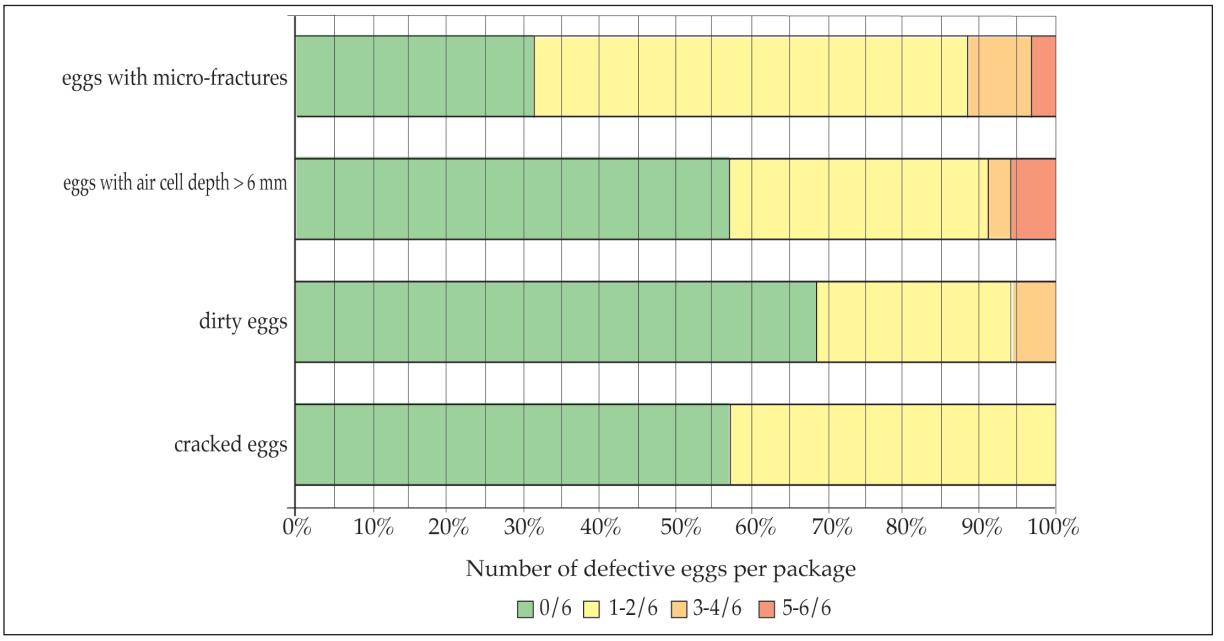

Figure 1
Percentage of packages with defective eggs

no eggs with micro-fractures (Figure 1). In 3% of samples, 5 eggs out of six had micro-fractures (Figure 1).

No relationship was found between the type of package and the presence of cracked eggs (χ^2=1.993, p=0.813: Fisher probability, p=0.785).

Shell cleanliness

In 69% of samples, all eggs were clean while the remaining 31% of the tested packages contained almost 1 dirty egg (Figure 1).

Shape

Shell inspection detected from 1 to 4 irregular eggs in 20% of the inspected packages (7 out of 35); 3 of them showed both rough surface and concretions, the others 4 only concretions.

Color

Each tested egg was free from stains or discoloration.

Candling

Yolk

Yolk was indistinct and appeared to blend into the surrounding albumen as the egg was twirled, with the exception of two eggs, where yolk was dislocated towards the wall.

Air cell

Forty-three percent of the samples contained at least one egg whose air cell depth exceeded 6 mm (maximum value accepted in the EU for grade A eggs) (7). Six percent of the analyzed packages contained 5/6 defective eggs with respect to air cell depth (Figure 1).

Albumen

Every sample showed clear albumen, but 8 eggs, where chalazae were evident.

Internal inspection (content)

Smell

All tested samples were free of foreign smells.

Germinal disc

The germinal spot was well defined in each inspected egg. An embryo, with a diameter of about 4 mm, was detected in one of them, and afterwards confirmed by stereomicroscope.

Albumen

Average pH value was 9.2 (minimum value 8.99, maximum value 9.28).

Based on the albumen thickness, 71% of samples were classifiable as Grade B (< 60 HU), the remaining 29% were grade A or AA (respectively 60-72 HU and > 72 HU). Albumen average thickness of the two chilled egg samples were respectively 68.32 (Grade A) and 73.71 HU (Grade AA).

No statistically significant correlation was detected between the HU and the air cell depth (Spearman correlation coefficient ρ=-0.104; p=0.554). Results are shown in Figure 2.

Should a stringent correlation between air cell thickness and HU number have been found, the distribution of samples as shown in Figure 2 would have been crowded in the "C" quadrant (fresh Extra or A grade eggs, with number of HU> 60 and air cell thickness ≤ 6 mm) and in the "A" quadrant (B or C grade not compliant eggs, (number of HU< 60 and air chamber height > 6 mm).

Chemical analysis

Nine samples out of the 35 tested samples were found positive for veterinary drugs residues:

- 7 due to the presence of only one active substance (6 for sulfadiazine, 1 for oxolinic acid);
- 2 due to the presence of two active substances (sulfadiazine and enrofloxacin).

Of the 9 positive samples, 6 were collected in Milan and 3 in Rome.

No statistically significant differences were found for the origin of the positive samples (Fisher's exact test, for all drugs: p = 0.712, for sulphonamides: p = 1.000).

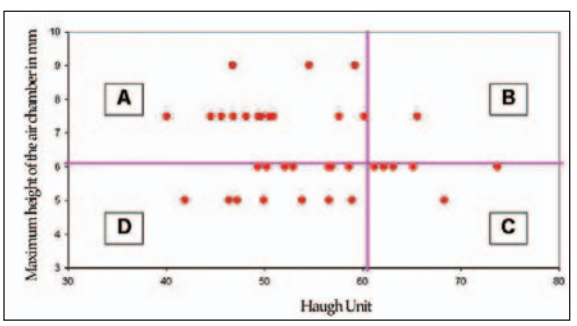

Figure 2
Correlation between HU average value and average value of the air cell depth

Statistical assessment of legal tolerances for quality defects

Quality defects up to a 7% threshold are tolerated by the European legislation (7) at the retail level; 4% of these defects can be represented by shell fractures or cracks and up to 1% by meat or blood stains.

Non-compliances (cracked shell, air cell thickness > 6 mm etc.) were mostly detected in only one of the 6 tested eggs that made up each sample.

Starting from the non-compliances frequency pattern detected in the tested samples, a Bayesian analysis was performed to calculate the probability distribution of irregular eggs in the batch.

Figure 3 shows the probability values of irregularity frequencies in the batch to which the tested eggs' package belonged, when only one egg out of 6 was found irregular.

In this case (1 irregular egg out of 6), the probability that all irregularities in the batch would not exceed the legal limit of 7%, is 8.1%. As far as the tolerated levels of shell cracks or micro-fractures (4%) or presence of meat or blood stains (1%) are concerned, probability values are respectively 2.9% and 0.2%.

Figure 4 shows probability values, if non compliances are detected in 2 eggs out of 6. In this case (2 irregular eggs out of 6), the probability that all irregularities in the batch would not exceed the legal limit of 7%, is 1%. As far as the tolerated levels of specific non-compliances (4% and 1%) are concerned, probability values are respectively 0.2% and 3×10^{-5}%.

Figure 5 shows probability values for non-compliance with the European legislation in the origin batch, when irregularities are detected in 3 eggs out of 6.

In this case (3 irregular eggs out of 6), the probability that all irregularities in the batch would not exceed

the legal limit of 7%, 4% or 1% is respectively 0.07%, 8 x 10^{-3}% and 3 x 10^{-5}%.

In order to be 95% confident that all irregularities in the batch would not exceed the legal limit of 7%, at least 7 packages containing six eggs per each lot should be examined with no detected irregularities or 11 packages with a maximum of 1 defective egg detected.

Discussion

Hygienic quality, freshness and residues of veterinary drugs, as tested in the 35 egg samples collected at the retail level, are the most relevant parameters as far as Public Health is concerned.

Freshness, determined by measuring air cell depth, was non-compliant with A grade standard in 15 samples out of 35. Such finding clearly indicates a qualitative standard of retailed product significantly lower than what declared, given that about 40% of the tested eggs belonged to grade categories inferior to what was reported on the label.

The decrease in quality might be due to difference in shell porosity (which was not tested in this survey) (16) or to different eggs' age, as demonstrated either by the presence of an embryonated egg and by the wide range in the air cell thickness.

Yolk position, as a further freshness parameter, regular in 33 samples out of 35, is a further proof, although less clear, of the decreasing quality detected in the tested eggs close to their best-before deadline.

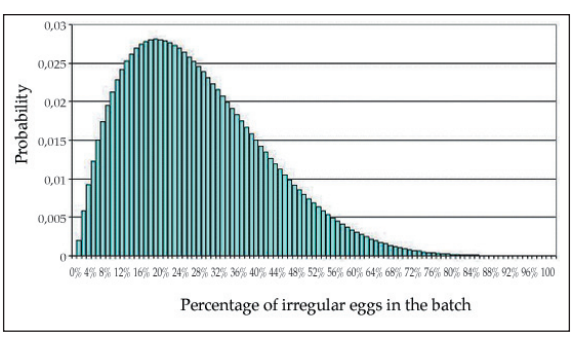

Figure 3
Probability of frequencies of non compliance with regulation in the package original batch, when 1 egg out of 6 is found irregular

Our findings do not support the use of albumen thickness measured in HU as a freshness parameter, due to a lack of correlation between air cell depth (Europe) and HU number (USA) (Figure 2).

In the USA, eggs must be stored at 45° F (7.2°C) (18), from the packing stage to the retail level. On the contrary, European legislation establishes that eggs must be stored at temperatures higher than 5°C (7) (with a few exceptions), and therefore, eggs are generally stored at room temperature until purchase. HU values are particularly affected by temperature (12). This parameter, therefore, cannot be used as a freshness indicator for shell eggs stored at not-controlled temperature.

As for freshness, results regarding shell cleanliness detected non-compliance with legal requirements, since 31.4% of packages contained between 1 and 3 dirty eggs. The presence of foreign material on the shell (traces of faecal matter, etc.), as it has already been reported in literature, increases both the probability that eggs could be directly contaminated by pathogens (*Salmonella* spp., etc.) (10, 17), and that they could cross-contaminate other food prepared in the household; especially cross-contamination is a relevant risk for ready-to-eat foods.

Shell inspection detecting fractures and cracks in about 43% of samples reveals a high level of non-compliance with legal requirements, as well.

It must be remembered, that, from the food safety point of view, USA legislation considers shell integrity more important than shell contamination (19). A cracked shell causes indeed exposure of the egg's content to pathogens, in particular *Salmonella* spp., increasing as well the risk of food-borne diseases occurrence, especially in the case

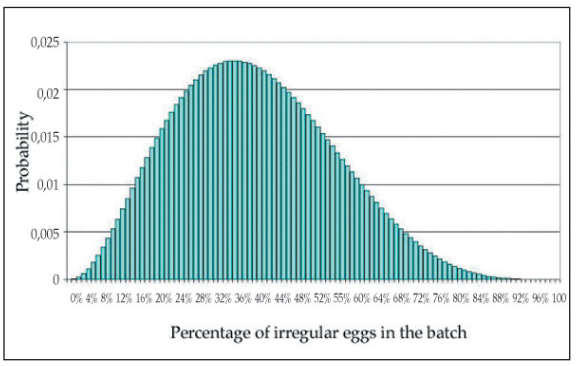

Figure 4
Probability of frequencies of non compliance with regulation in the package original batch, when 2 eggs out of 6 are found irregular

of consumption of food prepared with cracked eggs, as reported in a survey carried out in Canada in 1996 (17).

Official data available about residues of veterinary drugs in shell eggs are scarce, sparse and derive mainly from surveillance performed within National Residues Plans (NRPs).

Four hundred and one egg samples and poultry egg products, whose collection and analysis was prescribed by the NRP, were compliant with legal requirements (13). Similar results in the same year were reported by the Canadian Food Inspection Agency on a number of samples ranging from 60 to 103 according to the active substance being investigated (1). On the contrary the present survey, carried out in the same period, detected residues of veterinary drugs, exceeding the maximum

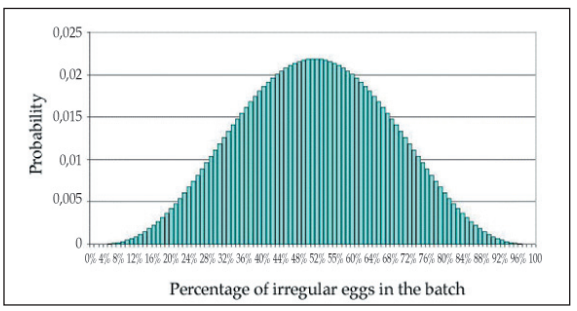

Figure 5
Probability of frequencies of non compliance with regulation in the package original batch, when 3 eggs out of 6 are found irregular

residue limits set in the EU (6, 15) in 9 samples out of 35 (25.7%). This is a very alarming finding from the food safety point of view, due to the fact that some detected substances were, and still, are prohibited.

Such lack of compliance, even if it has been detected on a limited number of samples, indicates that control systems implemented by the producers and packaging centers are probably inadequate; control systems, adopted by the competent Authorities, are probably inadequate as well, due to their low sensitivity.

Our findings, although not representative of the entire Italian territory, provide useful indications regarding the hygienic quality of shell eggs sold in Italy and define the necessity to carry out further surveys on statistically significant samples in order to clearly assess the quantitative levels of risk for consumers.

References

1. CFIA Canadian Food Inspection Agency 2004. Report on pesticides, agricultural chemicals, veterinary drugs, environmental pollutants and other impurities in agri-food commodities of animal origin. http://www.inspection.gc.ca/english/fssa/microchem/resid/2002-2003/anima_eoe.shtml
2. European Community 1999a. Commission Regulation (EC) No 508/1999 of 4 March 1999 amending Annexes I to IV to Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off J*, No *L 060*.
3. European Community 1999b. Commission Regulation (EC) No 804/1999 of 16 April 1999 amending Annexes I, II and III to Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in

foodstuffs of animal origin. *Off J*, No *L 102*, 17/04/1999, 58–63.

4. European Community 2001. Commission Regulation (EC) No 1651/2001 of 14 August 2001 amending Regulation (EEC) No 1274/91 introducing detailed rules for implementing Council Regulation (EEC) No 1907/90 on certain marketing standards for eggs. *Off J*, No *L 220*, 15/08/2001, 5 – 11.
5. European Economic Community 1990a. Council Regulation (EEC) No 1907/90 of 26 June 1990 on certain marketing standards for eggs. *Off J*, No *L 173*.
6. European Economic Community 1990b. Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off J*, No *L 224*.
7. European Economic Community 1991. Commission Regulation (EEC) No 1274/91 of 15 May 1991 introducing detailed rules for implementing Regulation (EEC) No 1907/90 on certain marketing standards for eggs. *Off J*, No *L 121*.
8. European Economic Community 1993. 93/257/EEC: Commission Decision of 15 April 1993 laying down the reference methods and the list of national reference laboratories for de-tecting residues. *Off J* No *L 118*.
9. Fagotti U. 1994. Norme sulla commercializzazione delle uova e degli ovoprodotti. Roma: Di Renzo Editore.
10. Hara-Kudo Y., Sakakibara Y., Konuma H., Sawada T. & Kumagai S. 2001. Laying season and egg shell cracks on the growth of Salmonella enteritidis in the egg albumen during storage. *J Food Protec,* **64** (8), 1134-1137.
11. Mantovani G. 1961. Ispezione degli alimenti di origine animale – Volume 2. Torino, UTET.
12. Mc Avoy K.C., Tharrington G.B., Keener K.M., Curtis P.A. & Anderson K.E. 2001. Comparison of methods and testing temperature for internal egg quality measurement. *In* Proceedings of Poultry Science Annual meeting, International Animal Agriculture and Food Science Conference. Indianapolis, Indiana, USA.
13. Ministero della Salute 2004. Vigilanza e controllo degli alimenti e delle bevande in Italia: anno 2000. www.ministerosalute.it/alimenti/resources/documenti/sicurezza/rapporto_integrale_2000.pdf
14. Pasquarelli R. 2004. Un'annata equilibrata. *Unavicolturam* **4** (1), 60-64.
15. Repubblica Italiana, Legge n. 283, del 30 aprile 1962, Disciplina igienica della produzione e della vendita delle sostanze alimentari e delle bevande. Gazzetta Ufficiale n. 139 del 04.06.1962.
16. Thapon J.L. & Bourgeois C. M. 1994. L'oeuf et les ovoproducts. Technique et Documentation. Paris: Lavoisier.
17. Todd E.C.D. 1996. Risk Assessment of use of cracked eggs in Canada. *Int J Food Microbiol,* **30,** 125-143.
18. USDA United States Department of Agriculture. Agriculture Marketing Service 1999a. Voluntary grading of shell eggs. *Fed Reg*, 7CFR56, 49-67.
19. USDA United States Department of Agriculture. Agriculture Marketing Service, Poultry Division. 1995. U.S. Standards, grades and weight classes for shell eggs. AMS 56 http://www.ams.usda.gov/poultry/pdfs/AMS-EggSt-1995.pdf.
20. USDA United States Department of Agriculture. Agriculture Marketing Service, Poultry Division 1999b. Food Labelling: Safe Handling Statements: Labelling Shell Eggs; Shell Eggs: Refrigeration of Shell Eggs Held for Retail Distribution. *Fed Reg*, **64** (128), 36491-36516.