Shelf life of fresh air packaged and precooked vacuum packaged quails

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Abstract

The shelf-life of 3 batches (Q1, Q2, Q3) of quail meat, were examined. Q1 were cut and seasoned with commercial olive oil, stoned green olive and sliced bacon. Q2 were divided into two subgroups: Q2.1 produced in the previously described conditions; Q2.2 seasoned also with rosemary. Quails were placed in low-density polystirene barrier trays and aerobically packaged. Q3 quails were boiled in salted hot water for 40 min, seasoned with myrtle leafs, placed in low density polyethylene bags and vacuum packaged. All samples were stored at +2 and +7°C. Analysis were conducted at 0, 3, 7, 9 and 14 days (T0, T3, T7, T9, and T14, respectively). For all the samples, pH measurement and microbial analysis [total viable count (TVC), *Enterobacteriaceae*, *E. coli*, *Lactobacillus* spp. (LAB), *Pseudomonas* spp., *Brochothrix thermosphacta*, coagulase-negative Staphylococci (CNS), *Enterococcus* spp., yeasts and moulds, *Salmonella* spp., *Listeria monocytogenes*] were performed. Initial TVC levels of fresh quails (*ca.* 4 log CFU/g) were rather high and this may be due to the microbial population of the raw material. In Q1 and Q2.1 samples, TVC reached the value of 7 log, which is considered as the upper acceptability limit for fresh poultry meat (after T9 under storage at +2°C and after T7 at +7°C). In Q2.2 samples such limit was reached earlier, after T3. In Q3 samples, lower TVC levels were recorded and did not reach the above mentioned limit, not even at the end of storage. However, mean counts >5 log were reached, maybe because of a post-cooking cross-contamination. *Salmonella* spp. prevalence was 33% in Q1, Q2.1 and Q2.2 samples.

Introduction

In the last years poultry meat has become increasingly popular worldwide due to its low cost of production, high nutritional quality, low fat content and low cholesterol level (Mexis *et al.*, 2012). For the same reasons, the consumption of game bird meat has gained increasing favour among consumers and typical game bird species such as quail (*Coturnix coturnix*), pheasant (*Phasianus colchicus*), and partridge (*Alectoris* spp.), are being produced in alternative poultry farms (Rojas *et al.,* 2009).

However, there is still a lack of data about microbial profile and shelf-life of meat products and meat preparations obtained from farm animals of these species, while some informations are available on hunted animals and a comparison can be done with analogue poultry products.

Poultry products is highly perishable food providing an almost perfect medium for microbial growth, including both spoilage and pathogenic microorganisms which represent a potential health hazard (Khanjari *et al*., 2013). Microbial conditions of poultry meat will depend mainly on the initial bacterial load and the microbial species carried on the skin, in the gastro-intestinal tract or in the muscle, which are influenced by farm practices and, most of all, by slaughtering procedures (Mexis *et al*., 2012). During slaughtering, carcass microflora increases progressively and there is a change in the microbial population which, in the living animals, is mainly represented by Gram positive bacteria. Subsequently, in the carcasses, these microrganisms are replaced by Gram negative species, as *Pseudomonas* spp., *Enterobacteriaceae* and *Acinetobacter* spp., wich are psychrotrophic and able to grow at refrigeration temperatures and to cause spoilage (Scarano *et al*., 2004). Poultry-based meat products and meat preparations are sold as either fresh or precooked, and after packaging they are usually stored under refrigeration (Patsias *et al*., 2008). Moreover, nowadays the distance between processing plants and retail distribution has become increasingly longer than in the past, because of the greater demands by retailers and consumers for retail products with longer shelf-life. For this reason, shelf-life of chicken and chicken products must be optimised to meet all marketing requirements.

The aim of the study was to determine the shelf-life of fresh air-packaged quails and precooked vacuum-packaged quails.

Materials and Methods

Samples obtained from 3 batches (Q1, Q2 and Q3) of quail meat were examined. All the quails were slaughtered in an avian slaughterhouse placed in Sardinia. The slaughtered quails were immediately chilled to a T<4°C and then transported, under controlled conditions, to the meat processing plant.

Shelf-life studies

Quails from Q1 were cut and seasoned with commercial olive oil, stoned green olive and sliced bacon. Quails from Q2 were divided into two subgroups: one (Q2.1) was produced in the previously described conditions; the other (Q2.2) was seasoned also with rosemary (*Rosmarinus officinalis*). The rosemary was previously treated with a 1% solution of sodium hypochlorite. Quails were placed in low-density polystirene barrier trays (two quails/tray), 75 µm in thickness having an oxygen permeability of 3800 cm^3/m^2/24 h/1 bar and a water vapour permeability of 150 g/m^2 day at 90% relative humidity (RH)/38°C, chilled with an air-chiller to a T<4°C and then aerobically packaged.

Quails from Q3 were prepared as a traditional Sardinian recipe, known as *grive*. Quails were boiled in salted hot water for 40 min and, afterwards, seasoned with myrtle (*Myrtus communis*) leafs, previously treated with a mixture of water and sodium bicarbonate. Afterwards, quails were placed in low density polyethylene bags (2 quails/bag) with an oxygen permeability of 52.2 mL/m^2 day atm at 60% RH/25°C and water vapour permeability of 2.4 g/m^2 day at 100% RH/25°C.

Samples coming from the three batches were divided into two groups and stored in the dark at two temperatures: +2±2°C (ideal retail condition storage) and +7±2°C (simulating a temperature abuse condition). During the storage period, the temperature was monitored by

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datalogger (Tynitag Plus; Tynitag, Chichester, UK).

Sampling and analysis were conducted at the following intervals: 0 (T0), 3 (T3), 7 (T7), 9 (T9) and 14 (T14) days. Overall, 72 samples were analysed: 36 fresh quail preparations air-packaged without rosemary (Q1, Q2.1); 18 fresh quail preparations air-packaged with rosemary (Q2.2); 18 precooked quail vacuum-packaged (Q3). Prior to lab analysis, the following organoleptic characteristics of the packaged samples were evaluated: i) package collapse; ii) drip loss; iii) meat colour.

Chemical analysis

Samples (10 g) were homogenised with distilled water (1:1) and the homogenate was used for pH determination with a pH-meter (Orion 420; Columbia Weather Systems Inc., Hillsboro, OR, USA).

Microbial analysis

All the samples were analysed for total viable count (TVC) (ISO 4833; ISO, 2013); *Enterobacteriaceae* (ISO 21528-2; ISO, 2004) and *E. coli* (ISO 16649; ISO, 2001); *Lactobacillus* spp. (LAB) count on Man Rogosa Sharpe medium Agar (Oxoid, Basingstoke, UK); *Pseudomonas* spp. count on Pseudomonas Agar Base (Oxoid); *Brochothrix thermosphacta* count on streptomycin Sulphate-Thallous Acetate-cycloheximide (actidione) Agar (Oxoid); coagulase negative Staphylococci (CNS) count on Mannitol Salt Agar (Oxoid); *Enterococcus* spp. count on Kanamycin Aesculin Azide Agar (Oxoid) base with; yeasts and moulds count on Oxytetracycline Agar Base (Oxoid); *Salmonella* spp. (ISO 6579/2002; ISO, 2002); *Listeria monocytogenes* (ISO 11290-1:1996 and 11290-2:1998; ISO, 1996, 1998).

For the isolation of *Salmonella*, the ISO method 6579/2002 (ISO, 2002) was used. Presumptive colonies were submitted to phenotypic identification with the API ID 32E system (bioMérieux, Marcy l'Etoile, France). Fifteen isolates were sent to the laboratories of the *Centro Nazionale di Referenza per le salmonellosi* in Legnaro (Padua, Italy), serotyped by agglutination tests with specific O and H antisera (Staten Serum Institute, Copenhagen, Denmark) and classified according to the Kauffmann-White scheme. Strains of serotypes Typhimurium and Enteritidis were phage typed according to the recommendations of the Health Protection Agency, London, UK. Moreover, precooked vacuum packaged quails were analysed for sulfite-reducing Clostridia on Perfringens agar base (Biolife, Bothell, WA, USA); *Clostridium perfringens* on Perfringens Agar Base (Biolife), and mesophilic aerobic sporulating bacteria on Tryptone Glucose Extract Agar (Oxoid). An analysis of variance (ANOVA) using the General Linear Model (GLM) procedure was performed for all considered variables, and when F-values were significant at the $P<0.05$ level, mean differences were separated by the least significant differences (LSD).

Results

Organoleptic characteristics of the samples

Fresh air-packaged quails (Q1, Q2.1, Q2.2): no collapse or drip loss were recorded during storage. The main odour throughout the entire storage time was ascribed to vegetal seasonings, particularly green olive in Q1 and Q2.1, samples and rosemary in Q2.2.

In samples stored at +2°C meat colour was dark brilliant red until T9 and, only at the end of storage, darkening of the muscle surface was recorded. Moreover, at the end of storage slime was detected both on skin and muscle surface. In samples stored at +7°C darkening of the muscle surface was recorded starting from T9 and become more evident at the end of storage irrespective of the temperatures.

Precooked quails under vacuum (Q3): meat colour remained unchanged and no slime was detected throughout the entire storage period. The main odour was that of myrtle until T9, whereas at the end of storage a moderate off-odour was registered.

pH analysis

Table 1 shows the pH evolution during storage of fresh quails air-packaged with rosemary, fresh quail air-packaged without rosemary and precooked quails [mean±standard deviation (SD)].

Changes in pH during storage of Q1, Q2.1, Q2.2 samples were not statistically significant ($P>0.05$). Initial pH values were similar in Q1, Q1.2 (6.23±0.10) and Q2.2 (6.15±0.08). A similar trend was also noticed during storage with an increase of the mean values. Final pH levels were 6.16±0.23 and 6.18±0.16, respectively in samples stored at +2 and at +7°C.

As expected, higher mean pH levels were recorded in Q3 samples. T0 mean levels were 6.31±0.03. A rise of the mean values were recorded during the storage, irrespective of the temperatures. T14 levels were 6.73±0.03 and 6.67±0.14, respectively in samples stored at +2 and at +7°C.

Microbial analysis

Tables 2, 3 and 4 show the results of the counts of the targeted microbial groups in fresh quails air-packaged with rosemary, fresh quail air-packaged without rosemary and precooked quails (log_{10} CFU/g; mean±SD).

Fresh quails air-packaged without and with rosemary (Q1, Q2.1, Q2.2)

In the samples without rosemary (Q1, Q2.1) stored at +2°C, initial mean values (T0) of TVC were *ca.* 3 log and increased progressively ($P<0.01$) during storage, attaining a final level >8 log. A similar trend was detected in samples stored at +7°C, for which higher counts were recorded throughout the entire storage period. In the samples seasoned with rosemary (Q2.2), initial levels were slightly higher (>4 log), compared to Q1 and Q2.1, with a progressive increase irrespective to temperatures. Final counts were >8 and >9 log, respectively in samples stored at +2 and at +7°C.

Mean levels of *Enterobacteriaceae* in Q1 and Q2.1 samples stored at +2°C showed a moderate increase and remained low, attaining a final level of *ca.* 3 log. On the contrary, in samples

Table 1. pH evolution of fresh quails air-packaged with and without rosemary and precooked vacuum packaged quails.

	Temperature (°C)	Storage time (days)*				
		0	3	7	9	14
Fresh quails air-packaged without rosemary	+2	6.23±0.10	6.23±0.24 x	6.25±0.07 x	6.29±0.58 x	6.16±0.23 x
	+7		6.25±0.11 x	6.31±0.09 x	6.20±0.39 x	6.18±0.16 x
Fresh quails air-packaged with rosemary	+2	6.15±0.08	6.18±0.30 x	6.23±0.06 x	6.29±0.10 x	6.15±0.23 x
	+7		6.21±0.18 x	6.24±0.01 x	6.25±0.40 x	6.27±0.21 x
Precooked vacuum packaged quails	+2	6.31±0.03	6.57±0.05 x	6.58±0.03 x	6.56±0.14 x	6.73±0.03 x
	+7		6.42±0.06 x	6.53±0.12 x	6.61±0.11 x	6.67±0.14 x

*Values are expressed as means±standard deviation. Means within columns with different capital letters (X and Y) are significantly different ($P<0.01$; $P<0.5$).

Table 2. Changes in microbial profile (mean±SD; log CFU/g) of fresh quails air-packaged without rosemary.

Microbial parameters	Temperature (°C)	Storage time (days)*,°				
		0	3	7	9	14
TVC	+2	3.86±0.16	4.90±0.94 D, x	7.50±0.56 C, x	6.17±0.86 B, x	8.76±0.27 A, x
	+7		5.52±0.75 C, x	6.66±0.75 B, x	7.38±0.86 B, x	8.94±0.18 A, x
Enterobacteriaceae	+2	2.02±0.65	0.89±1.03 C, x	2.51±0.95 BA, x	2.68±0.43 BA, y	3.70±1.54 A, x
	+7		2.21±1.45 B, x	3.68±0.29 B, x	5.34±0.90 A, x	5.65±1.04 A, x
LAB	+2	2.20±0.12	2.90±0.51 A, x	3.07±0.23 A, x	3.06±0.65 A, x	4.14±0.01 A, x
	+7		3.20±0.03 B, x	3.37±0.08 B, x	3.24±0.77 B, x	4.21±0.02 A, x
Pseudomonas spp.	+2	3.56±0.40	3.41±0.17 C, x	5.22±1.32 B, x	7.23±0.82 A, x	7.95±0.43 A, x
	+7		4.19±0.64 C, x	6.21±0.85 B, x	7.90±1.06 A, x	8.42±0.90 A, x
CNS	+2	3.70±0.47	3.33±1.75 C, x	4.33±0.71 BA, y	4.89±0.95 B, x	6.21±1.26 A, x
	+7		2.91±0.51 C, x	5.52±0.40 B, x	5.58±0.23 B, x	6.81±0.86 A, x
Yeasts	+2	3.08±0.15	4.38±0.47 C, x	5.08±0.57 B, y	5.31±0.38 B, x	6.52±0.56 A, x
	+7		4.04±1.07 C, x	5.93±0.22 B, x	5.64 0.19 B, x	7.20±0.28 A, x
Moulds	+2	2.18±0.21	2.83±0.57 A, x	2.50±0.53 A, x	2.41±1.61 A	3.51±2.60 A
	+7		3.33±0.19 x	1.92±1.35 x	-#	-#

TVC, total viable count; LAB, *Lactobacillus* spp.; CNS, coagulase negative staphylococci. *Means in rows with different capital case letters (A-D) are significantly different (P<0.01); °means within columns with different lower case letters (x and y) are significantly different (P<0.01; P<0.5); #not detected.

Table 3. Changes in microbial profile (mean±SD; log CFU/g) of fresh quails air-packaged with rosemary.

Microbial parameters	Temperature (°C)	Storage time (days)*,°				
		0	3	7	9	14
TVC	+2	4.50±0.81	4.33±0.61 C, x	7.27±0.01 B, x	8.28±0.08 BA, x	8.93±0.20 A, x
	+7		5.75±1.53 C, x	7.48±0.01 B, y	8.49±0.13 BA, x	9.56±0.27 A, x
Enterobacteriaceae	+2	1.45±0.21	1.65±0.49 CB, x	3.12±0.93 BA, x	3.53±0.14 A, x	4.21±0.75 A, x
	+7		2.09±0.35 B, x	5.05±0.27 BA, x	5.12±1.32 BA, x	5.48±1.84 A, x
LAB	+2	2.24±0.20	2.75±0.05 A, x	3.02±0.03 A, x	3.33±0.00 A, x	3.34±0.06 A, x
	+7		2.77±0.05 D, x	2.94±0.02 C, x	3.26±0.10 B, x	3.50±0.10 A, x
Pseudomonas spp.	+2	3.74±0.06	3.72±0.24 C, x	6.87±0.40 B, x	8.16±0.12 A, x	7.87±0.17 A, x
	+7		3.94±0.34 D, x	6.48±0.01 C, x	8.35±0.23 A, x	7.48±0.01 B, x
CNS	+2	4.30±0.68	0.86±1.22 C, x	4.39±0.44 B, x	5.47±0.58 BA, x	6.85±0.64 A, x
	+7		1.76±0.04 C, x	5.22±0.98 B, x	5.93±0.64 BA, x	7.57±0.33 A, x
Yeasts	+2	3.60±0.02	3.22±0.88 D, x	4.96±0.73 CB, x	5.30±0.19 BA, x	6.46±0.29 A, x
	+7		3.78±0.43 C, x	5.21±0.07 B, x	5.87±0.27 B, x	7.16±0.01 A, x
Moulds	+2	2.65±0.07	2.69±0.44 BA, x	2.89±0.58 A, x	-#	-#
	+7		3.00±0.01 A, x	3.55±0.07 A, x	-#	4.98±0.03 A

TVC, total viable count; LAB, *Lactobacillus* spp.; CNS, coagulase negative staphylococci. *Means in rows with different capital case letters (A-D) are significantly different (P<0.01); °means within columns with different lower case letters (x and y) are significantly different (P<0.01; P<0.5); #not detected.

Table 4. Changes in microbial profile (mean±SD; log CFU/g) of precooked vacuum packaged quails.

Microbial parameters	Temperature (°C)	Storage time (days)*,°				
		0	3	7	9	14
TVC	+2	2.28±0.14 C	3.07±0.11 B, x	5.0±0.05 A, x	3.48±0.01 B, x	5.39±0.46 A, x
	+7		1.65±0.07 D, y	4.42±0.11 C, y	5.71±0.20 B, x	6.43±0.16 A, x
Enterobacteriaceae	+2	-#	-	-	-	-
	+7		-	-	-	2.13±0.49
Pseudomonas spp.	+2	-	-	4.09±0.19	3.07±0.41	5.66±0.00
	+7		-	-	4.55±0.49	4.39±0.55
Coagulase negative staphylococci	+2	-	-	-	2.69±0.30	2.39±0.12
	+7		2.00±0.00	-	-	3.19±0.27

TVC, total viable count; LAB, Lactobacillus spp.; CNS, coagulase negative staphylococci. *Means in rows with different capital case letters (A-D) are significantly different (P<0.01); °means within columns with different lower case letters (x and y) are significantly different (P<0.01; P<0.5); #not detected.

stored at +7°C, higher levels were recorded during all the storage time and a progressive increase and final counts >5 log were recorded. Q2.2 samples showed higher levels with storage, particularly at +2°C, and an increase at both temperature was detected, with final levels of *ca.* 4 and 5 log, respectively in samples stored at +2 and +7°C.

In Q1 and Q2.1 samples T0 mean levels of LAB were *ca.* 2 log. A rise was detected during storage at both temperatures and final counts of 4 log were attained. A similar trend was noticed in Q2.2 samples, but with slightly lower final levels (*ca.* 3 log).

Pseudomonas spp. in Q1 and Q2.1 showed a significant (P<0.01) rise during storage irrespective of the temperature and represented the dominant bacterial species at the end of storage with mean levels >7 log in samples stored at +2°C and >8 log in those at +7°C. Q2.2 samples showed a similar trend with a progressive increase throughout the entire storage period, irrespective of temperatures with final levels >7 log.

CNS constituted part of the microflora of Q1 and Q2.1 samples and showed a progressive increase during storage at both temperatures, attaining final levels of *ca.* 6 log. In Q2.2 samples, mean counts were also high with a progressive increase at both temperatures and final levels of 6 and 7 log in samples stored at +2 and at +7°C, respectively.

Yeasts and moulds: mean counts were also high with a progressive increase irrespective of temperature. Moulds mean levels were higher in samples stored at +2°C than in samples stored at +7°C in which were isolated only until T7. Mean counts and trends were similar to those detected in analogue products air-packaged without rosemary.

B. thermosphacta and *L. monocytogenes* were not detected in any of the samples. *Salmonella* spp. was isolated in 12 out of 36 samples of Q1 and Q2.1 samples (33%) and in 6 out of 18 Q2.2 samples (33%). *Salmonella* was isolated throughout the storage time, and at both temperatures. Two *Salmonella* serotypes were detected: *S. Typhimurium* monophasic variant 1,4,[5],12:i:- and *S.* Kentucky. The prevalence of *S.*Typhimurium monophasic variant 1,4,[5],12:i:- was 60% (6/10) in Q1 and Q2.1 samples, and 80% (4/5) in Q2.2 samples. The prevalence of *S.* Kentucky was 40% (4/10) in Q1 samples and 20% (1/5) in Q2.2 samples. Phage typing of *S.* Typhimurium isolates resulted into 3 different phage types: in Q1 and Q2.1 samples DT7a (83%, 5/6) and DT20a (17%, 1/6), in Q2.2 samples U311 (75%, 3/4) and DT7a (25%, 1/4) were detected.

Precooked quails (Q3)

TVC mean values were low at the beginning of storage (*ca.* 2 log). Mean counts of samples stored at +2°C showed an irregular trend with significant changes (P<0.01) during storage and attained final levels >5 log, while those of samples stored at +7°C increased gradually (P<0.01) with higher final levels (>6 log).

Enterobacteriaceae were only detected in samples stored at +7°C at the end of the experiment (*ca.* 2 log).

Pseudomonas spp. in samples stored at +2°C was isolated after 7 days of storage with mean levels >4 log that increased to 5 at the end of the storage. A similar trend was noticed in samples stored at +7°C.

CNS mean levels remained low during storage at both temperatures (*ca.* 2 log). LAB, *Brochotrix thermosphacta*, *Enterococcus* spp., yeasts, moulds, solphite-reducing anaerobes, *Clostridium perfringens*, mesophilic aerobic sporulating bacteria, *Listeria monocytogenes* and *Salmonella* spp. were not detected in any of the samples.

Discussion

For TVC, the value of 7 log CFU/g is considered the upper acceptability limit for fresh poultry meat as defined the by International Commission on Microbiological Specifications for Foods (ICMSF, 1998). In samples Q1 and Q2.1 this limit was reached between T9 and T14 under storage at +2°C, and between T7 and T9 under storage at +7°. In Q2.2 samples such limit was reached earlier, between T3 and T7, irrespective of the temperature. Therefore, rosemary seems to influence the shelf-life of fresh quails packaged in air, even if no statistical difference could be attributed to its presence. TVC levels could be due to the initial microbial load and also to the contamination during preparation. Pseudomonas, CNS and, to a lesser degree, LAB constituted part of the microflora of fresh quails. The growth of yeasts and moulds was observed in fresh quails at T0 and, particularly yeasts attained high levels (>4 log at both temperatures of storage) starting from T7. These results are in agreement with those obtained in similar studies for various chicken products (Ismail *et al.*, 2000, Patsias *et al.*, 2006). Considering the organoleptic characteristics and the microbiological results, a shelf-life of 7 days could be appropriate of fresh quails packaged in air and could be improved by reducing the initial microbial load.

Salmonella prevalence in fresh quails air-packaged with and without rosemary was higher (33%) than in other studies and can represent a risk for public health. This can be caused by the presence of *Salmonella* at farm level, confirmed by the results of official (on faecal and dust samples) and on-check food business operator (FBO) controls (data not showed). As prevention measures, the FBO will implement vaccination and improve cleaning and disinfection procedures. It is important to highlight that the identified serotypes, *S. Typhimurium* monophasic variant and *S.* Kentucky, are among the 10 most frequent serovars isolated from confirmed cases of human salmonellosis in 2010-2011 (EFSA, 2013).

Conclusions

As expected, in precooked quails packaged under vacuum, TVC levels were lower and did not reach the above mentioned limit, not even at the end of storage. However, mean counts >5 log were reached in samples stored at +2°C at T14 and at +7°C at T9. This could be attributed to a post-cooking cross-contamination. In a related study, Patsias *et al.* (2006) reported similarly high initial TVC microbial load (*ca.* 4 log) in a chilled pre-cooked chicken product.

Vacuum packaging changes the microflora but does not inhibit bacterial growth. During spoilage, the species that predominate are those with the shortest generation times under the storage conditions. For example, LAB do not normally compete well, because of their longer generation times (Linton *et al.*, 2004). In Q3 samples, Pseudomonas has been isolated starting from T7 and reached quite high levels (>4 log), irrespective of the temperature of storage. Sawaya *et al.* (1993) reported that *Pseudomonas* grew slowly in vacuum packs but did not reach the high numbers achieved under aerobic conditions. For precooked vacuum packaged quails a shelf life of 9 days could be considered acceptable.

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