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Short Communication

Microbiological quality of vacuum-packed retail ostrich meat in Spain

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Abstract

The microbial levels (cfu/g and cfu/cm^2) of retail refrigerated vacuum-packed ostrich steaks was assessed in Spain. Samples were purchased within 3 to 7 days after packaging. Physicochemical parameters (pH, A_w and E_h) were also determined. Average counts ($\log_{10}$ cfu/g) were 7.32 (total aerobic counts–TAC, determined at 30°C), 7.09 (mesophilic), 6.62 (psychrotrophs), 6.05 (pseudomonads), 3.29 (fluorescent pseudomonads), 5.29 (*Enterobacteriaceae*), 0.86 (enterococci), 6.86 (lactic acid bacteria—LAB) and 4.90 (yeasts and moulds). *Staphylococcus aureus* strains were not detected. Average values of 6.00, 0.995 and 61.75 were found for pH, A_w and E_h, respectively. The high percentage of mesophilic populations in the TAC ($71.39 \pm 51.63\%$) and the low percentage of psychrotrophs ($30.47 \pm 19.70\%$) suggest high pre- or post-packaging storage temperatures. A significant influence of ostrich pH on microflora development is suggested from our results, because the lowest ($P < 0.05$) microbial loads were found at pH $\leqslant$ 5.8. The high microbial loads found in this study suggest an improvement of the microbiological quality of retail ostrich meat is convenient.

Keywords: Ostrich meat; Microbiological quality; Vacuum-packed meat; Spain

1. Introduction

Ostrich (*Struthio camelus*) breeding was started in Spain in 1990s (Carbajo, 2000), with interest focussing on using the ostrich as a meat producer. Spanish regulations have placed the ostrich among the animal species to be slaughtered as raised game birds (Anonymous, 1994).

Ostrich meat is now becoming increasingly popular in western society because of its nutritional value (Alonso-Calleja et al., 2002). The consumption of this type of meat in each European country is estimated to reach 750 t/year (FAO, 1999). In Spain the market for ostrich meat is growing and its production and consumption have increased 20-fold since 1996 (Carbajo, 2000). Clearly, the continued growth and prosperity of the ostrich meat industry will depend, to a large degree, on its ability to supply the consumer with wholesome and safe products.

Even though the nutritional value of ostrich meat is well documented, very little information is available worldwide on the microbiological aspects and keeping quality of this foodstuff, especially of the refrigerated vacuum-packed steak form, which is the most frequently purchased in developed countries.

Traditionally, pH has been considered as a fundamental parameter in the microbiological quality of the meat (Blixt and Borch, 2002). However, there is a lack of published research characterizing the influence of final pH on microbial loads of ostrich meat.

Taking into account the progressive increase of the production and consumption of this meat type and the lack of information in the scientific literature, the present study was designed to determine the microbiological quality of refrigerated vacuum-packed retail ostrich meat in Spain and to investigate whether the pH has a significant influence on bacterial levels of this foodstuff or not.

2. Materials and methods

2.1. Samples

Twenty samples of refrigerated vacuum-packed ostrich meat fillet steaks of approximately 0.5 cm thick

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were purchased from different retail outlets in León (Spain). Steaks were packed in plastic film with low oxygen permeability by the processor. All samples were purchased before the "sell-by" date, between 3 and 7 days after packaging.

2.2. Microbiological analysis

Meat samples (10 g) were blended (Stomacher 400, A.J. Seward, London) for 90 s in 90 ml of 0.1% (w/v) peptone water. Decimal dilutions were carried out using the same diluent. Culture media (all from Oxoid Ltd., Hampshire, UK) and incubation parameters used are shown in Table 1. Duplicate plates were incubated under aerobic conditions. Fluorescent pseudomonads on *Pseudomonas* agar containing CFC (cephaloridine, fucidin, cetrimide) supplement were enumerated under a 254 nm ultraviolet light (Palleroni, 1984).

One hundred colonies (five isolates for each ostrich sample) were randomly selected from VRBGA and from OGYEA plates. Characterization of presumptive *Enterobacteriaceae* was carried out on the basis of Gram stain and catalase and oxidase reactions. The microscopic examination was performed in order to visually confirm the presumptive yeast and mould isolates.

Colonies with a typical morphology of *Staphylococcus aureus* (presumptive strains) were not detected on Baird-Parker agar. A hundred atypical colonies were randomly selected, purified and tested for Gram stain, catalase activity, modified oxidase test, coagulase activity and thermo-stable nuclease activity, as previously described (Capita et al., 2001a; Álvarez-Astorga et al., 2002). Percentages of confirmed colonies were used to correct the results of the counts obtained in each agar plate.

The results were initially expressed as $\log_{10}$ cfu/g meat. The results were transformed into cfu/cm^2 to facilitate the comparison with data found by other authors and to values from criteria established for meat and poultry. Studies were then carried out to relate the weight with the surface area of ostrich steaks analysed (all fillets were approximately 0.5 cm thick, therefore the surface to volume ratio was similar for all samples). On average, it was found that 1 g meat corresponded to 4.296 cm^2 of meat surface. The following equation (Capita et al., 2001b, 2002) was used:

$$\log_{10}\text{ cfu/cm}^2\text{ ostrich fillets} = \log_{10}\text{ cfu/g ostrich fillets} - 0.633.$$

2.3. Physicochemical determinations

For the pH and E_h determinations, 10 g of ostrich meat were placed in a stomacher bag with 10 ml of sterile distilled water (MILLI RO^{TM}) and then blended for 2 min. The pH and E_h were measured after 10 min using a pH meter Crison MicropH 2001. The A_w was determined in an Aqua-Lab model CX2 system (Decagon Devices Inc., Washington, USA) following the manufacturer's instructions. All physicochemical data were obtained in duplicate.

2.4. Statistical analysis

In order to compare the $\log_{10}$ values of different microbial groups, the data were analysed using a one-way analysis of variance (ANOVA). Differences in microbial counts were determined using the Duncan's multiple range test. Samples were classified according to pH and divided into tertiles, with the lowest tertile (grouping samples with pH $\leqslant$5.8) categorized as "1", the middle (5.8 < pH $\leqslant$ 6.2) as "2" and the highest (pH > 6.2) as "3". Analysis of variance (ANOVA) was carried out first to detect for an overall difference of microbial counts ($\log_{10}$ cfu) and physicochemical determinations across the tertiles. If found significant, Newman–Keuls range test was performed to identify where differences occurred.

Table 1
Culture media, incubation times, temperatures and references for microbiological analysis

Microbial group	Culture medium	Incubation		Reference
		Temp. (°C)	Time	
Total aerobic counts[a]	Plate count agar (PCA)	30	72 h	Jay (2002)
Mesophilic[b]	Plate count agar (PCA)	37	72 h	ICMSF (1986)
Psychrotrophic[b]	Plate count agar (PCA)	7	10 d	Cousin et al. (2001)
Pseudomonads[b]	*Pseudomonas* agar with CFC supplement	25	24 h	Baird et al. (1987)
Enterobacteriaceae[b]	Violet red bile glucose agar (VRBGA)	30	24 h	Baird et al. (1987)
Enterococci[a]	Kanamycin aesculinazide (KAA) agar	42	24 h	Baird et al. (1987)
Staphylococcus aureus[b]	Baird-Parker agar with egg yolk emulsion supplement	35	24 h	Lancette and Tatini (1992)
Lactic acid bacteria[a]	Man, Rogosa, Sharpe (MRS) agar	30	3 d	Baird et al. (1987)
Yeasts and moulds[b]	Oxytetracycline glucose yeast extract agar (OGYEA)	25	4 d	Baird et al. (1987)

[a] Pour-plate technique.
[b] Spread-plate technique.

The degree of correlation between all microbial counts, pH, A_w and E_h was investigated using the linear regression continuous data. The "Statistica® 6.0" software package (Statsoft Ltd., Tulsa, OK, USA) was used to perform all the computations.

3. Results and discussion

Microbial loads and physicochemical data are given in Table 2. The high percentage of mesophilic ($71.39 \pm 51.63\%$) and the low percentage of psychrotrophs ($30.47 \pm 19.70\%$) over TAC populations do not agree with previous findings in refrigerated meat, where psychrotrophs are higher than mesophilic (Capita et al., 2001a, 2002; Jay, 2002) and suggest that a temperature abuse during processing, storage, transportation, distribution or retail display of product exist. The very short vacuum-packaged storage time (2–7 days) could also be partially responsible for the low relative loads of psychrotrophs observed.

In the present study, TAC were found to be higher than those by Harris et al. (1994) in Texas: 2.6–4.6 $\log_{10}$ cfu (determined at 25°C)/cm^2 of ostrich carcass. However, this low microbial load may be due to the fact that the analysis was carried out very shortly after slaughtering on non-packed meat. It must be taken into account that microbial levels present in the raw material can go up during processing, transportation and storage. Thus, as previously suggested (Álvarez-Astorga et al., 2002), most cutting installations increase bacterial loads. Moreover, cutting results in a rise of meat surfaces, which favours microbial growth. These facts could account for the higher microbial loads found in our study.

On the other hand, similar or even higher values of aerobic loads than that of the present study have been found by other authors in refrigerated vacuum-packed ostrich fillets. Thus, Angulo et al. (2001) in Spain observed mesophilic (37°C) counts as high as 8.30 $\log_{10}$ cfu/g in refrigerated retail samples. Otremba et al. (1999) obtained in the United States log counts between 2 and 7 (depending on the day of refrigerated storage) $\log_{10}$ TAC (32 ± 2°C)/cm^2 of vacuum-packed ostrich steaks. It must be pointed out that samples analysed by the previously mentioned authors were obtained from a commercial processor, and were frozen prior to refrigerated storage, which could have influenced the results obtained.

Levels of psychrotrophs fit in the wide range of values reported by Otremba et al. (1999) in vacuum-packed ostrich: from 2 to 7 $\log_{10}$ cfu/cm^2, depending on the sampling day.

At the sampling time pseudomonads accounted for $28.94 \pm 32.61\%$ of TAC and for $79.13 \pm 100.38\%$ of psychrotrophs. Data for fluorescent pseudomonads were $0.97 \pm 1.36\%$ and $2.10 \pm 3.35\%$, respectively. Fluorescent pseudomonads were only detected in 25%

Table 2
Microbial counts ($\log_{10}$) and physicochemical data in retail ostrich meat fillet steaks

Variable	Mean	SD[a]	Median	Range
Aerobic plate counts (30°C)	7.32[b]	0.63	7.53	6.13–8.12
	(6.69)[c]	(0.63)	(6.90)	(5.50–7.49)
Aerobic plate count (37°C)	7.09	0.57	6.93	6.20–7.78
	(6.45)	(0.57)	(6.30)	(5.57–7.26)
Psychrotrophs	6.62	0.82	6.59	5.48–7.78
	(5.98)	(0.82)	(5.95)	(4.84–7.15)
Pseudomonads	6.05	0.58	6.05	5.04–7.23
	(5.42)	(0.58)	(5.42)	(4.41–6.60)
Fluorescent pseudomonads	3.29	2.11	4.35	0.00–5.30
	(2.66)	(2.11)	(3.72)	(0.00–4.67)
Enterobacteriaceae	5.29	0.71	5.42	4.00–6.25
	(4.66)	(0.71)	(4.79)	(3.36–5.62)
Enterococci	0.86	0.86	0.70	0.00–2.02
	(0.22)	(0.86)	(0.37)	(0.00–1.39)
Lactic acid bacteria	6.86	0.71	7.14	5.74–7.69
	(6.23)	(0.71)	(6.51)	(5.10–7.06)
Yeasts and moulds	4.90	0.42	4.85	4.08–5.54
	(4.27)	(0.42)	(4.21)	(3.45–4.90)
pH	6.00	0.39	5.93	5.43–6.69
A_w	0.995	0.001	0.995	0.993–0.998
E_h	61.75	9.34	62.00	44.00–74.50

[a] Standard deviation.
[b] $\log_{10}$ cfu/g.
[c] $\log_{10}$ cfu/cm^2.

of tested samples, that is a value lower than that (75%) found in non-vacuum-packaged raw poultry (Capita et al., 2001a). This result is expected taking into account the decrease in percentage of pseudomonads throughout storage on vacuum-packed meat. Thus, according to Gram et al. (2002) in vacuum-packed meat the respiratory pseudomonads will be inhibited, while a shift in the microflora to LAB and *Enterobacteriaceae* (facultatively anaerobic micro-organisms) take place. The negative correlations between LAB and pseudomonads ($r = -0.510$; $P < 0.01$), and fluorescent pseudomonads ($r = -0.480$; $P < 0.01$) found in our study affirm this relationship. Significant negative correlations were also found between pseudomonads or fluorescent pseudomonads and total counts. The percentage of fluorescent pseudomonads with regard to total pseudomonads was $2.88 \pm 3.52\%$.

The *Enterobacteriaceae* were found in all examined samples and in relatively high numbers. Nevertheless, enterococci were only detected in 56.25% samples, with 1.00–2.02 $\log_{10}$ cfu/g in positive samples. *Enterobacteriaceae* counts in the present study were lower than those of Angulo et al. (2001), who found an average of 6.78 $\log_{10}$ cfu/g in refrigerated vacuum-packed ostrich steaks. It must be taken into account that some *Enterobacteriaceae* are capable of growing at refrigeration temperatures in vacuum-packed products (Silla and Simonsen, 1985; Gram et al., 2002).

No significant correlations between total aerobic counts and *Enterobacteriaceae* or enterococci were found. These results coincide with previous findings in raw poultry (Capita et al., 2001a, 2002) and could be explained considering the low percentage of these microbial groups within the TAC ($6.79 \pm 12.34\%$ and $< 0.01\%$, respectively), which implies that the changes in the number of *Enterobacteriaceae* do not lead to any parallel variations in the TAC populations.

Staphylococcus aureus strains were not detected. This finding does not agree with that of Angulo et al. (2001), who report more than 3 $\log_{10}$ cfu *Staphylococcus aureus*/g in retail refrigerated ostrich steaks in Spain. It must be taken into account, however, that the high degree of manipulation that the samples studied by Angulo et al. undergo is responsible, according to these authors, for the high bacterial levels observed for most microbial groups.

LAB (counts on MRS) levels (Table 2) are higher than those of 2–4 $\log_{10}$ cfu/cm^2 found by Otremba et al. (1999) in vacuum-packed ostrich streaks stored at refrigeration temperatures for 0–28 days.

Counts on MRS were the most abundant bacterial group in the ostrich samples analysed ($37.42 \pm 13.02\%$ of total counts). Studies on refrigerated vacuum-packed meat products carried out by other authors have demonstrated a similar dominance of this microbial group (Korkeala et al., 1987; Blixt and Borch, 2002). According to Gram et al. (2002) mainly LAB and also *Enterobacteriaceae*, *Brochothrix thermosphacta* and *Shewanella putrefaciens*, which are capable of growing on anaerobic atmospheres, are responsible for spoilage in vacuum-packed meat and meat products. Sour and acid odour observed in these products upon spoilage has been reported to be caused by lactic acid bacteria (Korkeala et al., 1987).

In our study four samples (all containing acid lactic bacteria at levels higher than 7 $\log_{10}$ cfu/g) showed a non-repulsive and slight acid odour when opening the bag. This observations coincides with findings of Korkeala et al. (1987), who indicate that 7 $\log_{10}$ lactobacilli/g is the limit for perception of off-odours in vacuum-packed meat. None of the samples with $< 7 \log_{10}$ cfu/g had any off-odours in the present study, even when higher than 8 $\log_{10}$ TAC/g were detected.

A significant positive correlation was found between pH and most microbial counts, including LAB ($r = 0.770$; $P < 0.001$). This positive correlation could indicate that high pH values favourably influences microbial growth. These results are congruent with those of Grau (1981), who found that no *Enterobacteriaceae* was able to anaerobically grow on beef muscle at lower than 5.8 pH values. Blixt and Borch (2002) observed in pork and beef vacuum-packed meat that LAB showed a growth pattern related to initial pH. Our finding does not coincide with those of Silla and Simonsen (1985), who did not observe any correlation between pH and lactobacilli levels in sliced vacuum-packed meat products. However, Otremba et al. (1999) found an increase in pH as well as in microbial loads (including lactobacilli) from day 7 of refrigerated storage in vacuum-packed ostrich meat steaks.

Yeast and mould counts in our study are highly correlated ($r = 0.547$; $P < 0.01$) to those of psychrotrophs. This fact may be explained by taking into account the relatively constant percentage of yeasts and moulds within the psychrotrophs ($1.02 \pm 1.24\%$), which implies that changes in the number of yeasts and moulds lead be to parallel variations in the total number of psychrotrophs.

The pH value of ostrich (Table 2) is similar to standard values observed in ostrich meat and other poultry muscles (Otremba et al., 1999) and higher than the final pH observed in beef, lamb or pork (Blixt and Borch, 2002). However, higher values than ours (as high as 7.5) were found by Angulo et al. (2001) in refrigerated ostrich steaks in Spain.

From Table 3, we can observe that samples with higher than 5.8 pH (tertiles 2 and 3) have the highest ($P < 0.05$) counts for most of microbial groups considered, and samples that have lower than 5.8 pH (tertile 1)

Table 3
Differences between microbial counts ($\log_{10}$ cfu/g) and physicochemical determinations for tertiles of pH

Variable	Tertiles		
	1 ($n = 6$)[a]	2 ($n = 7$)[b]	3 ($n = 7$)[c]
pH	5.52 ± 0.07a[d]	5.95 ± 0.13b	6.48 ± 0.18c
A_w	0.996 ± 0.001a	0.995 ± 0.002b	0.994 ± 0.003b
E_h	72.50 ± 2.73a	64.29 ± 4.13b	56.91 ± 12.61c
Total aerobic counts (30°C)	6.52 ± 0.24a	7.44 ± 0.51b	7.75 ± 0.34b
Mesophilic (37°C)	6.38 ± 0.45a	7.24 ± 0.49b	7.31 ± 0.59b
Psychrotrophs	6.03 ± 0.32a	6.74 ± 0.92b	6.89 ± 0.77b
Pseudomonads	6.31 ± 0.21a	5.57 ± 0.34b	6.35 ± 0.67a
Fluorescent pseudomonads	4.41 ± 1.60a	2.40 ± 1.94b	1.56 ± 2.51b
Enterobacteriaceae	5.56 ± 0.73a	5.03 ± 0.76a	5.38 ± 0.55a
Enterococci	1.00 ± 0.94a	0.83 ± 0.72a	0.74 ± 0.97a
Lactic acid bacteria	6.01 ± 0.21a	7.25 ± 0.45b	7.23 ± 0.49b
Yeasts and moulds	4.56 ± 0.56a	4.85 ± 0.38a	5.05 ± 0.41a

Data in the same row with no letters in common are significantly ($P < 0.05$) different.
[a] Samples with pH ⩽ 5.8.
[b] Samples with 5.8 < pH ⩽ 6.2.
[c] Samples with pH > 6.2.
[d] Data are mean ± standard deviation.

have the lowest. The association between pH and microbial counts are corroborated by the positive significant correlations observed. The influence of pH on microbial loads of vacuum-packed chilled ostrich meat suggest the benefit of achieving low final pHs in order to improve the microbiological quality of this foodstuffs.

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