Sensory implications of chicken meat spoilage in relation to microbial and physicochemical characteristics during refrigerated storage

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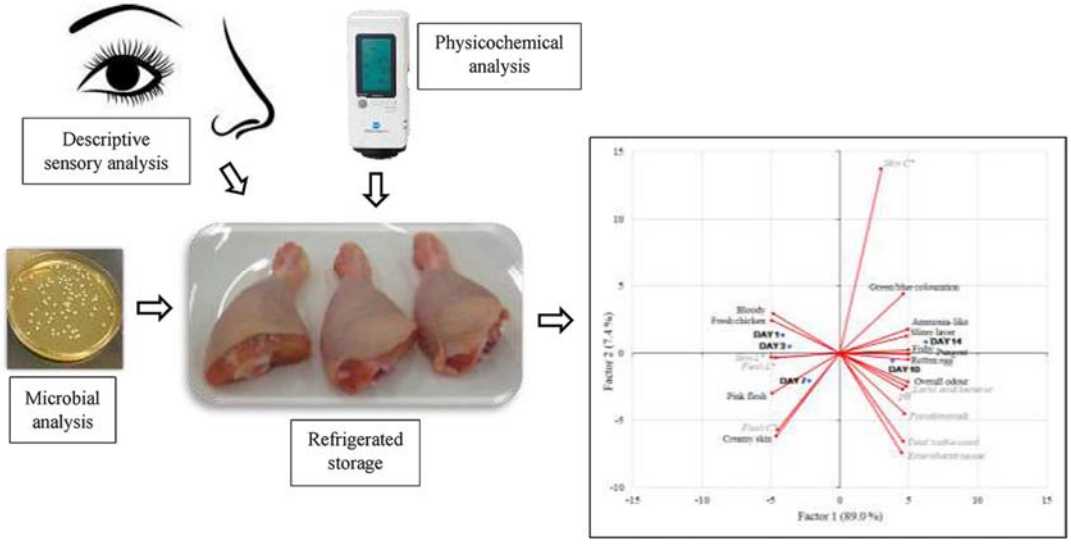
Graphical abstract



ABSTRACT

Consumer perception of chicken meat spoilage is linked to sensory, microbial and physicochemical changes of raw chicken during storage. The objective of this study was to characterise the sensory attributes of raw chicken meat and to establish the relationship with the microbial and physicochemical changes during refrigerated storage under aerobic packaging. Chicken legs obtained from a commercial poultry processing plant were stored at 4 °C and microbiological (total viable counts, *Pseudomonas* spp. Enterobacteriaceae, lactic acid bacteria), pH, colour and descriptive sensory (odour and appearance) analyses were conducted during storage for 14 days. Principal components analysis (PCA) biplot indicated that chicken meat stored for 1, 3 and 7 days was characterised by having pink flesh and creamy skin, with a bloody and fresh chicken smell, and high skin L*, flesh L* and flesh C* values. Chicken meat stored for longer than 7 days was described as having all the negative sensory attributes (green/blue colouration, slimy, pungent, fishy, rotten egg, ammonia-like and intense overall odour), total viable counts higher than 8 log CFU/g and pH beyond 7. The chroma of chicken skin did not differentiate well chicken meat samples stored for different days, hence would be a poor indicator of spoilage. Odour attributes of chicken meat deteriorated at a faster rate than instrumental colour and appearance attributes and were highly correlated ($r > 0.8$) with microbial growth. In contrast, no correlations were found between instrumental colour (except for skin L*) and appearance attributes and microbial growth in chicken meat. The findings suggest that, to consumers, the smell of raw chicken meat would be a more reliable signal for microbial spoilage than appearance, which may correlate with the presence of spoilage bacteria.

Keywords: Chicken meat, Microbial quality, Odour, Appearance, Correlation

1. Introduction

Chicken meat is the most commonly consumed animal protein source in many countries (OECD, 2019). Besides relatively low cost, factors that have been cited for the increased demand for chicken meat include changes in consumers' dietary preferences, consumers' perception of chicken meat as a healthy alternative to red meat due to its low fat content, the versatility of chicken meat (Henchion, McCarthy, Resconi, & Troy, 2014; Tan, De Kock, Dykes, Coorey, & Buys, 2018) and the limited religious restrictions related to its consumption (Mehta & Nambiar, 2007). As is the case with other meats, fresh chicken meat is highly perishable and it has a limited shelf life regardless of refrigerated storage. Deterioration in quality or freshness of refrigerated chicken meat is largely due to psychrotrophic microbial growth and physicochemical changes (Rukchon, Nopwinyuwong, Trevanich, Jinkarn, & Suppakul, 2014). The ready availability of proteins, free amino acids, fats, vitamins, mineral salts and moisture makes chicken meat an ideal medium for the survival and growth of microorganisms during processing, storage and distribution, and at retail and consumer level (Muchenje et al., 2009). Although research has proven that vacuum and modified atmosphere packaging result in a marked extension of the shelf life of refrigerated chicken meat, conventional aerobic packaging using material such as polyvinyl chloride (PVC) film continues to be the dominant type of packaging for fresh chicken meat (McMillin, 2017). During aerobic storage of chicken meat at refrigeration temperatures, the most frequently isolated psychrotrophic bacteria contributing to spoilage include *Pseudomonas* spp., Enterobacteriaceae, *Brochothrix thermosphacta* and lactic acid bacteria (LAB) (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015; Doulgeraki, Ercolini, Villani, & Nychas, 2012; Ercolini et al., 2010; Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015). Their metabolic activities result in the formation of metabolites which bring about physical and chemical changes in the chicken meat, sensorially perceived as off-odours, discolouration and

slime (Dave & Ghaly, 2011). At the consumer level, the sensory aspect of raw chicken meat is of paramount importance because it is the most apparent and hence linked to consumer acceptance during purchasing or preparation (Troy & Kerry, 2010). Chicken meat spoilage is not always evident though and perception of sensory spoilage may be influenced by the severity of meat spoilage and sensory acuity of the individual (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

Many studies have been conducted to analyse storage dependant microbial growth processes and the associated chemical and physical changes in raw chicken meat (Balamatsia, Paleologos, Kontominas, & Savvaidis, 2006; Balamatsia, Patsias, Kontominas, & Savvaidis, 2007; Doulgeraki et al., 2012; Guevara-Franco, Alonso-Calleja, & Capita, 2010; Wang et al., 2017). However, the relationship between microbial growth, pH, changes in instrumentally measured colour and sensory attributes of raw chicken has not yet been established. Therefore, the specific objective of this study was to characterise the odour and appearance attributes of raw chicken meat during refrigerated storage under aerobic packaging and to establish the relationship with the microbial and physicochemical quality changes. The aim of this study was to understand how the implications of microbial spoilage of chicken meat relate to consumers' sensory perception of chicken meat spoilage.

2. Materials and methods

2.1. Sample collection and storage conditions

Raw chicken legs were collected from the production line of a processing plant in Gauteng province, South Africa, immediately after aerobic packaging with PVC film (oxygen transmission rate - *c*. 5000 mL/m^2 per 24 h atm at 22 °C and 75 % relative humidity). At each sample collection date, 20 packs of chicken meat were collected, each with 6 chicken legs. The chicken was transported to the laboratory under chilled conditions within 6 h after slaughtering

and stored at 4 ± 0.5 °C. Samples were analysed after 1, 3, 7, 10 and 14 days of storage. Two independent trials of storage experiments were carried out over a period of 1 month.

2.2. Bacteriological analysis

Raw chicken leg samples were prepared for microbial analysis as described by Mikš-Krajnik, Yoon, Ukuku, and Yuk (2016). Total viable counts (TVC) were determined on Plate Count Agar (PCA) incubated at 25 °C for 3 days, *Pseudomonas* spp. on selective Cetrimide-Fucidin-Cephaloridine (CFC) agar incubated at 25 °C for 2 days, Enterobacteriaceae on Violet Red Bile Glucose (VRBG) agar incubated at 37 °C for 1 day and lactic acid bacteria (LAB) on De Man, Rogosa and Sharpe (MRS) agar incubated at 25 °C for 3 days.

2.3 pH determination

The pH of chicken samples was measured as described by Zhang, Wu, and Guo (2016), using a calibrated digital pH meter (Hanna pH meter 211, Hanna Instruments Inc., USA).

2.4 Colour measurements

Colour of the flesh and skin of raw chicken meat during storage were assessed at room temperature (25 °C) using a colorimeter after calibration with a white ceramic tile as per the manufacturer's instructions (Chroma Meter CR-400, Konika Minolta Inc., Japan). The colour was measured as CIE L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) colour coordinates. The a* and b* colour coordinates were reported as C*(Chroma) after calculation using the formula, $\sqrt{a^{*2} + b^{*2}}$ (Buys, 2004). Chroma measures the colour intensity (Buys, Nortjé, Jooste, & Von Holy, 2000). Measurements were made perpendicular to the surface of the flesh and skin of the chicken legs at 3 different locations on each leg (Choo et al., 2014). Areas on the chicken surface that were selected for colour measurements were free from obvious defects such as bruises, blood clots or scalding and defeathering damage. Images of chicken meat samples were taken using a digital camera (Canon PowerShot SX 50 HS, 12.1 megapixels).

2.5 Quantitative descriptive sensory analysis

Quantitative descriptive sensory analysis of the odour and appearance of refrigerated raw chicken meat was carried out by a trained panel of 10 members (3 males and 7 females), aged between 24 and 42 years. The panel was composed of people who regularly bought raw chicken and prepared it in their households. The sensory acuity of the panellists was assessed prior to training through a 12 plates colour test by Ishihara (1987) and aroma identification test as per the International Organisation for Standardisation (2014). Chicken samples stored for different time periods (1, 6 and 12 days), using a reversed storage design as described by Hough (2010), were used during 4 h of panel training. Panel training was conducted as described by Lawless and Heymann (2010). During training, the panel developed a vocabulary of terms with which to describe the odour and appearance of the range of chicken samples in the study. A group discussion was then held to agree upon the descriptors, their definitions and references to use in order to calibrate the panellists' judgements. The finalised list included 4 terms describing the appearance (pink flesh, creamy skin, green/blue colouration and slimy layer) and 6 terms describing the odour of chicken meat (fresh chicken, bloody, pungent, fishy, rotten egg and ammonia-like) (Table 1). Evaluation of the test samples was conducted in the Department of Consumer and Food Sciences, University of Pretoria food preparation pilot plant, at room temperature (25 °C) under white fluorescent light. The chicken samples were placed in their original packaging on white, plastic trays and served monadically, directly from the cold room along with the selected reference standards. However, the two standard references, rotten egg and spoiled tilapia, were excluded from the final sensory evaluation tests as these resulted in carryover effects. Instead, the panellists used mental references of rotten egg and fish (Franke, Höll, Langowski, Petermeier, & Vogel, 2017). To prevent assessor recognition bias, each chicken sample was labelled with a randomly selected 3-digit code. The order of sample presentation to each panellist was selected following a Williams design. A blind control sample

that was previously frozen at -20 °C and thawed at 4 °C for 18 - 24 h prior to evaluation was presented at each test session. After evaluating the appearance of raw chicken, panellists used stainless steel tongs to open the chicken packages for odour assessment. A 60 s rest period was given between samples for panellists to smell the back of their hands to neutralise their sense of smell. The chicken samples were rated for intensities of odour and appearance attributes on an unstructured 10-cm line scale anchored at both ends with words describing the extremes of each attribute. The sensory tests were run using Compusense® Cloud Saas software (Compusense Inc., Guelph, ON., Canada). The research protocol was approved by the ethics committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa (EC161205-087). Panellists provided informed consent prior to participating in the study.

2.6 Statistical analysis

Experiments were replicated three times. The effect of storage period on microbial growth, pH, instrumental colour and descriptive sensory characteristics of raw chicken meat was determined using repeated measures analysis of variance (ANOVA) followed by the Fisher's Least Significant Difference (LSD) test. SPSS software (version 20.0, IBM SPSS Statistics Inc., Armonk, NY, USA) was employed for the analyses. Principal component analysis (PCA) was used to visualise correlations between the descriptive sensory attributes, microbiological levels, pH and instrumental colour characteristics of raw chicken meat. Pearson's correlation coefficients were calculated to examine the significance of the relationships between the data. XLSTAT software (version 2016, Addinsoft XLSTAT, NY, USA) was employed for the analyses. The above analyses were carried out at 95 % confidence level. The rate of change of the intensity of odour and appearance of chicken meat during storage was determined by fitting linear equations to the data.

3. Results and discussion

A lexicon developed to describe the sensory characteristics of raw chicken meat during refrigerated aerobic storage is presented in Table 1. Changes in microbial levels, pH, instrumental colour and descriptive sensory characteristics (odour and appearance) of raw chicken meat during storage at 4 °C are shown in Fig. 1. A visual display of the changes in the appearance of the chicken over the storage period is shown in Fig. 2.

3.1 Microbial growth versus descriptive odour changes and slime formation

The results show that the initial (day 1) and final (day 14) TVC in the chicken samples was 5.00 and 9.13 log CFU/g, respectively (Fig. 1a). *Pseudomonas* spp. were the predominant bacteria present in raw chicken meat throughout the storage period, followed by Enterobacteriaceae and LAB. As storage progressed, microbial levels increased significantly ($p < 0.05$) at each test interval, except for Enterobacteriaceae and LAB. There was no difference between the Enterobacteriaceae population in chicken stored for 10 and 14 days, and that for LAB in chicken stored for 3 and 7 days. Generally, raw chicken meat that is stored aerobically at refrigeration temperatures develops a microflora confined to the surface of the meat that is usually dominated by *Pseudomonas* spp., most often *P. fragi*, *P. fluorescens* and *P. putida* (Hinton Jr, Cason, & Ingram, 2004; Rouger, Tresse, & Zagorec, 2017; Wickramasinghe, Ravensdale, Coorey, Chandry, & Dykes, 2019). It has been proposed that *Pseudomonas* spp. gain a strong competitive advantage over other spoilage bacteria partly due to their ability to metabolise glucose to 2-oxo-gluconate and gluconate through the Entner-Doudoroff pathway (Dainty, 1996). These compounds are not readily assimilated by other spoilage bacteria and thus *Pseudomonas* spp. build up an energy reserve for use when glucose is depleted (Dainty, 1996). In addition, the proteolytic activity of *Pseudomonas* spp. assists their penetration into the meat, enabling them to gain access to new sources of nutrients that are not available to other spoilage bacteria with weaker or no proteolytic properties (Nychas et al., 2008). Consequently,

Pseudomonas spp. grow rapidly and constitute up to 50 - 90 % of the overall bacterial population.

In the present study, slime on the surface of the chicken legs was detected by all the panellists only after 10 and 14 days of storage (Table B.1.) when the TVC were 8.66 and 9.13 log CFU/g, respectively (Fig. 1a). At high cell counts (> 8 log CFU/g), spoilage bacteria secrete exopolysaccharides which gradually form a layer on the surface of the meat, thereby acting as a protective diffusion barrier against desiccation and predation (Vihavainen & Björkroth, 2010). Russell (2010) described slime as translucent, moist bacterial colonies that increase in size and eventually coalesce and form a layer on the surface of the chicken skin.

As the microbial levels increased, the intensity of the odours also changed, with significant changes ($p < 0.05$) after storage for 7 days (Fig. 1b). While the intensity of the positive odours (fresh chicken and bloody) decreased with storage time, the negative odours (pungent, fishy, rotten-egg and ammonia-like) and overall odour intensity increased. Storage for 7, 10 and 14 days resulted in significant increases ($p < 0.05$) in the intensity of all the negative odours, except for ammonia-like which significantly increased only after 10 and 14 days. However, chicken meat stored for 7 days smelled significantly different ($p < 0.05$) from that refrigerated for 10 and 14 days for all odour descriptors, including the overall odour. In the present study, the TVC and *Pseudomonas* spp. levels in the chicken meat reached 7.54 and 6.49 log CFU/g, respectively, after 7 days of storage (Fig. 1a). It is generally agreed that the first signs of off-odours in poultry stored under refrigerated aerobic conditions occur when superficial TVC reach about 7 log CFU/g (Alexandrakis, Downey, & Scannell, 2012; Balamatsia et al., 2006; Lin et al., 2004). It was proposed that at this point glucose, the most preferred energy substrate by bacteria, would have been depleted. The depletion of the glucose supply results in the sequential utilisation of other substrates such as lactate, pyruvic acid and gluconic acid until amino acids are utilised. The sulphur containing amino acids cysteine, cystine and methionine

were identified as responsible for the formation of malodorous sulphur containing volatile compounds such as hydrogen sulphide, dimethyl sulphide and dimethyl disulphide at TVC levels higher than 8 log CFU/g (Ellis, Broadhurst, Kell, Rowland, & Goodacre, 2002). Malodorous biogenic amines such as dimethylamine and trimethylamine were also reported to be formed through microbial enzymatic decarboxylation of amino acids (Lázaro et al., 2015). It is reasonable to assume that these chemical compounds were the ones described by the panel in this study as fishy, rotten egg, ammonia-like and pungent. The volatile amines, dimethylamine and trimethylamine, have been detected as giving off the characteristic odour of decomposing fish at low concentrations and an ammonia-like odour at higher concentrations (Byrne, Lau, & Diamond, 2002; Morsy et al., 2016). Hydrogen sulphide has been described as reminiscent of over-boiled or rotten eggs (Guidotti, 1996).

Vasconcelos, Saraiva, and de Almeida (2014) reported that at TVC between 7 and 8 log CFU/g, the chicken meat could be categorised as semi-fresh and was still considered as acceptable because the off-odours were slight. Only after the cell counts exceeded 8 log CFU/g could the off-odours be readily perceived, and the meat was categorised as spoiled. This way of categorising meat samples under aerobic refrigerated storage was also applied by Papadopoulou, Panagou, Tassou, and Nychas (2011), but for pork meat. Using microbial levels and the intensity of odours in the present study, chicken legs stored for 7 days could be categorised as semi-fresh. This suggests that it is possible for some consumers, especially those with low olfactory sensitivity, to perceive chicken meat at this stage of microbial growth as still fresh, and only detect spoilage when the microbial levels are too high. Balamatsia et al. (2006) pointed out that consumer perception of meat spoilage is subjective and hence there is no general agreement on the point of incipient spoilage of meat. Some countries, such as South Africa and Australia, do not have regulatory requirements regarding the maximum TVC for raw chicken meat for sale.

3.2 Microbial growth, pH and instrumental colour versus descriptive appearance changes

There was a significant difference ($p < 0.05$) in the pH of chicken meat during the first 3 days of storage from 6.70 to 6.92, and then it remained stable up to day 7 (Fig. 1c). However, there was no significant change ($p > 0.05$) in the lightness values (L*) of both flesh and skin of chicken legs during the first 7 days of storage. After storage for 10 days, the pH increased significantly to 7.08 and finally to 7.28 after 14 days. This increase coincided with darkening of the chicken, with significantly lower ($p < 0.05$) lightness values (L*) of both skin and flesh. At this point, there were more than 8 log CFU/g TVC in the chicken meat. According to literature, during extended storage of refrigerated meat, the accumulation of alkaline nitrogenous compounds such as ammonia and amines results in an increase in meat pH, which leads to unacceptable darkening of the meat (Allen, Russell, & Fletcher, 1997; Zhang et al., 2016). This usually occurs at advanced stages of meat spoilage (about 9 log CFU/g). Consistent with this study, Zhang et al. (2016) also found that the pH of aerobically packaged chicken breast fillets increased significantly during storage for 15 days at 4 °C. In their study, the increase was observed after 9 days of storage. Likewise, del RÍO, Muriente, Prieto, Alonso-Calleja, and Capita (2007) reported a significant pH increase in aerobically packaged chicken legs refrigerated at 3 °C after 5 days of storage. Differences in pH values reported in various studies are most probably due to different chicken portions under study, storage periods, storage temperatures and initial microflora in the chicken meat.

A similar trend to the instrumental colour results (L*) was observed for the changes in the intensity of the pink colour of chicken flesh during storage (Fig. 1d). Chicken meat stored for 10 and 14 days was significantly less pink ($p < 0.05$) than the rest of the samples. Likewise, the chroma values of chicken flesh (flesh C*) decreased significantly only after 10 and 14 days of storage. Another way in which microbial growth is thought to affect the colour of packaged fresh meat is by reducing the level of oxygen at the surface tissue leading to the oxidation of

myoglobin, resulting in the formation of metmyoglobin which gives the meat a brownish colour (Mancini & Hunt, 2005; Suman & Joseph, 2013). Further utilisation of oxygen by aerobic bacteria decreases the oxygen partial pressure at the meat surface to essentially zero and hence promotes the formation of deoxymyoglobin, resulting in meat that is purplish in colour (Mancini & Hunt, 2005). However, these phenomena are more obvious in red meat than chicken meat muscle because myoglobin concentration in chicken is reported to be relatively low, even in the dark muscle (thighs and legs). Kranen et al. (1999) and Miller (2002) found myoglobin contents of only 1.17 and 1.50 mg/g in chicken leg muscle, respectively. Regarding skin colour, the creamy appearance of chicken legs stored for 1 day did not differ with that of chicken stored for 3 and 7 days (Fig. 1d). The colour of the chicken skin deteriorated significantly after storage for 10 and 14 days. On the other hand, lower chroma values (skin C*) were observed up to 7 days of storage and then the values increased (Fig. 1d). The increase in skin C* could be as a result of the apparent discolouration on day 10 and 14 samples (Fig. 2). The panel described it as green/blue colouration on both the chicken flesh and skin and it was apparent to all only after 10 and 14 days of storage. The observed blueish hue could have been due to the formation of deoxymyoglobin, as aforementioned. However, the colour of deoxymyoglobin is commonly described as purplish in red meat (Mancini & Hunt, 2005). Previous studies have also indicated that some bacterial species such as *Shewanella putrefaciens*, *Pseudomonas* spp. and bacteria from the Enterobacteriaceae family produce large amounts of hydrogen sulphide from sulphur containing amino acids under restrictive conditions of very low oxygen concentration and high meat pH (Kameník, 2013). Hydrogen sulphide subsequently reacts with myoglobin in the meat tissue forming sulphmyoglobin, which causes green discolouration. Besides contributing to the formation of sulphmyoglobin, early studies have reported that certain *Pseudomonas* strains synthesise greenish pigments during the logarithmic growth phase (Meyer, 2000; Wasserman, 1965). Pigment products such

as pyoverdine by *P. fluorescens* and *P. putida* could possibly have also contributed to the greenish tint observed on the chicken meat by the panel. Green/blue discolouration on the chicken legs was observed when TVC, *Pseudomonas* spp. and Enterobacteriaceae levels were at 8.66, 7.47 and 7.10 log CFU/g, respectively. The chicken legs were however not analysed for the presence of deoxymyoglobin, specific hydrogen sulphide-producing bacterial species or microbial pigments in the present study. It is important to mention that the discolouration on the chicken skin and flesh was faint and not uniform and the colorimeter measurements, a* (red/green) and b* (yellow/blue) (results not shown), did not reflect the green/blue colour assessment by the human panel. Thus, for chicken colour, the human panel was more informative than the colorimeter measurements in this regard.

3.3 Descriptive odour changes versus instrumental colour and descriptive appearance changes

It is apparent from Fig. 3 that PCA factor 1 (89.0 %) explained the variance in the chicken samples more clearly than factor 2 (7.4 %). The total variance explained by the two factors for the chicken stored for different days was 96.4 %. Chicken meat stored for 1 and 3 days was more similar (left side of biplot) but different from samples located to the right side of the biplot (day 10 and 14). Chicken meat stored for 7 days was clearly different from all the other samples but relatively more similar to day 1 and 3 samples. Chicken meat stored for 1, 3 and 7 days was characterised by having pink flesh and creamy skin, with a bloody and fresh chicken smell, and high skin L*, flesh L* and flesh C*values. Chicken meat stored for 10 and 14 days was described as having all the negative sensory attributes, high microbial levels and high pH. The PCA results also suggest that although chicken meat stored for 7 days still exhibited colour and appearance attributes of fresh chicken, it may not have been as fresh smelling as that stored for 1 and 3 days. Computation of the rate of change of intensity of odour and appearance during chicken storage also showed that odour deteriorated at a faster rate than appearance, except for the attributes bloody and ammonia-like (Fig. 4). Overall, this indicates that odour is a critical

characteristic of chicken meat during refrigerated storage. Of the descriptive odours under study, overall odour and pungent intensity changed at the fastest rate (Fig. 4). The results confirm the findings of our earlier study whereby consumers (n = 863) were asked to indicate how important intrinsic and extrinsic attributes of chicken meat are when judging the quality and safety of raw chicken during purchasing and preparation (Katiyo et al., in press). The consumers indicated that the smell of raw chicken was one of the most important attributes that they consider both at retail and home. The colour of chicken meat was considered as significantly less important. Franke et al. (2017) also reported that the formation of off-odours preceded discolouration, based on sensory analysis of chicken breast packaged in two different modified atmospheres and stored at 4 °C for 8 and 14 days. Thus, off-odours are the signal for microbial spoilage of chicken meat. Colour could be a more discriminating factor for red meat such as beef due to the relatively high myoglobin content.

3.4 Correlations between microbial growth, pH, instrumental colour and descriptive sensory characteristics

There were generally high correlations (r = 0.80 - 1.00) between microbial levels, pH, instrumental colour and human panel intensity ratings of odour and appearance of chicken meat during storage (Table 2). As discussed earlier, microbial growth in aerobically packaged and refrigerated chicken meat results in the production of volatile catabolites which affect the odour quality (Casaburi et al., 2015). Colour deterioration during storage is usually attributed to an increase in meat pH and biochemical reactions between oxygen, volatile microbial catabolites and meat colour pigments (Mancini & Hunt, 2005; Suman & Joseph, 2013). Nevertheless, it is important to highlight that although meat spoilage is almost always due to microbial growth, other mechanisms such as lipid oxidation and enzymatic autolysis are responsible as well (Iulietto, Sechi, Borgogni, & Cenci-Goga, 2015). There also could be interactions between microbial growth and enzymatic reactions (Nychas et al., 2008). While there were many

moderate to very high microbial quality-appearance-colour correlations, the results showed that most correlations were not significant (Table 2). This may be due to the non-significant changes observed in most of the appearance attributes and colour measurements of chicken meat after 1, 3 and 7 days of storage (Fig. 1c and d). Correlations between microbial quality and odour attributes were however significant. These results suggest that the appearance attributes and colour of chicken meat may be less reliable indicators of microbial spoilage during storage than odour.

Non-significant low to moderate correlations (r = 0.21 - 0.67) were observed between chroma of chicken skin (skin C*) and microbial levels, pH, skin L*, flesh L* and intensity of odours of chicken meat (Table 2). Moreover, skin C* values did not differentiate well the chicken stored for different days (Fig. 3). Skin C* would have been a reliable indicator of skin discolouration if there was an accumulation of greying on the skin as storage progressed. Instead, according to the panellists, the skin turned blue/green. These results indicate that the chroma of chicken skin may not be a reliable indicator of microbial spoilage in chicken meat. Brewer, Zhu, Bidner, Meisinger, and McKeith (2001) reported that instrumental colour measures are affected differently by meat colour changes during storage, and L* and a* are typically related to meat muscle colour. However, it can be argued that b* measurements could also be appropriate in the case of corn-fed chicken meat which is naturally yellow-hued (Kennedy, Stewart-Knox, Mitchell, & Thurnham, 2005).

4. Conclusions

Overall, microbial growth is significantly correlated with odour quality of chicken meat during refrigerated storage, but not colour and appearance. For consumers, the smell of raw chicken meat is a more reliable indicator of microbial spoilage than changes in appearance, and detection of pungent, fishy, rotten egg and ammonia-like odours would be warning signals.

However, consumers should be wary of semi-fresh or slightly spoiled chicken as this could be an indication of a broken chicken meat cold chain, thereby posing a food safety risk. Retailers are encouraged to follow good meat retailing practices, such as stock control and maintaining the cold chain, to ensure microbial quality of chicken meat.

Declaration of competing interests

None

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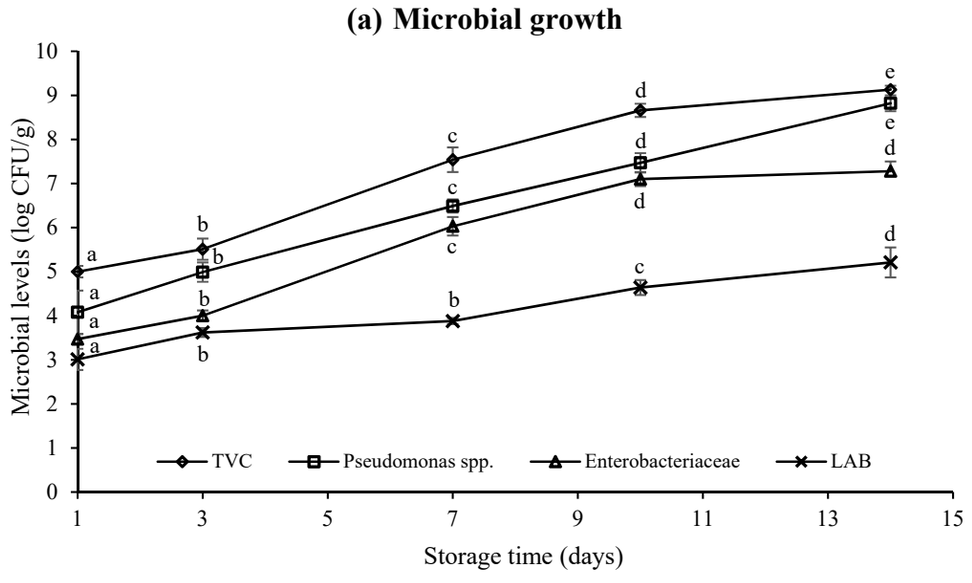
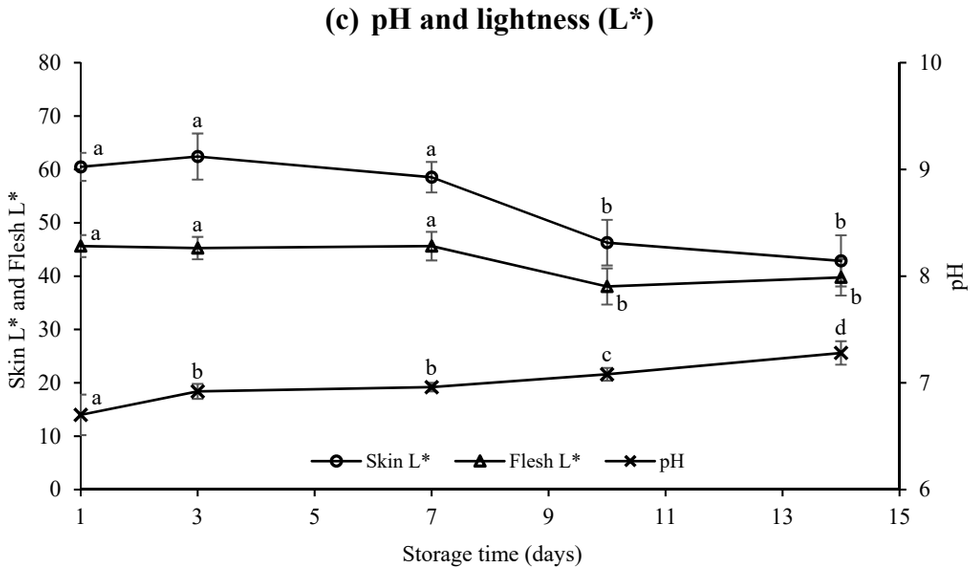
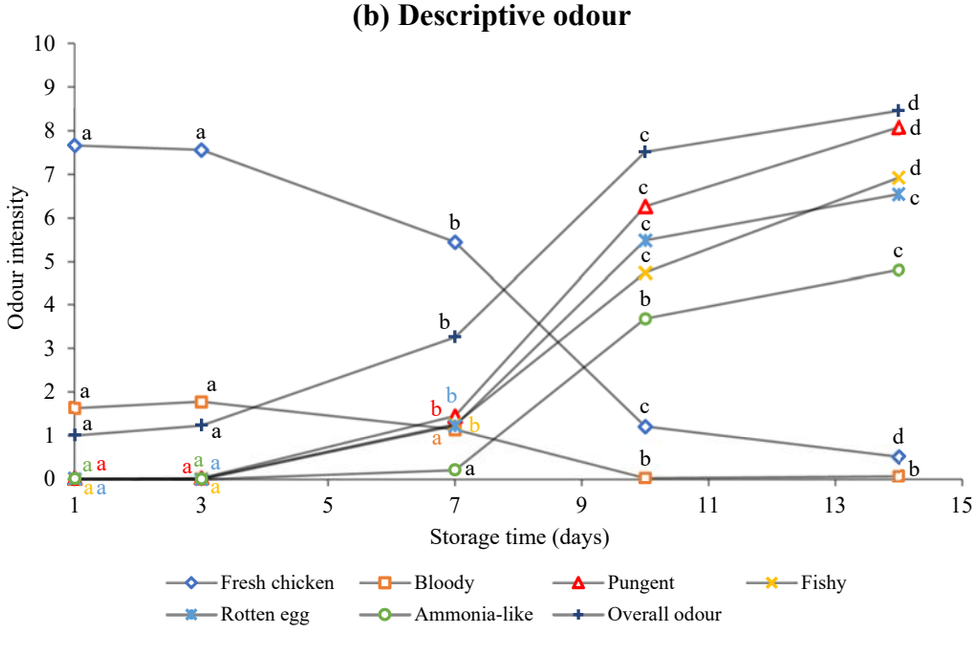

(a) Microbial growth
Microbial levels (log CFU/g)
Storage time (days)
TVC
Pseudomonas spp.
Enterobacteriaceae
LAB


(c) pH and lightness (L*)
Skin L* and Flesh L*
pH
Storage time (days)
Skin L*
Flesh L*
pH


(b) Descriptive odour
Odour intensity
Storage time (days)
Fresh chicken
Bloody
Pungent
Fishy
Rotten egg
Ammonia-like
Overall odour

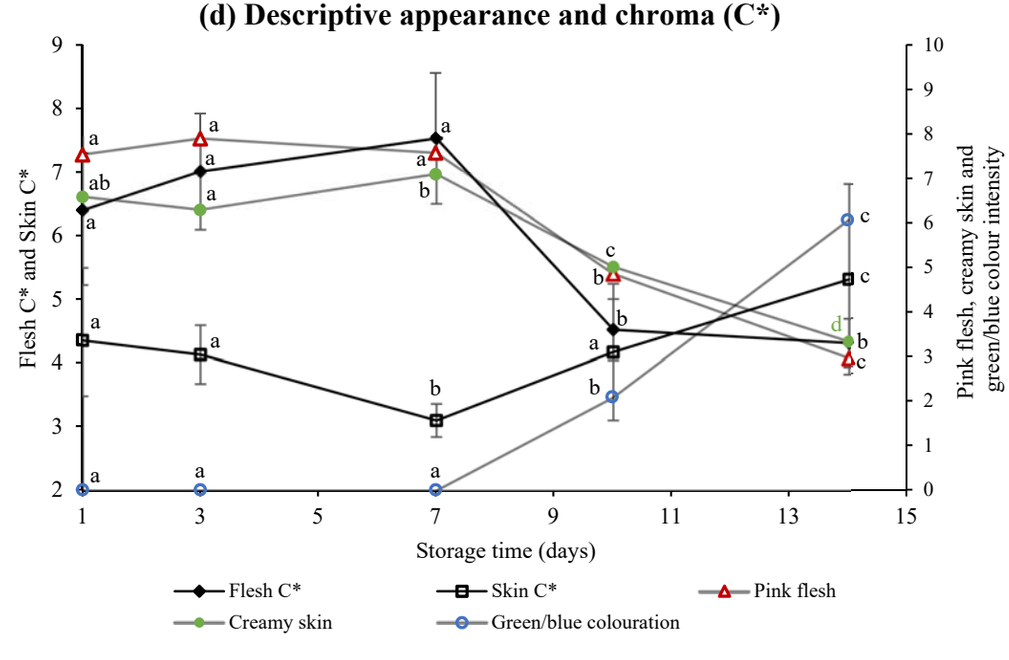

(d) Descriptive appearance and chroma (C*)
Flesh C* and Skin C*
Pink flesh, creamy skin and green/blue colour intensity
Storage time (days)
Flesh C*
Skin C*
Pink flesh
Creamy skin
Green/blue colouration

Fig. 1. Effect of refrigerated storage (4 °C) on microbial levels, pH, instrumental colour and descriptive sensory characteristics (odour and appearance) of chicken meat.

TVC - total viable count; LAB - lactic acid bacteria. Values are expressed as mean ± standard deviation. Values with different letters during storage are significantly different ($p < 0.05$). Descriptive sensory data are shown in colour. For visibility purposes, standard deviations for descriptive sensory data are not shown but presented in Table B.1. The 10-cm scale anchors (0 = absent, 10 = intense) for the different odour and appearance attributes are shown in Table 1

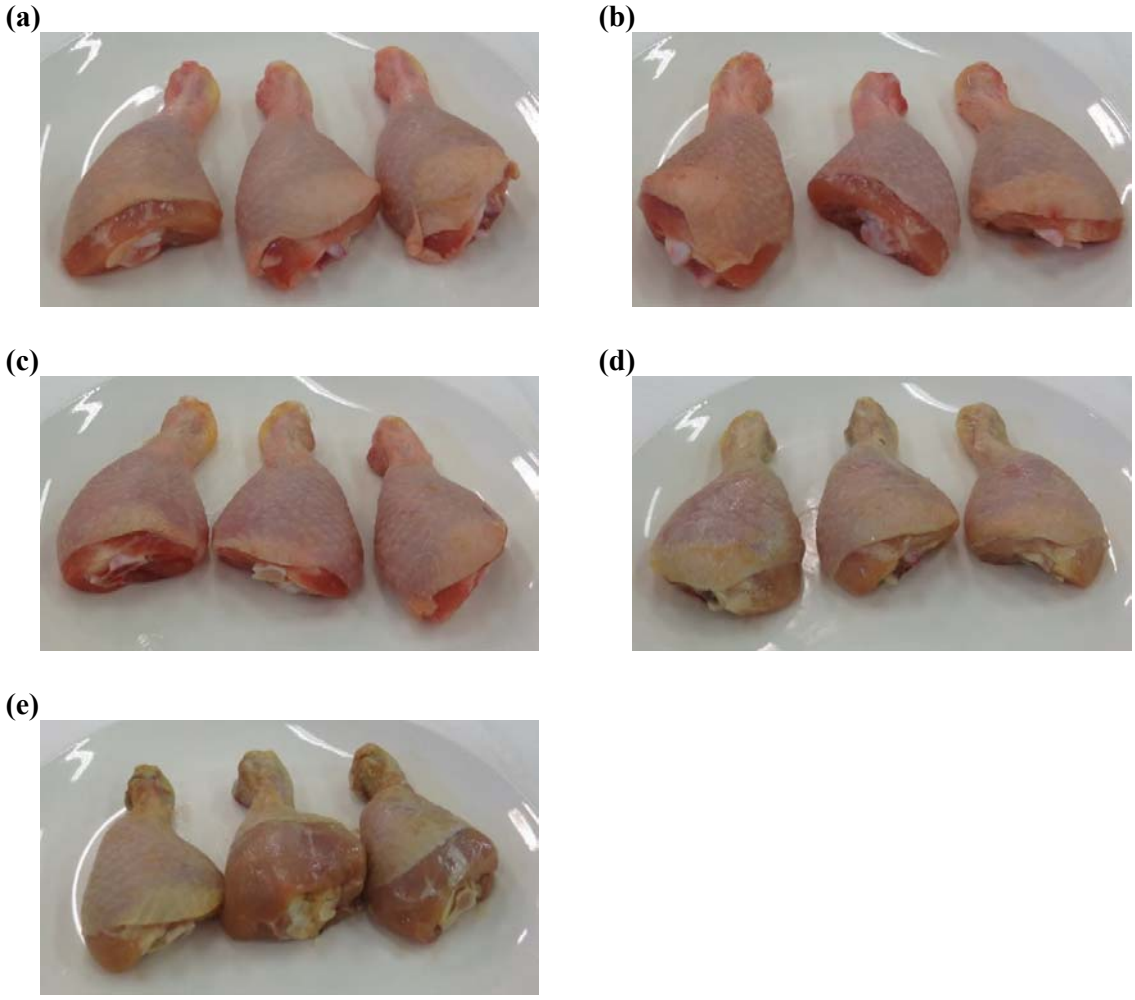


Fig. 2. Chicken meat samples stored at 4 °C for 1 (a), 3 (b), 7 (c), 10 (d) and 14 days (e).

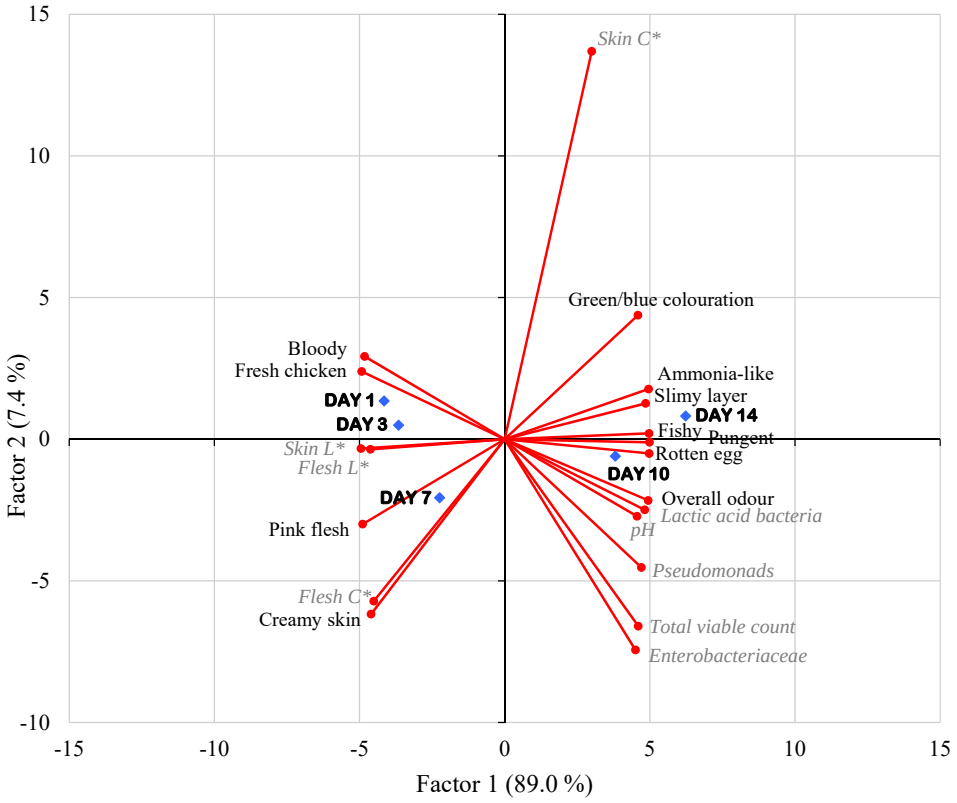


Fig. 3. Principal component analysis biplot of factors 1 and 2 representing chicken meat sample scores and microbial, physicochemical and descriptive sensory characteristics loadings.

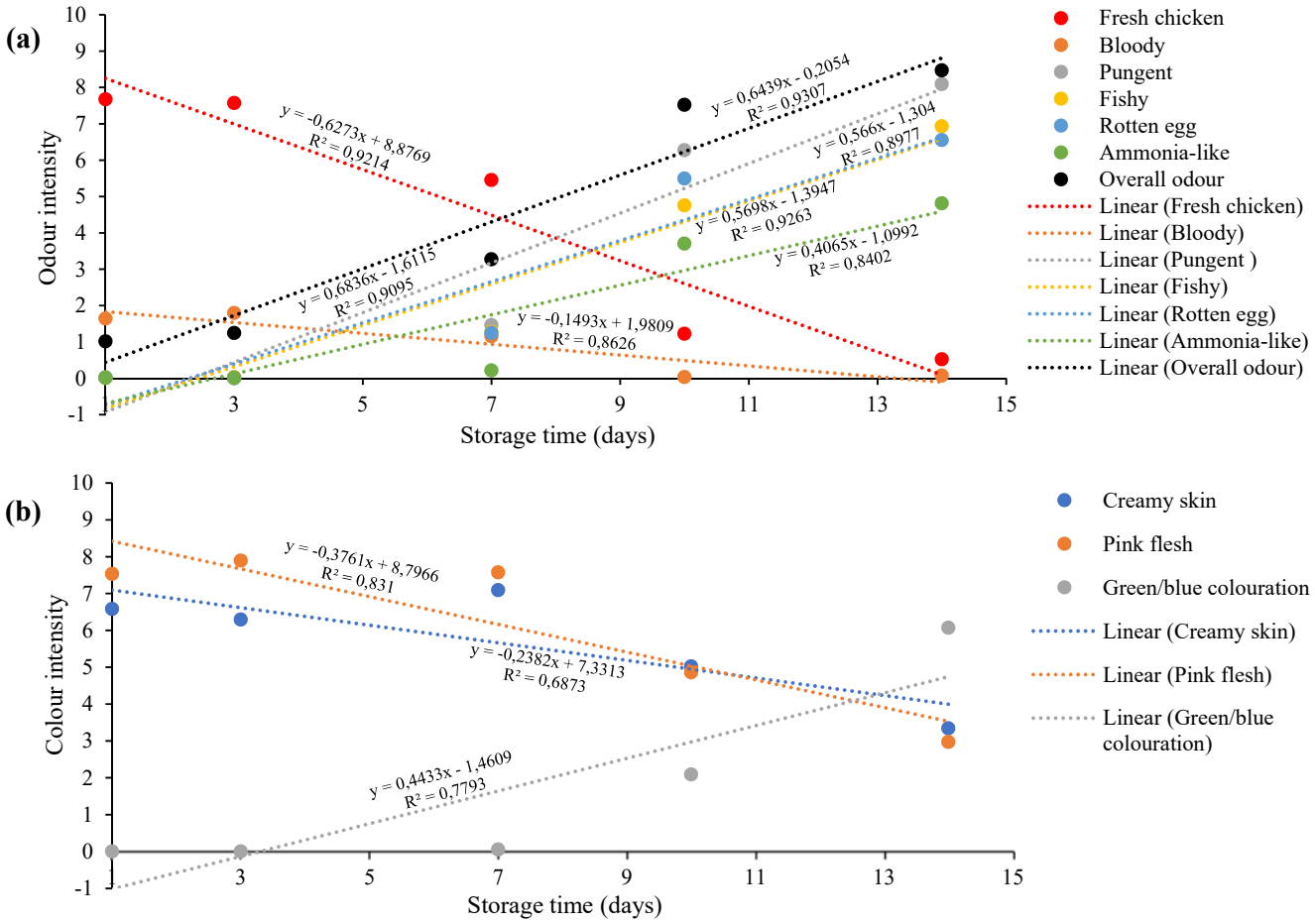


Fig. 4. Rate of change of intensity of odour (a) and appearance (b) attributes of chicken meat during aerobic storage at 4 °C for 14 days.

The 10-cm scale anchors (0 = absent, 10 = intense) for the different odour and appearance attributes are shown in Table 1.

Appendix A

(a)

(b)[1]

Fig. A.1. Standard references for creamy skin, pink flesh (a) and blue/green colouration (b) attributes of chicken meat during refrigerated aerobic storage.

[1]https://en.wikipedia.org/wiki/Chromis_viridis

Appendix B

Table B.1

Appearance and odour descriptive profile of raw chicken meat during refrigerated aerobic storage

	Storage time (days)[1]				
Descriptor	**1**	**3**	**7**	**10**	**14**
Odour					
Fresh chicken	$7.67^{a} \pm 1.81$	$7.57^{a} \pm 1.87$	$5.45^{b} \pm 2.49$	$1.22^{c} \pm 1.59$	$0.52^{d} \pm 0.94$
Bloody	$1.64^{a} \pm 3.27$	$1.79^{a} \pm 3.16$	$1.15^{a} \pm 2.22$	$0.03^{b} \pm 0.11$	$0.07^{b} \pm 0.25$
Pungent	$0.02^{a} \pm 0.06$	$0.03^{a} \pm 0.14$	$1.46^{b} \pm 2.00$	$6.27^{c} \pm 2.57$	$8.09^{d} \pm 1.61$
Fishy	$0.00^{a} \pm 0.00$	$0.02^{a} \pm 0.09$	$1.27^{b} \pm 2.33$	$4.75^{c} \pm 2.97$	$6.93^{d} \pm 2.47$
Rotten egg	$0.01^{a} \pm 0.03$	$0.00^{a} \pm 0.00$	$1.24^{b} \pm 2.32$	$5.49^{c} \pm 2.90$	$6.55^{c} \pm 2.95$
Ammonia-like	$0.01^{a} \pm 0.03$	$0.00^{a} \pm 0.00$	$0.21^{a} \pm 0.70$	$3.70^{b} \pm 3.45$	$4.81^{c} \pm 3.76$
Overall odour	$1.01^{a} \pm 1.03$	$1.24^{a} \pm 1.66$	$3.27^{b} \pm 2.64$	$7.52^{c} \pm 1.92$	$8.47^{d} \pm 1.34$
Appearance					
Slimy layer[2]	0^{a}	0^{a}	0^{a}	100^{b}	100^{b}
Pink flesh	$7.53^{a} \pm 1.58$	$7.89^{a} \pm 1.81$	$7.57^{a} \pm 2.02$	$4.86^{b} \pm 2.91$	$2.97^{c} \pm 2.38$
Creamy skin	$6.58^{ab} \pm 2.55$	$6.29^{a} \pm 2.80$	$7.09^{b} \pm 2.35$	$5.02^{c} \pm 3.25$	$3.34^{d} \pm 3.07$
Green/blue colouration	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$2.09^{b} \pm 0.39$	$6.07^{c} \pm 0.47$

[1]Values are expressed as mean ± standard deviation. Means with different superscripts within a row were significantly different (repeated measures ANOVA, $p < 0.05$). The 10-point scale anchors (0 = absent, 10 = intense) for the different odour and appearance attributes are shown in Table 1.

[2]Percentage of panellists who observed a slimy layer on the surface of raw chicken meat.

Table 1

Descriptors, definitions, ratings and standard references used in descriptive sensory analysis of raw chicken meat during refrigerated storage

Descriptor	**Definition**	**Rating scale**	**Standard reference** (extreme level)
Appearance			
Pink flesh	Pink colour intensity in the flesh of raw chicken legs	0 = no pink flesh 10 = very pink flesh	Photograph of fresh raw chicken legs from abattoir, taken on day zero (Appendix A)
Creamy skin	Cream colour intensity in the skin of raw chicken legs	0 = no creamy skin 10 = very creamy skin	Photograph of fresh raw chicken legs from abattoir, taken on day zero (Appendix A)
Green/blue colouration	Visible green/blue colouration on parts (or all) of raw chicken legs	0 = no green/blue colouration 10 = intense green/blue colouration	Picture of naturally green-blue fish (*Chromis viridis*) (Appendix A)
Slimy layer	Visible growth with a slippery appearance on parts (or all) of the surface of raw chicken legs	0 = absent 1 = present	-
Odour			
Fresh chicken	Distinct aromatic associated with fresh raw chicken muscle	0 = no fresh chicken odour 10 = very fresh chicken odour	Intensity of very fresh raw chicken odour
Bloody	Aromatic associated with raw lean meat, blood, serum or metal/iron	0 = no bloody odour 10 = intense bloody odour	Intensity of an odour of raw beef liver
Pungent	Very strong, sharp smell	0 = no pungent odour 10 = intense pungent odour	Intensity of an odour reminiscent of a rotten freshwater fish
Fishy	Aromatic associated with spoiled fish	0 = no fishy odour 10 = intense fishy odour	Intensity of an odour reminiscent of a rotten freshwater fish
Rotten egg	Aromatic associated with rotten eggs	0 = no rotten egg odour 10 = intense rotten egg odour	Intensity of an odour reminiscent of a rotten egg
Ammonia-like	Aromatic associated with ammonia	0 = no ammonia-like odour 10 = intense ammonia-like odour	Intensity of the odour of 0.2 % ammonia in water
Overall odour	-	0 = no odour 10 = intense odour	Intensity of the overall odour perceived whether positive (fresh) or negative (spoiled)

Table 2

Pearson's correlation coefficients (*r*) between microbial, physicochemical and descriptive sensory characteristics of chicken meat[1]

Variables	TVC	Pseudomonads	Enterobacteriaceae	LAB	pH	Skin L*	Flesh L*	Skin C*	Flesh C*	Pink flesh	Creamy skin	Green/blue colouration	Slimy layer	Fresh chicken	Bloody	Pungent	Fishy	Rotten egg	Ammonia-like
Pseudomonads	**0.981**																		
Enterobacteriaceae	**0.998**	**0.968**																	
LAB	**0.949**	**0.984**	**0.933**																
pH	**0.911**	**0.968**	**0.893**	**0.985**															
Skin L*	**-0.907**	**-0.914**	**-0.888**	**-0.930**	-0.859														
Flesh L*	-0.819	-0.798	-0.813	-0.853	-0.762	**0.937**													
Skin C*	0.261	0.387	0.207	0.490	0.468	-0.599	-0.528												
Flesh C*	-0.693	-0.711	-0.668	-0.779	-0.685	**0.927**	**0.937**	-0.767											
Pink flesh	-0.845	**-0.890**	-0.815	**-0.920**	-0.868	**0.979**	**0.881**	-0.731	**0.930**										
Creamy skin	-0.719	-0.805	-0.681	-0.872	-0.843	**0.905**	0.827	-0.852	**0.914**	**0.966**									
Green/blue colouration	0.773	0.860	0.732	**0.888**	0.874	**-0.901**	-0.744	0.788	-0.837	**-0.967**	**-0.969**								
Slimy layer	0.853	0.855	0.838	**0.901**	0.822	**-0.979**	**-0.985**	0.613	**-0.960**	**-0.949**	**-0.899**	0.845							
Fresh chicken	**-0.960**	**-0.952**	**-0.949**	**-0.956**	**-0.893**	**0.986**	**0.932**	-0.474	0.867	**0.943**	0.849	-0.855	**-0.963**						
Bloody	**-0.949**	**-0.921**	**-0.943**	**-0.918**	-0.838	**0.978**	**0.941**	-0.424	0.867	**0.916**	0.803	-0.804	**-0.960**	**0.993**					
Pungent	**0.924**	**0.940**	**0.905**	**0.958**	**0.901**	**-0.996**	**-0.927**	0.592	**-0.907**	**-0.980**	**-0.915**	**0.915**	**0.973**	**-0.990**	**-0.973**				
Fishy	**0.920**	**0.948**	**0.898**	**0.963**	**0.915**	**-0.990**	**-0.896**	0.614	**-0.891**	**-0.987**	**-0.927**	**0.941**	**0.954**	**-0.980**	**-0.957**	**0.997**			
Rotten egg	**0.928**	**0.935**	**0.911**	**0.953**	**0.890**	**-0.997**	**-0.942**	0.567	**-0.910**	**-0.971**	**-0.899**	**0.893**	**0.980**	**-0.994**	**-0.982**	**0.999**	**0.991**		
Ammonia-like	0.873	**0.899**	0.851	**0.936**	0.875	**-0.992**	**-0.942**	0.670	**-0.946**	**-0.988**	**-0.946**	**0.923**	**0.985**	**-0.970**	**-0.952**	**0.993**	**0.988**	**0.991**	
Overall odour	**0.959**	**0.957**	**0.947**	**0.963**	**0.903**	**-0.987**	**-0.929**	0.490	-0.869	**-0.949**	-0.861	0.867	**0.963**	**-1.000**	**-0.990**	**0.992**	**0.985**	**0.995**	**0.974**

[1]TVC - total viable count; LAB - lactic acid bacteria. Values in bold were significant ($p \leq 0.05$). The magnitude of correlation coefficients was interpreted according to Hinkle, Wiersma, and Jurs (2003): 0.00 to 0.30 little if any correlation; 0.30 to 0.50 low correlation; 0.50 to 0.70 moderate correlation; 0.70 to 0.90 high correlation; 0.90 to 1.00 very high correlation.