Title: APPLICATION OF MULTIVARIATE STATISTICAL ANALYSIS TO QUALITY CONTROL SYSTEMS. RELEVANCE OF THE STAGES IN POULTRY MEAT PRODUCTION

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ABSTRACT

The poultry meat production includes several stages. In this study, the whole process has been evaluated in order to determine the stages or processes which must be specially taken into account in the control system, and which other could be out of the routine controls. The study has been carried out in reverse by studying the relevance of every stages starting from the end of the process towards the initial point

A sequence of operations and consecutive statistical analyses has been performed to finally state the stages and/or operations that must be controlled

Based on the result of statistical studies, the plucking, gutting, washing and classifying stages should be considered Process Control Points. Air chilling and packaging stages are not considered checkpoints in the process verification system, although they should be included within the Good Hygiene Practices, since factors such as temperature, time, cleaning, disinfection or appropriate conditions of handling should be monitored.

Keywords: HACCP; poultry meat; statistical process control

1. INTRODUCTION

Nowadays, food industries apply an important part of their resources to ensure the quality of their manufactured products, mainly with regard to the hygienic-sanitary quality, due to the great economical losses produced as a consequence of the microbiological alteration, both in the foods and the consumers. Poultry meat is one of the main foods commonly involved in food infections (Forsythe & Hayes, 2002).

The process of obtaining poultry meat is very similar in all the slaughterhouses, with only few differences in some stages. It involves common phases, named slaughtering (consisting of hanging, stunning, neck cutting, and bleeding), scalding, plucking, gutting, inside/outside carcass washing, chilling and classifying (Vaquerizo, 1991; Buncic & Sofos, 2012). After this, the carcasses can be sent to the market or to another food industry to be used as raw material.

Some of these stages have hygienic-sanitary (mainly microbiological) risks for the consumers such is the case of the cutting operation, which involves the handling of the product, or the chilling, which needs adequate ambient (humidity and temperature) conditions. In cutting and chilling areas the product is mainly contaminated by cross contamination (by transferring bacteria from one to another product), This can be due to the use of unwashed cutting boards, countertops or knives, or even hands. Therefore, it is necessary to know the prevalence of different microorganism along the food production chain, from the raw material to consumption, to prevent their occurrence (Vitas, Aguado & García-Jalon, 2004).

Approaches for contamination control must primarily be based on

application of good manufacturing practices (GMP), good hygiene practices (GHP), and the principles of hazard analysis critical control points (HACCP). Thus, the process of obtaining poultry meat must be done in approved facilities with established prerequisite programs including GMP and GHP, and managed under the HACCP principles (Buncic & Sofos, 2012). General considerations include sanitary facility and equipment design, sanitary and hygienic conditions in the slaughterhouse, written and validated cleaning and sanitation programs using technologies and operations appropriate for the plant and equipment, control of humidity, moisture, aerosols and condensation, positive air pressure, appropriate air flow, and control of cross contamination (Bolder, 2007; FAO/WHO, 2009). The European Union Regulation 853/2004 describes requirements for slaughterhouses, relative to design, construction, cleaning and sanitation of equipment.

The quality control systems during the production in food-processing industries are based on a preventive concept of the methodology of control, that is to say, the knowledge of the risks to avoid theirs appearance or to reduce their effects when they take place. To achieve this aim, it is necessary to identify and locate the stages, processes or practices of the productive chain which involve any risk. The relevance of each step can be assessed by determining whether exerts significant effects (increases or decreases) on the microbial content of the product by counting different microorganisms. Then, these relevant stages can be identified and proposed as CCP's (Critical Control Points).

Nevertheless, the food industry aspiration is to obtain the total quality of their products. Thus, it can be developed a *Continuous Control System during*

the Production based on the HACCP systems, but having wider aims of quality. It consists of identifying the process stages that can cause an effect (beneficial or detrimental) on the quality of the final product, and then exert a systematic monitoring on them, in order to favour the positive effects and minimize or eliminate the risks as far as possible (González-Miret, Alonso & Heredia, 1998). To get these aims, not only the points affecting the food safety (Critical Control Points, CCP) but also those in which it is possible to improve the quality of the product must be controlled.

In a quality control process, taking decisions should be made based on accurate research data. In this sense, the application of statistical techniques has great importance. Thus, general industry, and most especially food industry, incorporates statistical techniques as part of their Quality Control programs. Statistical patterns can be effectively applied in each stage of the HACCP system (Hayes, Scallan & Wong, 1997; González-Miret, Alonso & Heredia, 1998; 2000; González-Miret, Escudero, Alonso & Heredia, 2001; Tsola, Drosinos & Zoiopoulos, 2008). Multivariate statistical techniques such as Multiple Analysis of Variance (MANOVA), Cluster Analysis or Stepwise Discriminant Analysis (SDA) have been used to select parameters of validation (González-Miret, Coello, Alonso & Heredia, 2001), by identifying the most useful variables among all the variables involved, avoiding parameters giving redundant information.

The experimental studies for the control system design can be stated in two ways according to the reasoning sequence: direct (forward), or reverse (backward), starting from the beginning or the end of the production chain, respectively.

In this study we propose an inverse performance to design the control system by studying the relevance of every process stages, deciding the steps points or processes which have to be specially taken into account in the routine control system. The proposed method involves carrying out a sequence of consecutive operations and statistical analysis aimed at search for the stage and/or operation of the production chain that must be considered in the quality control system.

2. EXPERIMENTAL

2.1. Process

The poultry meat production includes several stages: 1) slaughtering and total bleeding; 2) scalding by immersion into hot water (aprox. 52 °C, 2 minutes); 3) plucking to remove mechanically the feathers; 4) automatic gutting; 5) internal and external washing with pressurised water to remove any dirt on the skin coming from feathers, paws and faeces (Thomas & McMeekin, 1980), to reduce the superficial contamination (Siragusa, 1995; González-Miret, Alonso & Heredia, 1998; Escudero-Gilete, González-Miret & Heredia, 2005), and to avoid drying in the freezers (Buxadé, 1985; Vaquerizo, 1991); 6) chilling in airing tunnel (cold air, around 0 °C, for 100 min) to avoid the growth of non-psychrotrophic flora and slow down the psychrotrophic one; 7) classifying according to the weights and quality; 8) refrigerated storage (4°C) for a variable time before being processed; and then 9) packaging. In this study the whole process has been assessed.

2.2. Samples

838 samples were analysed, corresponding to 70 carcasses sampled at six different stages of the production chain (Table 1). In each sampling one carcass was specially labelled with identification purposes. Samples of skin fron this same carcass were taken before and after each stage, in order to determine the evolution of the microbial content occurred due to the process of this stage.

Breast skin was selected for sampling since it is a very homogeneous and extensive surface which allows taking several samples from the same carcass. Samples were aseptically taken with sterile tweezers and scalpels, placed into Petri plates and immediately analysed.

2.3. Analytical methods

For each sample, 10 g of skin were taken aseptically, placed into a sterile Stomacher® bag containing 90 ml of 1% Peptone Water solution (PW, Oxoid, Basingstoke, Hampshire, England), and stomached for 2 min. Decimal dilutions (10^{-2}, 10^{-3}, 10^{-4}) were prepared with 1% PW.

The microbiological variables must give significant information about the control system in every stage to be used as indices of contamination. In this sense, previous studies (González-Miret, Coello, Alonso & Heredia, 2001; González-Miret, Escudero-Gilete & Heredia, 2006 Escudero-Gilete, González-Miret & Heredia, 2005;Escudero-Gilete, González-Miret, Moreno & Heredia, 2007) have assessed and proved by means of uni and multivariate statistical techniques the usefulness of Total count, *Pseudomonas*, *Enterobacteriaceae* and *Staphilococcus aureous* as higyenic-sanitary parameters in quality control systems (APHA, 1992; AOAC, 1978, 1995, 2004-2005; Forsythe & Hayes,

158 2002). Although Salmonella is also important in poultry meat hygiene analysis,
159 under a global point of view for a routine checking of a process the main Total
160 count and *Enterobacteriaceae* analysis is usually made, additionally
161 *Pseudomonas* for refrigerated products as the poultry meat is. Hence, in this
162 study the samples were analysed by counting these microbiological parameters:
163 - *Total count* (Tc): Nutrient Agar (Oxoid, Basingstoke, Hampshire, England).
164 Incubated at 30 °C for 72 h (ISO:4833, 2003)
165 - *Pseudomonas* (Ps): Pseudomonas Isolation Agar (Difco, Detroit, Michigan,
166 USA). Incubated at 25 °C for 48 h (ISO:13720, 2010)
167 - *Enterobacteriaceae* (Eb): Violet Red Bile Glucose Agar (Oxoid, Basingstoke,
168 Hampshire, England). Incubated at 37 °C for 24 h (ISO:21528-2, 2004)
169 - *Staphylococcus aureus* (St): Baird-Parker Agar (Scharlau, Barcelona, Spain).
170 Incubated at 37 °C for 48 h (ISO:6888-1, 1999)
171 Also ambient and carcasses temperature were measured and taken into
172 account in the study.

173 **2.4. Experimental design**

174 The general objective of this study was optimizing the quality of the final
175 food product. For this purpose, first we need to define the characteristics of the
176 product when it is ready to be consumed. These characteristics are
177 consequence of those having the product before this step and the factors
178 conditioning them. In this sense, the conditions of the carcasses at the end
179 (final point [FP]) of a stage result from what occurred during the process as well
180 as the conditions having the product at the beginning of the stage, which are the
181 same that at the immediately previous point (initial point [IP]).
182 The evaluation of the relevance of each process stage have been

183 performed by assessing the significance of their effects (increase or decrease)
184 on the microbiological levels (Tc, Ps, Eb, St) of the products after the
185 corresponding step. A backward study system was applied consisting of the
186 evaluatoin of the production chain in inverse sense, i.e., starting from the end of
187 the chain up to the initial stage. Samples were taken immediately before and
188 after each studied step of the process. Also, factors related to process (stages)
189 and product (carcasses) were measured in every sampling point. The relevance
190 of every step or process was determined based on all this information by
191 applying uni and multivariate statistical techniques.

192 **2.5. Statistical analysis**

193 Transformed microbiological variables consisted on taking log 10 of the
194 original data (*Tc* = Log (Tc); *Ps* = Log (Ps); *Eb* = Log (Eb) and *St* = Log (St)) to
195 obtain the normal data needed to carry out statistical analysis.

196 Repeated-measures statistical techniques were applied to determine
197 whether significant differences ($p<0.05$) exist among the different groups of
198 samples in each stage (Figure 1). A *t*-Student test for the related groups (normal
199 distribution of samples) and Wilcoxon test (non-parametric test for samples that
200 do not fulfil the normal distribution) were carried out to compute the effect of the
201 stage (initial/final) on each microbiological variable analyzed. Also, repeated
202 measures Analysis of the Variance (ANOVA) was carried out in order to
203 determine the effect of each stage on the microbiological variables (dependent
204 variables) simultaneously.

205 The Kruskal-Wallis test was applied to check the variables showing
206 significant differences among several independent groups. It is a non
207 parametric test for samples that do not fulfil the normal distribution.

These statistical analyses of the data were performed using the Statistica® V 8.0 software (StatSoft, 2007).

3. RESULTS AND DISCUSSION

The descriptive analysis of the data gives a previous general view and complementary to the confirmation by multivariate statistics (Martín, 2001). The microbiological counts of each analyzed bacteria, in the initial and final points of each stage, are shown graphically in Figure 2.

Following the established inverse order, it can be observed that the contamination level of the carcasses almost was not affected in packaging stage, showing slight increases in the case of *Pseudomonas* and *Staphylococcus* (0.07 and 0.14 log units, respectively). However, an increase of the four microbiological variables occurred during classifying. The air chilling tunnel (before the classifying process) showed similar average levels of microorganisms at the end and at the beginning. The pressurised water exercised an important effect on the superficial pollution of the carcasses (González-Miret, Alonso & Heredia, 1998; Escudero-Gilete, González-Miret & Heredia, 2005). *Tc*, *Ps*, *Eb* and *St* decreased after this stage (0.53, 0.34, 0.64 and 0.37 log units, respectively). So, the washing stage is a very important operation within the poultry meat production. However, the opposite occurred in the gutting process, showing increases in counts of superficial pollution for the four microbiological variables. Finally, the behaviour among the different analyzed microorganisms in the plucking process varied. The *Total Count* and *Staphylococcus* pollution descended after feathers removing (0.90 and 0.92 log

232 units, respectively), *Pseudomonas* increased (0.28 log units) and
233 *Enterobacteriaceae* showed a slight rise of values (0.04 log units).
234 Therefore, this information indicates that the stages most affecting the
235 superficial pollution of the carcasses in negative way are classifying and gutting.
236 The carcasses are handled by workers in both stages, so high risk of cross-
237 contamination exists. In addition, gutting area show very high environmental
238 humidity and have several points of showers that can disperse pollutants from
239 some carcasses, or even from the environment (soil, air, machines), to other
240 carcasses. And in the classifying stage, the carcasses are closely in contact
241 other carcasses and with surfaces of containers (bins, crates, and plastic
242 boxes), increasing the cross contamination risk.
243 Having in mind the objective of not only prevent hazards and risks but
244 also increase the positive effects, and based on the previous results, washing
245 with pressurised water stage and, in certain way, plucked stage could be
246 considered in the system as beneficial stages. These steps have two common
247 characteristics: shower with pressurised water to remove the dirt from the
248 surface of carcasses, and lack of worker manipulation. The cleaning in the
249 plucking stage is less effective than in the washing stage since the first one has
250 less suitable ambient and process conditions (humidity and temperature). Also,
251 the internal side of the plucking machine, consisted of many rolling rubber
252 fingers, can contain and disperse the dirt. On the other hand, it is necessary to
253 bear in mind that the carcasses come to the plucked stage having high
254 contamination level (with feathers, entrails and just slaughtered).
255
256 **3.1. Statistical evaluation of the relevancy of each stage**

257 The Kolmogorov-Smirnov-Lilliefors test was used to evaluate the
258 normality of every microbiological variable transformed at each stage for each
259 sampling point. Table 2 shows the values of $|D_{max}|$ which indicate the difference
260 between the sampled and theoretical distribution (Martín, 2001; StatSoft, 2012;
261 Vives-Rego, Resina, Comas, Loren & Juliá, 2003). These values were
262 significant (*p<0.01*) for Log St in all sampled points, for Log Tc in [C] and [K]
263 points (packaging stage), [W] point (washing stage), [P] point (gutting stage)
264 and [S] point (plucking stage), for Log Ps in [T] point (classifying stage), in [W]
265 and [T] points from air chilling stage, in [W] point from washing stage, [P] point
266 from gutting stage and [S] point from plucking stage, and for Log Eb in [T] from
267 classifying stage, and [P] from gutting stage, meaning that these data do not
268 showed a normal distribution.
269 Suitable statistical techniques were then carried out, for groups of related
270 samples, to verify the significance of the effect that every stage exercises on the
271 superficial pollution of the carcasses. The t-Student test (for normal distribution)
272 and the Wilcoxon test (non-parametric for not normal distribution) were applied
273 in order to compare two dependent samples (Table 3). It can be observed that
274 classifying, washing, gutting and plucking stages exert significant effect
275 (*p<0.01*) on the superficial pollution of the carcasses for all the microbiological
276 variables studied, except for the plucking stage on *Eb* (not significant effect).
277 Other stages such as packaging and air chilling do not exert significant effect on
278 the superficial microbiological count of carcasses. These results corroborate the
279 information obtained from the initial descriptive study of the data, and reveal the
280 importance of some stages of the fresh poultry meat production, some having
281 negative effect and some others positive ones.

Repeated measures MANOVA was carried out with the purpose of establishing differences between dependent sampling groups (Norman & Streiner, 1996). A 2x4 design with two factors of repeated measures (sample point (SP) and microbiological variable (MV)) was performed. Four microbiological variables (Log Tc, Log Ps, Log Eb and Log St) were determined for the same sample unit, before and after each studied stage ([initial]/[final]) (Figure 2). Results of the repeated measures MANOVA are shown in Table 4.

In the case of **packaging** stage (PK) (Table 4 and Figure 2a), significant differences among the sampling points were found ($p<0.05$), as well as among microbiological variables ($p<0.01$), although there was no significant difference in the interactions between both factors. The size of the effect ($\eta^2=0.69$) was higher for the "microbiological variable" factor. Pair comparisons using Bonferroni test indicated that no significant difference existed between points [C] and [K]. Comparisons between pairs of microbiological variables revealed significant differences ($p<0.05$) between the average values of *Tc* and the rest of the microbiological variables, also between average values of *Ps* and *St*; however, there was not significant difference between *Ps* and *Eb* means. When comparing pairs of points ([C]/[K]) regarding each microbiological variable, no significant differences between any pair were found, as it was verified by t-Student or Wilcoxon test.

Regarding to **classifying** stage (CL) there were significant differences ($p<0.01$) respect to the effect of the sampling point (stage), microbiological variables and both factors interaction (Table 4 and Figure 2b). The contribution of "sampling point" factor was 74%. Bonferroni test indicated significantly ($p<0.05$) lower mean for point [T] than point [C]. The comparison between

microbiological variables showed significantly (*p<0.01*) higher means of *Tc* and *Eb* than *Ps* and *St*. All other possible comparisons were not significant. On the other hand, the differences between the initial and final points, for every microbiological variable, were significant in all cases except for *Tc*. This result does not agree with that obtained in *t*-Student test, which found significant difference between the initial and final point for *Tc*. This can be due to the severe correction of α-level applied by Bonferroni test, obtaining a more strict new value of α-level ($\alpha=\alpha/$nº comparisons) (Norman & Streiner, 1996; Martin & Luna, 2004).

In the case of the **air chilling** stage (CH), significant differences (*p<0.01*) between microbiological variables were found (Table 4 and Figure 2c). The size of the effect was $\eta^2=0.63$; however, there were not significant differences between the sampling points. When considering both factors simultaneously, significant differences were not found. These results revealed that the microbial proliferation is stopped in the tunnel of cold air since the levels of superficial pollution of the carcasses on the initial point ([W]) are kept.

On the basis of these results it can be stated that the cold air applied on the carcasses during a short period of time (100 minutes) does not induce changes on the levels of superficial pollution, but slows down the development of microbial flora. This greatly prevents from increasing the levels of pollution. Moreover, a previous study performed by González-Miret, Alonso & Heredia(2000) revealed the decontaminating effect of the low temperature, principally on *Eb*, when it is applied for a longer period of time (24 hours).

The **washing** with pressurised water stage (WH) showed significant differences (*p<0.01*) for both factors of repeated measures (sampling point (SP)

and microbiological variable (MV)), as well as for their interaction (SP x MV) (Table 4 and Figure 2d). The washing stage is one of the most relevant processes of the production line. It divides the whole process in two basic areas: the "*dirty zone*", including slaughtering, bleeding, scalding, plucking and gutting stages, and the "*clean zone*", including the rest of the stages where processes occur at controlled low ambient temperature and under strict hygienic controls.

In the **gutting** stage (GT) (Table 4 and Figure 2f) the product is highly handled and significant differences between the SP and among the MV were found ($p<0.01$). Post hoc pair comparison test was carried out observing that the average of data at plucking point [P] was significantly lower than the average at gutting point [G]. Also, the means of the MV were significantly different among them ($p<0.01$), having *Tc* the higher mean value followed in order by *Eb*, *Ps* and *St*. Bonferroni test between the initial and final point found significant differences ($p<0.01$) for all the microbiological variables, showing increments in all cases. This was in accordance with the t-Student and the Wilcoxon tests.

The **plucking** stage (PL) (last stage included in this study) (Table 4 and Figure 2g) shown significant values ($p<0.05$) for the two factors studied and for the interaction between them, obtaining a 67% contribution of "microbiological variable" factor. However, significant differences ($p<0.05$) were not found between sampling points by the Bonferroni test. The comparisons between the microbiological variables were always significant, with a higher mean value for *Tc*, followed by *Eb*, *St* y *Ps,* respectively. The differences between pairs of sampling points for every microbiological parameter were significant, with

357 negative sign for *Tc* and *St*, and positive for *Ps*. *Eb* did not shown significant
358 difference between [S] and [P]. The decontaminating effect of removing the
359 feathers is confirmed, especially for *Tc* and *St* variables. The results for *Ps* were
360 similar to those found by Escudero-Gilete, González-Miret & Heredia (2005) in a
361 study of the washing stage, in which a light increase of *Ps* occurred when the
362 time of washing diminished from 8 to 4 seconds. In the case of the plucking
363 stage, the carcasses are shortly washed at the end, even shorterly than in the
364 washing stage. So, the behaviour of *Ps* could indicate that a reduction of the
365 superficial contamination of this microorganism might need longer washing
366 time.

367 Stepwise Discriminant Analysis (SDA) was applied to explore the extent
368 to which the microbiological variables are able to discriminate between stages
369 (Tabachnick & Fidell, 1983; Johnson, 2000; StatSoft, 2012). The results
370 indicated the usefulness of *Eb* for this purpose, showing high significant levels
371 between [T]/[C] (p=0.047) and between [G]/[W] (p=0.00, r=0.576). *Tc* had the
372 highest contribution to the discrimination in the [P]/[G] relationship, and *Tc*, *Ps*,
373 and *St* were able to discriminate between [S]/[P] points (plucking stage).

374 The increments of the microbiological variables between the final [FP]
375 and the initial point [IP] of each stage were used to determine whether
376 differences exist between the six studied stages: (PK), (CL), (CH), (WH), (GT)
377 and (PL). Transformed log-10 microbiological variables were used to approach
378 normal distribution: $\Delta Tc = (\pm)\ \mathrm{Log}\ |(Tc_{[FP]} - Tc_{[IP]})|$

379 $\Delta Ps = (\pm)\ \mathrm{Log}\ |(Ps_{[FP]} - Ps_{[IP]})|$

380 $\Delta Eb = (\pm)\ \mathrm{Log}\ |(Eb_{[FP]} - Eb_{[IP]})|$

381 $\Delta St = (\pm)\ \mathrm{Log}\ |(St_{[FP]} - St_{[IP]})|$

The Kolmogorov-Smirnov-Lilliefors test was used to assess the normality of every transformed microbiological variable in each group of studied samples ((PK)/(CL)/(CH)/(WH)/(GT)/(PL)), obtaining significant values in all cases (Table 5). Since variables ΔTc, ΔPs, ΔEb and ΔSt do not have a normal distribution, the Kruskal-Wallis test (called non-parametric ANOVA) was carried out to establish whether significant differences exist for all the microbiological variables among the six groups of samples (PK), (CL), (CH), (WH), (GT) and (PL). Significant differences ($p<0.01$) were found between the six groups for all the microbiological variables. Pairs of groups were taken to establish whether one variable shows significant differences between two independent groups of samples ((PK)/(CL); (PK)/(CH); (PK)/(WH); (PK)/(GT); (PK)/(PL); (CL)/(CH); (CL)/(WH); (CL)/(GT); (CL)/(PL); (CH)/(WH); (CH)/(GT); (CH)/(PL); (WH)/(GT); (WH)/(PL); (GT)/(PL)). The superficial contamination of the carcasses (Table 6) decreased for all the studied microorganisms in the washing stage (WH) (ΔTc=-5.00, ΔPs=-3.30, ΔEb=-3.98, ΔSt=-3.180), for Tc and St in the plucking stage (PL) (ΔTc=-6.77, ΔSt=-4.94) and for Ps and St during the air chilling stage (CH) (ΔPs=-2.36, ΔSt=-3.17). In general, this decrease is significantly higher in washing stage than in the rest, highlighting the importance of this stage in the production of poultry meat is revealed. Tc and St decrease in the plucking stage (PL) (ΔTc=-6.77 y ΔSt=-4.94). This decrease was significantly different ($p<0.05$) from the increases occurring in the stages of the "cold zone". The microbiological variables always increased in gutting stage (ΔTc=5.36, ΔPs=3.13, ΔEb=4.05, ΔSt=3.43), being significant ($p<0.05$) for Tc and Eb. ΔPs shown significant differences between (CL) and (CH). These results indicate that low temperatures affect mainly the decrease of the superficial

407 contamination caused by Ps and St.

408

409 **3.2. Conclusions**

410 The microbiological variables usually considered as indices of
411 manipulation suffered significant increases of counts in the classifying and
412 gutting stages, both being operations which involve high handling of the product
413 by the workers. The stages that clean the carcasses surface produce significant
414 decreases of the majority of the studied parameters, which can be caused by
415 removing feathers (in plucking stage) or dirt with pressurised water (in washing
416 stage).

417 Based on the statistical results, plucking, gutting, washing and classifying
418 stages should be considered process control points since significant differences
419 were found. However, although air chilling and packaging stages are not
420 checkpoints in the process verification system, they should be included within
421 the Good Hygiene Practices (GHP) protocol since factors such as temperature,
422 time, cleaning, disinfection or appropriate conditions of handling should be
423 monitored.

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Highlights

- Stages of poultry meat production have different relevance on the final quality
- Stages can exert positive or negative influence on the quality of the product
- Multivariate statistics can be applied to determine the influence of each stage
- Plucking, gutting, washing and classifying stages must be considered control points

Figure 1

Figure 1.

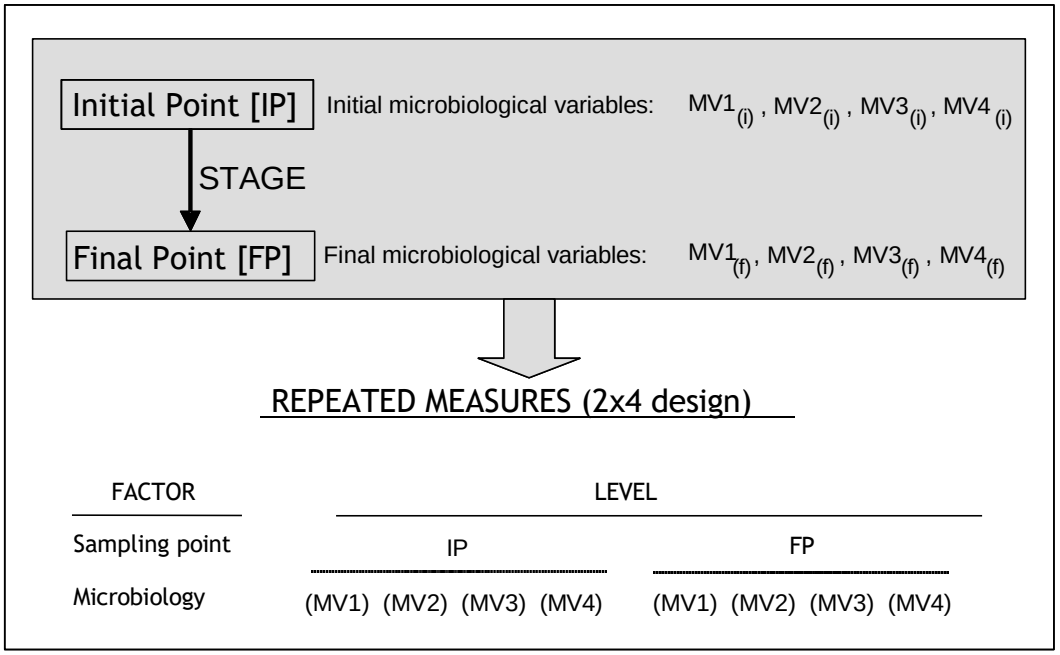

Figure 2

Figure 2.

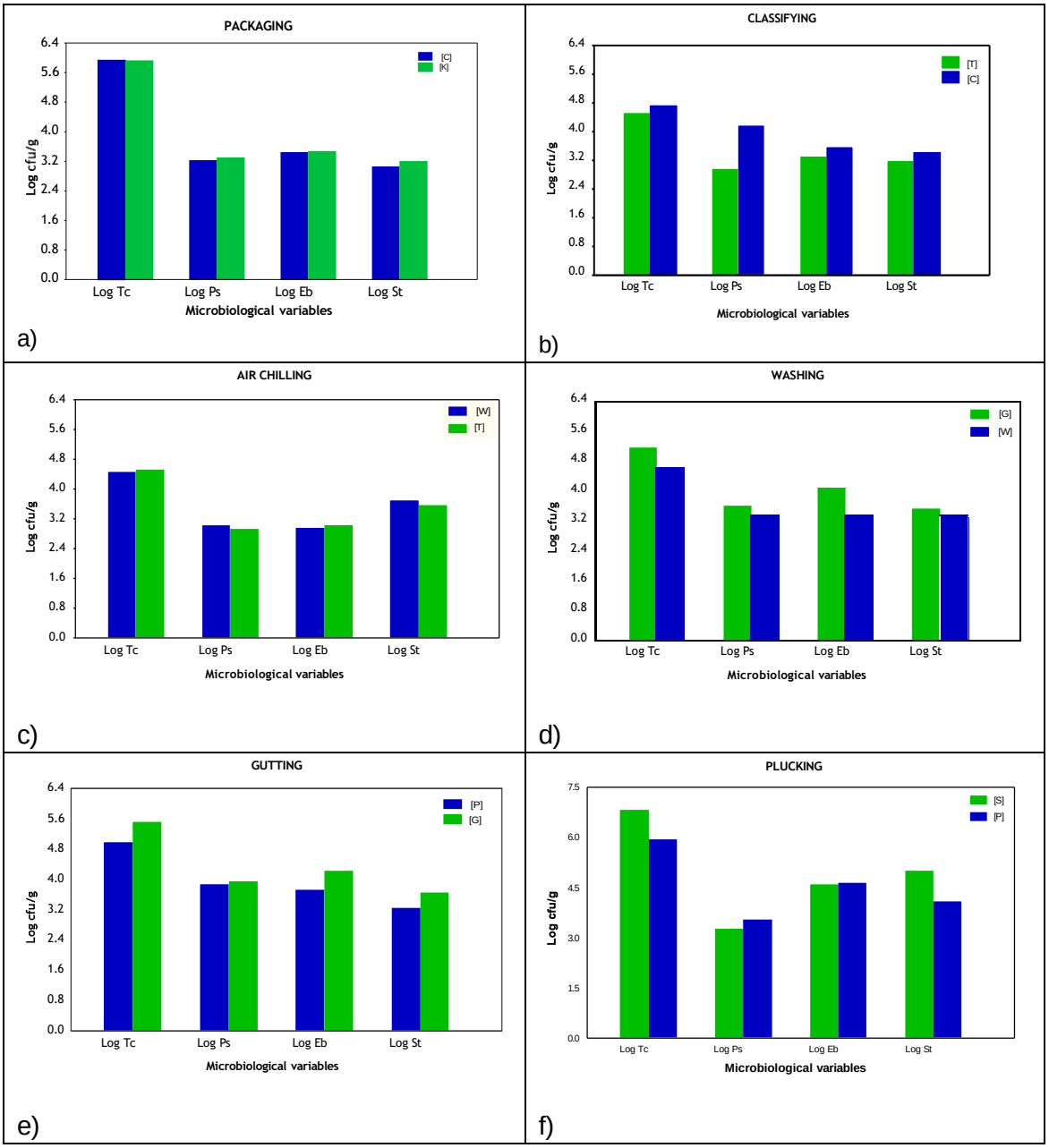

Table 1

Table 1. Description of sampling points at the studied stages of the poultry meat process chain.

Stage	Carcasses number	Sampling points	Code	N
Packaging (PK)	70	After classifying	[C]	70
		After packaging	[K]	70
Classifying (CL)	70	After air chilling tunnel	[T]	70
		After classifying	[C]	70
Air chilling (CH)	70	After washing with pressurised water	[W]	70
		After air chilling tunnel	[T]	68
Washing (WH)	70	After gutting	[G]	70
		After washing with pressurised water	[W]	70
Gutting (GT)	70	After plucking	[P]	70
		After gutting	[G]	70
Plucking (PL)	70	After scalding	[S]	70
		After plucking	[P]	70

Table 2

Table 2. Kolmogorov-Smirnov-Lilliefors normality test ($|D_{max}|$) results

Stage	Sampling point	Microbiological variable			
		Log Tc	Log Ps	Log Eb	Log St
Packaging	[C]	0.159**	0.052	0.067	0.202**
	[K]	0.187**	0.066	0.057	0.191**
Classifying	[T]	0.102	0.138**	0.153**	0.209**
	[C]	0.042	0.088	0.088	0.176**
Air chilling	[W]	0.078	0.125**	0.063	0.156**
	[T]	0.061	0.157**	0.101	0.179**
Washing	[G]	0.070	0.083	0.095	0.168**
	[W]	0.151**	0.135**	0.098	0.175**
Gutting	[P]	0.117*	0.135**	0.109*	0.202**
	[G]	0.091	0.066	0.080	0.202**
Plucking	[S]	0.281**	0.185**	0.089	0.173**
	[P]	0.088	0.056	0.072	0.138**

* $p<0.05$
** $p<0.01$

Table 3

Table 3. Results of *t*-Student and Wilcoxon test of related groups [initial]/[final] for microbiological variables

Stage	[initial]/[final]	Variable	Test-*t* dependent groups	Wilcoxon Test
Packaging	[C]/[K]	Log Tc	-	0.835
		Log Ps	0.127	-
		Log Eb	0.254	-
		Log St	-	0.147
Classifying	[T]/[C]	Log Tc	0.000	-
		Log Ps	-	0.000
		Log Eb	-	0.000
		Log St	-	0.000
Air chilling	[W]/[T]	Log Tc	0.268	-
		Log Ps	-	0.587
		Log Eb	0.336	-
		Log St	-	0.398
Washing	[G]/[W]	Log Tc	-	0.000
		Log Ps	-	0.000
		Log Eb	0.000	-
		Log St	-	0.000
Gutting	[P]/[G]	Log Tc	-	0.000
		Log Ps	-	0.000
		Log Eb	-	0.000
		Log St	-	0.001
Plucking	[S]/[P]	Log Tc	-	0.000
		Log Ps	-	0.000
		Log Eb	0.630	-
		Log St	-	0.000

Table 4

Table 4. Results of ANOVA with two repeated measures factors: stage and microbiological variable

Stage	Factor	F	p	η^2
Packaging	Sampling point	4.997	0.029	0.068
	Microbiological variable (MV)	152.96	0.000	0.689
	Sampling point x (MV)	1.067	0.364	0.015
Classifying	Sampling point	291.501	0.000	0.739
	Microbiological variable (MV)	44.736	0.000	0.393
	Sampling point x (MV)	94.023	0.000	0.577
Air chilling	Sampling point	0.022	0.883	0.000
	Microbiological variable (MV)	114.683	0.000	0.631
	Sampling point x (MV)	1.088	0.355	0.016
Washing	Sampling point	71.290	0.000	0.512
	Microbiological variable (MV)	123.900	0.000	0.646
	Sampling point x (MV)	5.711	0.001	0.077
Gutting	Sampling point	70.911	0.000	0.507
	Microbiological variable (MV)	144.572	0.000	0.677
	Sampling point x (MV)	2.153	0.095	0.030
Plucking	Sampling point	5.504	0.022	0.074
	Microbiological variable (MV)	139.162	0.000	0.669
	Sampling point x (MV)	46.350	0.000	0.402

Table 5

Table 5. Kolmogorov-Smirnov-Lilliefors normality test ($|D_{max}|$) results

Variable	(PK)	(CL)	(CH)	(WH)	(GT)	(PL)
Log (ΔTc)	0.255**	0.331**	0.285**	0.353**	0.366**	0.375**
Log (ΔPs)	0.229**	0.288**	0.230**	0.261**	0.283**	0.293**
Log (ΔEb)	0.268**	0.260**	0.221**	0.230**	0.314**	0.267**
Log (ΔSt)	0.176**	0.133**	0.193**	0.255**	0.181**	0.224**

** $p<0.01$

Table 6

Table 6. Log of the mean of the increments of the microbiological variables between the final [FP] and the initial point [IP] for each stage.

Microbiological Variable	Stage					
	(PK)	(CL)	(CH)	(WH)	(GT)	(PL)
(±)Log (ΔTc)	-3.889[a]	4.343[a]	3.512[a]	-5.008[b]	5.360[c]	-6.768[b]
(±)Log (ΔPs)	2.484[a,b]	4.139[b]	-2.356[a]	-3.302[a]	3.128[b]	3.208[b]
(±)Log (ΔEb)	2.003[a]	3.187[a]	2.207[a]	-3.976[b]	4.050[c]	3.593[a]
(±)Log (ΔSt)	2.643[a]	3.092[a]	-3.169[a]	-3.180[b]	3.431[a]	-4.935[c]