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Original article

The effects of processing and packaging parameters on the occurrence of sulphide black in canned meat

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Summary The aim of this study was to evaluate the packaging and processing parameters that produce sulphide black in corned beef. Twenty experimental batches were prepared, and in order to evaluate the effect of the packaging manufacturing process, variations were introduced in the tin layer, in the cure of lacquer applied inside and in the presence or absence of superficial scratches in the lacquer. Processing parameters, such as sterilising time and temperature as well as filling temperatures, were evaluated. The visual evaluation carried out to check the occurrence of black spots and the statistical analysis of variance with 95% confidence interval showed that the lids that were produced with a greater total tin layer (5.6 g m^{-2}), with normal cure (200 °C) and without scratches had a lower occurrence of the problem. The analyses also showed that the use of filling temperatures lower than 40 °C decreased the presence of black spots.

Keywords Black spots, canned meat, corned beef, metallic packaging, sulphide black.

Introduction

Brazil is one of the world's largest exporters of beef. In 2011, 1 097 000 tons was shipped abroad, 10.8% less than the previous year. However, the revenue with total exports increased 11.65%, which was caused by 25.17% increase in the prices of the average exported beef, enabling Brazil to return to the levels of 2008. The decline in the exports may have been caused by the EU crisis, the Russian boycott to Brazil's beef exports and the US market, which imports processed meat from Brazil, and where residues of vermifuge above the permitted levels were found in 2010. Nevertheless, the prediction of growth in the exports of beef this year – 10% in the volume and 20% of the revenue – is based on the perspective of sales increase to the main markets such as Russia, which is expected to enable more slaughterhouses (Associação Brasileira Das Indústrias Exportadoras de Carnes, 2012).

Canned meat or corned beef has low acidity and subjected to a severe thermal treatment inside the package (commercial sterilisation) to make sure that it is preserved (Augusto *et al.*, 2010) . During the sterilisation process, which can last minutes or hours (for canned meat), high temperatures are used and may reach 125 °C for many minutes and the package must be resistant to such temperatures so as not to compromise its hermetic seal (Tribst & Faria, 2010). For thermoprocessed products, the metallic packaging is considered the most suitable. One of its most important characteristics is the hermetic seal, that is, this package ensures that the product does not interact with the external environment, preventing contact with light, oxygen and the penetration of microorganisms, preserving the commercial sterilisation and its contents after it is processed (Anjos, 1991).

High-protein food such as canned food, poultry, fish, legumes and leafy vegetables may be a source of sulphide black. The free hydrogen sulphide released by the degradation of proteins, when in contact with the metal from the packages, results in metal sulphides. The formation of tin sulphides (sulphide staining) produces the colours violet and brown, whereas iron sulphides (sulphide black) results in stains or black spots. The occurrence of sulphide black does not depend only on the packaging characteristics, such as the kinds of metal sheets and lacquer, but also on the product and the production process (Dantas *et al.*, 1999). According to Marsal (1989), the thermal treatment increases the sulphide staining or sulphide black in marine products. The long treatment produces a much greater quantity of hydrogen sulphide compared to the treatments carried out in short periods of time.

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The phenomenon of sulphide staining or sulphide black is more likely to happen with products with lower acidity, such as processed meat, as the dissociation of mercaptans (SH_2) in SH^- or S^{2-} depends on the pH. The more acid the pH is, the smaller the dissociation is. Furthermore, the thin sheet and the kind of lacquer are important factors as the steel offers conditions for the reaction of iron (Fe) with sulphur (S) to occur. As a rule, this occurs in deformity points, in the weld, in scratches in the lacquer or in protective layers.

The first stage of sulphide black formation involves sulphur-containing amino acids and takes place during the thermal processing. The thioproteins are degraded and release sulphur-containing amino acids, such as cystine, cysteine and methionine. In the second stage, the amino acids dissociate into sulphur compounds (SH^-, SH_2, mercaptans) for a later dissociation in S^{2-}. The FeS formed by the reaction between S^{2-} released in the dissociation and the Fe^{2+} ion, which is the oxidised form of the metallic material due to corrosion (Piggot & Dollar, 1963; Gruenwedel & Patnaik, 1971; Johnson & Vickery, 1964 in Khayat, 1977; Dantas *et al.*, 1999), precipitates in a dark compound located in some regions, either on the product or on the surface of the package (Fig. 1).

Mohan *et al.* (2008) observed a slight decrease in cysteine and methionine between raw and processed shrimp in aluminium cans processed at 121.1 °C for 52.53 min. The authors reported that the decrease was due to the heat-sensitive nature of these amino acids. Losses of sulphur-containing amino acids were detected by Kadidlova *et al.* (2010) in three types of ready-to-eat entrées with increasing storage time and temperature.

Corned beef is the processed product obtained exclusively with beef, which is cured, cooked, packaged, hermetically sealed, commercially sterilised and quickly cooled down. Corned beef can be obtained without adding cure agents to meet the commercial and legal requirements, as long as it does not compromise the food safety of the final product (Ministério Da Agricultura, Pecuária E Abastecimento, 2003). Beef, the main ingredient of corned beef, is one of the main products susceptible to the reaction, as it consists of about 3% of sulphur-containing amino acids such as cysteine and methionine. Brazil is a great exporter of canned food. However, the Brazilian product has been sometimes rejected by the agricultural authorities of such countries due to the presence of sulphide black, which makes new research into this relevant area.

The objectives of this research were to evaluate the parameters that give rise to sulphide black in corned beef and to find technological strategies that reduce or prevent its occurrence by analysing the influence of factors related to the processing and packaging conditions.

Materials and methods

Product

The corned beef was produced by using frozen industrialised meat from slaughterhouses under the federal inspection, and by adding liquid fat (approximately 1%), salt (2%), sugar (1%) and nitrite solution (0.06%). The meat was previously cooked and then grounded in discs with holes of 4 mm diameter.

The processing consisted of the following stages: cooking, mincing the raw material, formulation and homogenisation, packaging, double seaming, thermal treatment and the final product packaging.

The frozen meat was evaluated with regard to its centesimal composition and coliform count, *Escherichia coli*, psychrotrophic and *Staphylococcus aureus* according to the AOAC methods (2010).

Cans

Trapezoidal welded tin cans with easy-open bottoms were used (Fig. 2). The production consisted of two stages: preparation of tinplates by cutting coils, lacquering and curing and then the manufacturing of each can component (lid, body and bottom), as well as double seaming. The variables established for the can lids were the following: the tin layer of the internal face with 2.8 and 5.6 g m^{-2}, lids with superficial scratches and normal lids, as well as internal cured

$$\underset{\text{(amino-acids)}}{\text{R- SH}_2 \text{ or R- S- S- R ou R- S-R}} + H_2O \longrightarrow \underset{\text{(sulphur compounds)}}{SH_2 \text{ e } SH^-} + \underset{\text{(radical)}}{ROH}$$

$$SH_2 \longrightarrow SH^- + H^+$$

$$SH^- \longrightarrow S^{2-} + H^+$$

$$Fe^{2+} + S^{2-} \longrightarrow FeS$$

Figure 1 Amino acids dissociation.

Figure 2 Photographs showing the can (a), the bottom (b) and the lid (c) used in this study.

Table 1 Description of the experimental batches

Batches	Tin layer (g m^{-2})	Lacquer cure	Scratches	Filling temperature	Thermal treatment
A	2.8	Normal	No	35 °C	121 °C per 80 min
B	2.8	Normal	No	45 °C	125 °C per 65 min
C	2.8	Normal	Yes	35 °C	121 °C per 80 min
D	2.8	Normal	Yes	55 °C	125 °C per 65 min
E	2.8	Normal	No	45 °C	121 °C per 80 min
F	2.8	Super	Yes	45 °C	121 °C per 80 min
G	2.8	Super	Yes	35 °C	125 °C per 65 min
H	2.8	Super	No	55 °C	121 °C per 80 min
I	2.8	Super	No	35 °C	125 °C per 65 min
J	2.8	Super	No	45 °C	121 °C per 80 min
K	5.6	Normal	Yes	45 °C	121 °C per 80 min
L	5.6	Normal	Yes	35 °C	125 °C per 65 min
M	5.6	Normal	No	55 °C	121 °C per 80 min
N	5.6	Normal	No	35 °C	125 °C per 65 min
O	5.6	Normal	No	45 °C	121 °C per 80 min
P	5.6	Super	No	35 °C	121 °C per 80 min
Q	5.6	Super	No	45 °C	125 °C per 65 min
R	5.6	Super	Yes	35 °C	121 °C per 80 min
S	5.6	Super	Yes	55 °C	125 °C per 65 min
T	5.6	Super	No	45 °C	121 °C per 80 min

lacquer (200 °C) and supercured lacquer (220 °C).The internal face of the body and the bottom were made of 2.8 g m^{-2} tin layer, and the lacquer was epoxy phenolic with aluminium, cured under normal conditions. Powder lacquer was applied as side stripe.

Processing

After homogenising the ingredients, the product was disposed into the cans at 35, 45 and 55 °C, and the packed product was processed in vertical retorts under two time conditions and thermal treatment temperatures, 121 °C per 80 min and 125 °C per 65 min (company standards) with a minimum sterilisation value (F_0) of 8 min, meeting the requirements of safety and microbiological product stability, both according to the Brazilian and American legislation.

Experimental batches

Twenty experimental batches were prepared considering the effects of the packaging production and the processing conditions according to Piggot and Dollar (1963) and Marsal (1989). Table 1 shows the experimental batches prepared for the evaluation.

Characterisation of the metallic material used in the can production (tinplate with coating of 2.8 and 5.6 g m^{-2})

Tests for the characterisation of the metallic material (thickness, hardness, tin layer and roughness) were applied to six test pieces, according to the methods described in NBR NM ISO 6508-1 (2008), ABNT NBR 8481 (2008) and ABNT NBR 7407 (2009), respectively. The roughness of the internal metallic surface was evaluated in relation to the average roughness 'Ra', according to the methodology described in Dantas *et al*. (1996).

Characterisation of the internal lacquer used in the can production (cured at 200 °C and supercured at 220 °C)

The tests for the characterisation of the internal lacquer were carried out in the side stripe and in tinplates, previously lacquered and intended for the manufacturing of the can components (body, lid and bottom). Five test pieces were evaluated from each region, according to the methodology described by Faria *et al*. (1993), Dantas *et al*. (1996), ABNT NBR 15660-1 (2008) and ASTM D 3359 (2008). The porosity was determined by the electrochemical method (WACO Enamel Rater Test, Chicago, IL, USA), and the lacquer was identified by Fourier transform infrared spectrophotometer (Perkin Elmer, Spectrum 100, Beaconsfield, BUCKS, UK).

Evaluation of the cans with the product

Thirty-two cans and the respective product blocks were visually evaluated regarding the presence of sulphide black in different regions, according to the classification presented in Table 2.

The filling condition was evaluated in relation to its net weight, volume of gas and the gaseous composition in the headspace in thirty-two cans, eight cans and five cans, respectively. The net weight was determined according to the methodology described in Zenebon and Pascuet (2005), and the volume in the headspace and dissolved in the product was analysed according to the methodology described in Dantas *et al*. (1996). The gaseous composition in the headspace (oxygen and hydrogen) was determined by using a chromatograph (Agilent, model 7890, Waldbronn, Germany), and the results were expressed in volume of gas (% v/v) (Dantas *et al*., 1996). The gaseous composition was determined in nine experimental batches (A, E, G, I, K, M, Q, R, and S), considering at least one determination for each processing condition.

The results obtained in the visual evaluation of the can with the product were submitted to analysis of variance (ANOVA) with 95% confidence interval, by using the program called MINITAB version 15.1.20.0 [S.I.]; Minitab-Inc., State College, PA, USA. The results of the net weight and the vacuum were statistically analysed by analysis of variance, and the averages were then compared with Tukey's test, considering 95% confidence interval, through the MINITAB program, version 15.1.20.0 [S.I.] (Minitab-Inc.), and STATISTICA(data analisys software system) version 5 (StatSoft. Inc., Tulsa, OK, USA). Where applicable, results are presented as mean ± SD.

Table 2 Classification of the sulphide condition on the internal faces of the can lids and/or the product

Degree	Description
0	Absence of spots or stains
1	Presence of up to three small spots and/or a dark line
2	Between four and nine small spots and/or one big spot
3	Between ten and fifteen small spots and/or up two smaller stains or one big stain

Results and discussion

Product

The meat used in the production of the experimental batches was in accordance with the resolution RDC n. 12, 2 January 2001, by National Health Surveillance Agency. As regards the microbiological analysis, it showed hygienic and sanitary conditions and adequate product handling (Brasil, 2001). The centesimal composition remained close to the reference values, and the fresh meat's intrinsic features were preserved (73% of water, 21% of protein and 6% of lipids) (Lambert *et al*., 1991 in Nishi, 2008), as well as the parameters of water activity, pH, humidity, fat and ash. Therefore, the raw material used in the production of the experimental batches had characteristics that met the requirements demanded in the corned beef production.

Characterisation of the metallic material and internal lacquer used in the can production

The samples were within the permitted tolerance in accordance with the specific norms for each sample (ABNT NBR 6508-1: 2008; ABNT NBR 8481: 2008; ABNT NBR 7407: 2009; and Almonacid *et al*., 2012; ABNT NBR 6665: 2010). Similarly, regarding roughness, the body sample may be classified into bright finish, and the lid and bottom samples into stones finish.

The lacquer present in the can components were identified as epoxy phenolic aluminium for the lid and the body, and organosol aluminium for the bottom and the side stripe. The adhesion of the internal lacquer on the lid, body, bottom and side stripe samples was excellent. However, the supercured samples had detachment of the lacquer, and the lid with the biggest coating (5.6 g m^{-2}) had the worst performance. The cure degree evaluated by the absorption of dyes showed that the higher cure temperature results in a larger grouping of lacquer molecules, reducing the dye absorption (Fig. 3). The porosity of the supercured cans was superior to that of the normal cure cans, with average values 162.6 ± 51.5 mA per can and 127.7 ± 56.7 mA per can, respectively, which can be attributed to a bigger susceptibility to the discontinuity of the lacquer caused by reduced flexibility. Dantas

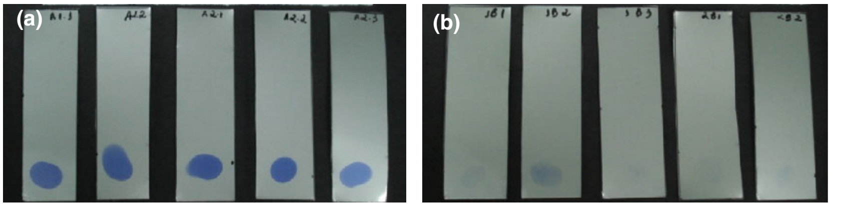

Figure 3 Photographs showing the test pieces after the test of the cure degree by absorbing the dyes (methyl violate): (a) lacquer with normal cure (200 °C), (b) super-cured lacquer (220 °C).

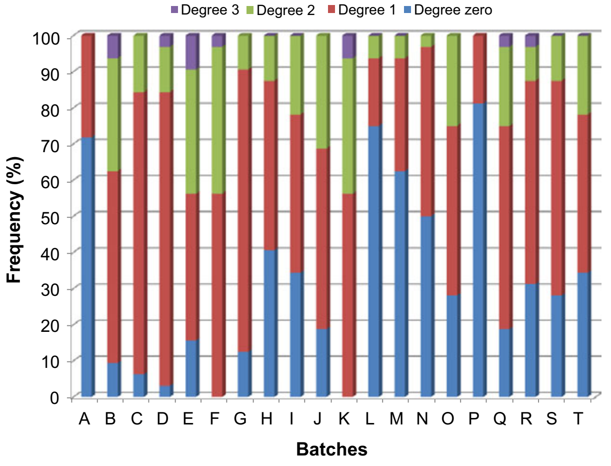

Figure 4 Frequency of sulphide black occurrence in each degree established in Table 2.

et al. (2011) found porosity in the internal lacquer in pea cans of 63.2 ± 5.3 mA per can for the lacquer identified as oleoresinous and with a suitable cure degree, showing that the results found for the trapezoidal tin cans were high. For aggressive products such as tomato sauce, Dantas *et al.* (2012b) found internal lacquer porosity of 47.6 ± 13.9 mA per can for the lacquer identified as epoxy phenolic with an adequate cure degree.

Evaluation of cans with the product

The visual evaluation of the product and the cans after the thermal processing made it possible to check that the twenty experimental batches showed sulphide black in different degrees, as presented in Figs 4 and 5.[1] The experimental batches with higher frequency of zero degree cans, that is, with the absence of black spots or black stains, were the batches identified as P, L and A, with more than 70% of evaluated units without the presence of black spots, followed by batch M with more than 60% of intact units. The batches identified as L, M and P have in common the tin layer used in the lid production of 5.6 g m^{-2}, and the batches A, L and P were filled at 35 °C. Sulphide black in Degree 3 was checked in batches identified as B, D, E, F, K, Q and R, which, with exception of batch R, were filled at 45 °C (5 batches) and 55 °C (1 batch), indicating that the intermediate filling temperature presented the worst performance. This fact can be accounted for the denaturation of myofibrillar proteins that start at temperatures between 30 and 32 °C, go on to the grouping stage between 30 and 40 °C and achieve the stage of gelation between 45 and 50 °C (Lantto, 2007 in Montezuma, 2010), that is, probably at temperatures between 40 and 45 °C, the sulphur ion from the sulphur-containing amino acids presents more availability. The lid scratches had a more significant influence on the batches produced with 2.8 g m^{-2} tin sheets, in which the coating thickness is directly proportional to the weight per area, that is, steel is more easily hit by the friction of the sharp material. In the cans produced with 5.6 g m^{-2} tin, the influence of the scratches was also observed. However, the frequency of the occurrence of zero degree units was higher compared to the cans produced with a small layer.

The good performance observed in batch A cans with regard to sulphide black may be accounted for a failure during its production. Unlike the other nineteen experimental batches, sodium chloride (NaCl) was not added to the batch A product. Thus, the dry mixture that should have sodium chloride and sugar was prepared only with sugar. This fact indirectly provided us new and relevant information about the salt influence on the problem under study. Adding sodium chloride is important because it works directly in the microstructure of the meat, in the solubilisation of myofibrillar proteins and consequently, in the water-binding capacity (WBC). Montezuma (2010) reported that the restructuring method applied in the corned beef and the previous cooking without adding salt result in denaturation and insolubilisation of proteins, as well as the gelation of the connective and collagenous muscle tissue, and aimed to reduce the mechanical effort in the compacting of the product in cans. Montezuma (2010) also claimed that the product restructuring starts at this stage and that it will be complete in the thermal process with the gelation of tendons and the distribution of gelatin formed in the pieces of the muscle.

[1]The frequency of sulphide black occurrence in each degree provided in Table 2 was calculated based on the evaluation of thirty-two cans and product blocks for each batch.

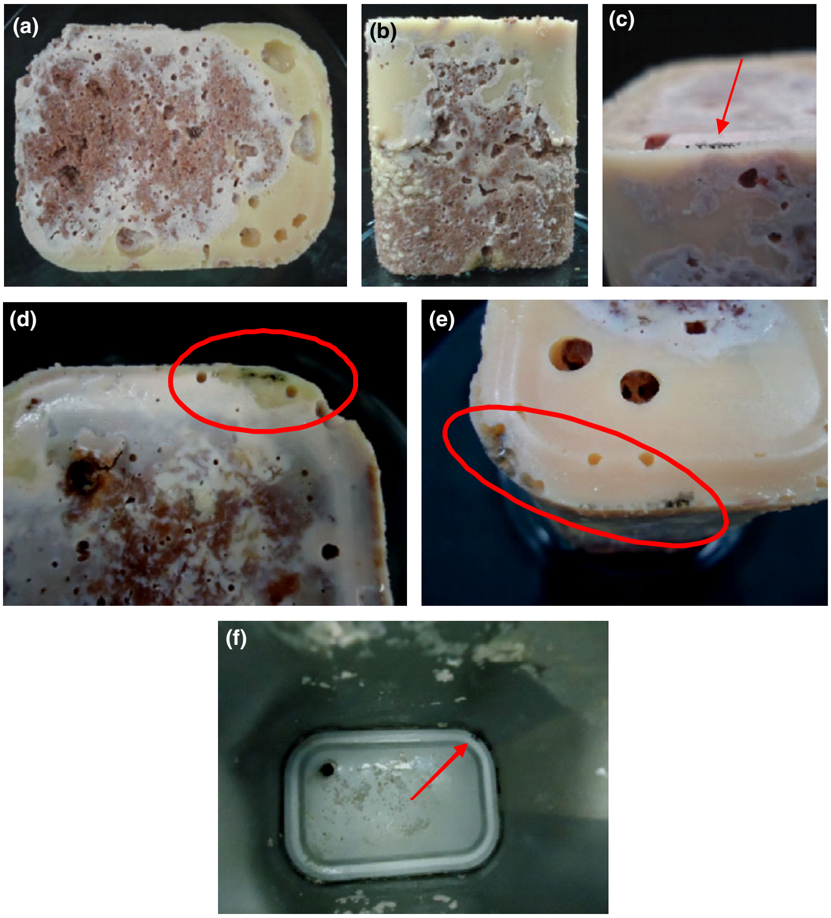

Figure 5 Photographs showing the degrees given to stains or black spots: (a) and (b) zero degree, (c) Degree 1, (d) Degree 2, (e) Degree 3, (f) impression of stain Degree 3 on the can.

Almonacid *et al.* (2012) claimed that salt increases the solubilisation of proteins and the water-binding capacity increases protein–protein and water–protein interactions.

Without adding salt, the proteins stay away from the isoelectric point and remain not soluble in the mixture as they are soluble only in saline solutions. Hamm e Hofmann (1965) in Khayat (1977) found that nearly all H_2S produced during the thermal treatment come from the structural muscle proteins, and not from water-soluble compounds, such as cysteine. It is likely that the nonsolubilisation of proteins continued during the thermal treatment and avoided the release of sulphur–containing compounds, which are necessary for the sulphide black to occur. On the other hand, this is not a valid resource to avoid sulphide black as sodium chloride is an ingredient that is in the identity standards and product quality. Currently, several studies are being carried out with a view to reducing the quantity of sodium in foodstuffs due to its negative effects on health, such as the increasing risk of high blood pressure. However, the total substitution faces challenges due to the change in taste in processed meat

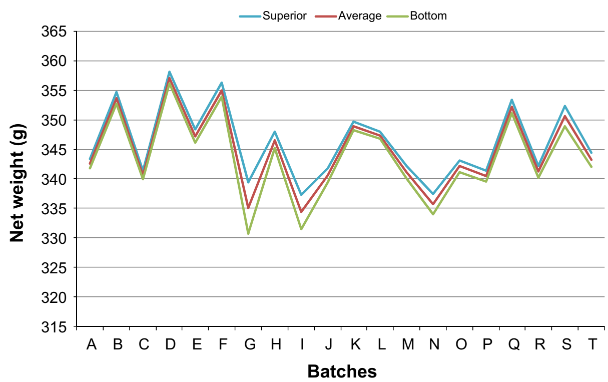

Figure 6 Average results and the interval of 95% reliability in the net weight determined in the twenty experimental batches.

(Jimenez-Colmenero *et al.*, 2001). Anyway, the effect of sodium chloride stems from the negatively charged Cl^- ions, which are more strongly linked to proteins, rather than the positively charged Na^+ ions.

Another fact that influenced the development of stains or black spots was the net weight. The graph in

Fig. 6 presents the average results obtained for each experimental batch, as well as the interval with 95% of confidence. It can be noted a difference higher than 20 g in the results of the averages in the batches, while for batch G this difference was higher than 40 g. Montezuma (2010) found a variation of only 3 g, between 340 and 343 g, in batches produced with the same product in the same can. The batches with a higher frequency of cans with stains or black spots classified into Degree 3, namely B, D, E, F, K, Q and R, also presented high product weights, differently from batches A, L, M and P, which had lower weights. The ANOVA ($P < 0.05$) and the Tukey test ($P < 0.05$) showed no significant difference in the results of batches B, D, E, F and K and in the results of batches A, L, M and P. A larger quantity of the product may be able to reduce the headspace, although it can introduce a larger quantity through the oxygen incorporated in the product in the mincing and mixing process. In addition, it can hinder the product compression in the canning process and, consequently, the removal of occluded air in the product mass.

Almonacid *et al.* (2012) analysed the loss of water and, consequently, loss of weight and nutrients in canned mussels. They found that a shorter period of time in the thermal processing (precooking and sterilisation) increased significantly the capacity of the processing plant as well as the weight of the commercialised product, which reduced losses.

The results of oxygen concentration in the cans' headspace showed that batches G and I (filling at 35 °C and processing at 125 °C per 65 min) had lower concentrations on average, respectively, 1.15 ± 0.38 and 1.86 ± 0.27 in gas percentage (v/v). Batches A (35 °C and 121 °C per 80 min), E and K (45 °C and 121 °C per 80 min) showed average concentrations of $2.20 \pm 0.45\%$, $2.33 \pm 0.70\%$ and $2.72 \pm 0.15\%$, respectively. Batches R (35 °C and 121 °C per 80 min), Q (45 °C and 125 °C per 65 min), S (55 °C and 125 °C per 65 min) and M (55 °C and 121 °C per 80 min) showed the highest average results: $3.10 \pm 1.19\%$, $3.60 \pm 1.32\%$, $3.76 \pm 1.57\%$ and $4.27 \pm 1.28\%$, respectively. Thus, these results may indicate that the filling at 35 °C resulted in lower residual oxygen inside the cans. The oxygen depolarises, accelerating the corrosion process and, consequently, the sulphide black reaction. The percentage of hydrogen found in all evaluated batches was below what is considered in other gases in the average air composition (1%), indicating that there was no corrosion at an advanced stage inside the evaluated cans.

Figure 7 shows the results of gas volume in the headspace and dissolved in the product. Considering the concentration of oxygen, in gas percentage (v/v), and the determined volume of air, it is possible to calculate the volume of oxygen inside the cans (Table 3).

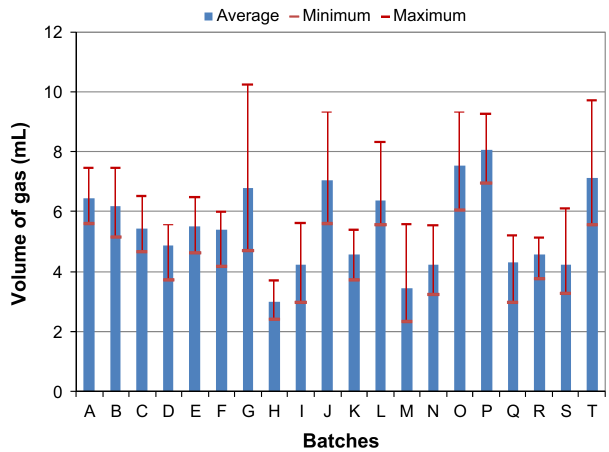

Figure 7 Average results, minimum and maximum volume of gas determined in twenty experimental batches.

Table 3 Average results of air volume and oxygen in corned beef cans

Batches	Air volume (mL)	O_2 volume (mL)
A	6.44	0.14
E	5.50	0.13
G	6.78	0.08
I	4.23	0.08
K	4.59	0.12
M	3.45	0.15
Q	4.32	0.16
R	4.58	0.14
S	4.22	0.16

Considering that during the production of the experimental batches, the whole mass of the product was minced and mixed into the ingredients in the same period of time (7 min), it is possible to conclude that the parameter of greater influence is the filling temperature as the smaller volumes of oxygen inside the cans were checked in batches G and I, both of which had filling temperatures of 35 °C.

The vacuum determined in cans of all experimental batches had a variation of −0.09 psi (0.0006 MPa or 0.18 pol Hg) for batch I to + 0.12 psi (0.0008 MPa or 0.24 pol Hg) for batch J; in other words, there is no statistically significant difference of 95% confidence interval in the results. The formation of vacuum relies mainly on the product temperature during the filling-up to the closing of the package, meaning that the higher the temperature, the higher the vacuum. In addition, the filling level of the package has also an influence. In summary, for the same product temperature, the same quantity and the same closing condition, the higher the vacuum, the smaller the volume of headspace will be, as long as a minimum value is kept. In this study, there was no headspace in the analysed cans, which is a common practice in the companies so as to eliminate the oxygen and avoid problems such as the fat darkening.

The visual evaluation has also allowed us to observe that the region with higher incidence of stains or black spots is the curvature or cover radius (65% of the occurrences). This result indicates that the double seam area, where there is greater mechanical stress lacquer, should receive more attention during the stages of lacquer specification, application to the metal substrate and closing. 59% of cans that presented black stains had the scratches on the lids.

The thermal treatment of canned products, whose heat transfer happens by conduction, results in superprocessing of its edges for security assurance of the cold point (Augusto *et al*., 2010). This severe treatment, or rather, overcooking, of the edges can also explain the higher incidence of stains or black spots in the curvature of the cover. Thus, the thermal treatment carried out in a shorter period of time may minimise such effect.

Dantas *et al*. (2012a) analysed canned cubed meat in 6 lb (2 kg) packages and found that high temperatures of the brine and a small headspace decreased the occurrence of sulphide black. Unlike the corned beef, the product evaluated by Dantas *et al*. (2012a) shows cover liquid or brine, whose heat transfer behaves initially like a convective heating system. Therefore, the difference in the effect on the sulphide black in the filling temperature in both studies shows the relevance of such research for the various kinds of products.

Conclusion

This study was undertaken in order to evaluate the effect of processing and packaging on the occurrence of sulphide black in corned beef cans and showed that the higher total tin layer (5.6 g m^{-2}) and the standard cure condition of the lacquer had a positive influence on the reduction in the problem. However, the presence of superficial scratches in the lacquer, the failure to control the product weight and the milder temperature in the sterilisation increased its occurrence. Using filling temperatures below 40 °C decreased the presence of black spots, did not influence the vacuum or resulted in a defect called Flipper, showing that for this product it is possible to reduce the filling temperature. Thus, to avoid sulphide black in canned food containing sulphur amino acids, it is necessary to ensure low tin layer porosity in metallic material, the correct specification and quality application of the lacquer, a minimum vacuum in the head space and the use of HTST thermal treatment.

Acknowledgments

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