Natural levels of nitrites and nitrates in San Daniele dry cured ham PDO, and in meat, salt and sugna used for its production

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Summary

The aim of the study was to determine the level of the “natural” nitrite and nitrate concentration in raw meat, salt and *sugna* (soft pork fat) used to produce San Daniele dry cured ham (SDDCH) and in SDDCH (PDO) that has been ripened over 14 months under controlled environmental conditions. The average natural nitrite content in meat, salt, *sugna* and dry cured ham was approximately 2, 1, 5 and 1 mg/kg, respectively. The natural nitrate content was 8, 6, 8 and 4 mg/kg. Data allowed to determine threshold value for both compounds: the nitrite and nitrate concentrations in San Daniele PDO ham must be considered “natural and not intentional added” when they are less than 4 and 22 mg/kg, respectively.

The underlying aim of the research was to enable producers to prove no additives were deliberately added during the ham production and to help authorities to identify SDDCH not compliant with the rules.

Key words: Nitrite, Nitrate, Natural Concentration, Threshold Value.

Introduction

San Daniele dry cured ham (SDDCH) is a typical meat product, made with Italian pork (thigh), which is salted and ripened for at least 13 months (Comi & Iacumin, 2012), when it reaches a particular delicate aroma and flavor (Kim *et al.*, 2016; Neethling *et al.*, 2016; Comi & Iacumin, 2012). In many areas of the world its popularity is consistently increasing. Italy has promoted the organic protection of this product since 1970, and in 1990 approved a new protection law - No. 30 (February, 14th 1990; Denominazione di origine del prosciutto di San Daniele, GU n. 45 del 23.2. 1990). Subsequently, the European Union registered the San Daniele ham as PDO with Reg. (CE) n. 1107/1996. Currently, the Reg. (UE) n. 1151/2012, establishing community protection for agri-food products with a designation of origin, has reinforced, adapted and developed schemes to identify quality of European products and foods. The processing phases, which derive from ancient artisan tradition, are reported in the PDO regulations and in the Ministerial Decree of February, 16 1993, No. 298. The processing stages consist of: the choice of meat, cooling, trimming, massaging, salting, pressing, pre-ripening and rest, tempering and washing, drying, pre-ripening and ripening (Comi & Cattaneo, 2007). The fresh thighs of heavy pig (150-180 kg) include the “zampetto” (foot), as codified in art. 25, Co. 1 of Law n. 30/1990, which is left on the ripened product and constitutes one of the characteristics of the SDDCH. The SDDCH Consortium monitors the most suitable thighs and applies a pre-mark and the complete date of production start (Comi & Iacumin, 2005, 2012). The thighs are then processed and salt and the dehydration/ripening phases are the only parameters influencing their stability and safety. Nitrite and nitrate which are commonly used to produce and maintain the red color of meat, to produce characteristic flavors, prevent fat oxidation and the development of pathogenic and spoilage microorganisms in order to improve the organoleptic, sensorial and hedonic characteristics in cooked and dry cured meats over time (Comi & Iacumin, 2012; Comi & Cattaneo, 2007; Toldrá, 2007), are not allowed to be used in SSDCH. Salt is the only other ingredient permitted to achieve stability and safety.

In dry cured ham, the loss of moisture, ripening and salt prevent the development of any spoilage or pathogenic microorganisms (Comi & Iacumin, 2012, 2005; Comi & Cattaneo, 2007). The lack of nitrites does not affect the typical red color of the meat, which remains stable because of the negative redox potential of the meat.

Given that nitrates have been detected in some commercially PDO SDDCHs, the aim of our work was to verify if this undue presence could be attributable to raw meat and to the other permitted ingredients. So, we determined both in SDDCH ripened over 14 months and in the only ingredients used in the production of SDDCH, meat, salt and *sugna*, the levels of nitrites and nitrates. *Sugna* is the typical paste prepared with rice flour, soft pork fat, and ground peppercorn (pepper), spread on the muscular area not covered by rind, which promotes homogeneous dehydration during the last phase of the production of San Daniele dry cured ham. An additional purpose was to define the threshold values of nitrite and nitrate concentrations naturally present, and to identify any deviations from the permitted standards. This is because, over the years and especially at the level of foreign markets, hams sold as "SDDCH" sometimes showed to have similar levels of both compounds to those of sausages in which their use is allowed.

Materials and methods

The natural content of nitrites and nitrates in SDDCH processed according to the strict protocol of the SDDCH Consortium and in the ingredients used in its production was determined. 'Natural' means that both compounds were not deliberately added by the producers during the ham production. The analyzed samples were strictly taken from PDO branded hams ripened over 13 months of different companies located in San Daniele (a municipality in north east Italy).

The investigated samples thus included:

50 slices (about 500 g each) corresponding to fifty SDDCH hams of different ripening times (22 hams with a ripening period of 14 months, 10 of 15 months, 10 of 16 months, 7 of 17 months and 1 of 19 months).

50 samples (about 500 g each) of pork meat derived from thighs used in SDDCH production;

3 samples of *sugna* (about 300 g each);

10 samples of food salt (300 g each) taken from different production facilities and belonging to 10 different lots.

Moisture was determined in meat and SDDCH samples according to AOAC (1995) in order to express the nitrites and nitrates concentrations on dry weight. Aw was determined on the SDDCH samples, to verify the conformity of the product to the Consortium standards, using an AquaLab CX-2 Steroglass (Pullman, WA, USA).

The nitrite and nitrate detection was carried out following Mirna and Schutz (1972), modified according to AOAC (1990). This method is widely used for the determination of nitrites and nitrates in food products (meat and meat products, milk and cheese, vegetables, and drinking and waste water). It is more productive than other colorimetric methods because it has a detection limit in meat of 0.05 mg/kg. The United States Environmental Protection Agency (EPA, 1993) recommends a similar method for nitrite and nitrate determination based on a colorimetric reaction using a cadmium reduction column, which has been validated by various international standardization organizations (Table 1). It is a spectrophotometric method, which is therefore cheap, easy to use, does not require special or expensive equipment (such as HPLC) and can be applied to various matrices (plants, meat products, baby foods, dairy products and surface waters).

In brief, the method involves a hot extraction of the sample with water, which prevents any interference due to ascorbic acid or other reducing agents. Subsequently, as stated by EPA (1993) "the sample is filtered and passed through a column containing granulated copper - cadmium to reduce nitrate to nitrite. The nitrite (that was originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically at 540 nm. Separate, rather than combined nitrate-nitrite, values are readily obtained by carrying out the procedure first with, and then without, the Cu-Cd reduction step”. A calibration curve is also periodically performed. Nitrites and nitrates were expressed in mg/kg as sodium nitrite and sodium nitrate.

Results

The results of the moisture determination in meat, ham and *sugna* samples are summarized in Table 2. These values were investigated in order to formulate the nitrite and nitrate concentrations with respect to dry weight. Figures 1 and 2 show the percentage of moisture and the Aw correlated to the ripening period. As shown, all the samples had Aw levels lower than 0.92. The moisture values were variable and were not correlated to the different ripening ages. All were below the maximum limit allowed by the SDDCH Consortium (63%).

Salt and *sugna* can naturally contain nitrites and nitrates, the former as impurity, the latter mainly due to ground peppercorns, and consequently can potentially "contaminate" meat and hams.

Therefore, the presence of both compounds was investigated in three *sugna* and in ten salt samples, obtained from different ham factories (Tables 3 and 4). In *sugna*, the average nitrite and nitrate concentrations were 5 ± 0.30 mg/kg and 8 ± 5,2 mg/kg, respectively; nitrates showed a greater variability compared to nitrites. In fact, the average nitrate concentration varied from a minimum of 4 to a maximum of 14 mg/kg (Table 3). The concentrations of nitrites and nitrates in food salt were also low (Table 4), on average 1 ± 0.8 mg/kg and 6 ± 2.9 mg/kg, respectively. Therefore, the contribution of salt to the concentrations of both compounds in SDDCH was theoretically very low, even considering that at the end of ripening the salt concentration can vary from 4.4% to 7.1%, as calculated on the basis of the limit values of the salt/humidity ratio imposed by PDO legislation.

The average concentrations of natural nitrites and nitrates of fresh meat were 2 ± 0.9 mg/kg and 8 ± 3.4 mg/kg, respectively; the range was quite wide (Table 5). Finally, the average nitrite and nitrate concentrations in SDDCH were 1 ± 0.5 mg/kg and 4 ± 3.1 mg/kg, respectively (Table 6). Furthermore, the values found in the 50 samples of SDDCH were lower than those observed in fresh meat.

This could be explained by the fact that the analyzed meat was not the same as that used in the production of the sampled SDDCH and that the natural levels depend on different factors such as feeding, water and farming conditions. Moreover, it could be hypothesized that during SDDCH production, some nitrites and nitrates are lost when exudates and residual blood drip from the meat surface, and some link to myoglobin to form nitrosyl-myoglobin.

The values obtained in fresh meat are also similar to those determined in farmed salmon meat (1-2 mg/kg of nitrites and 4-6 mg/kg of nitrates, unpublished data). Given that the ingredients can provide, albeit minimally, nitrites and nitrates, that their content can be concentrated by dehydration and that the loss of water and exudates can partially eliminate them, we thus investigated the threshold values of natural (non-added) nitrite and nitrate.

Table 6 shows the variability of nitrite and nitrate concentrations, observed in SDDCH. The data include minimum and maximum values, mean, median, standard deviations and fiducial confidence intervals of the means ($p < 0.05$ and $p < 0.01$). The maximum limit of the fiducial interval is about 1 mg/kg for nitrites in SDDCH ($p < 0.01$) and is below the maximum value observed in the 50 SDDCH samples (2 mg/kg). For nitrates, the maximum limit of the fiducial interval is about 4 mg/kg ($p < 0.01$) and it is below the maximum value observed in the 50 SDDCH samples (11 mg/kg).

We thus decided to double the observed maximum values and to consider them as threshold values. The threshold values, we proposed, appear to be mostly below the levels found in some counterfeit raw hams products of unknown origin and marked as SDDCH PDO, collected worldwide by the SDDCH Consortium. Therefore, accepting these threshold values, there is no risk of negatively judging either raw meat or SDDCH products with concentrations higher than the nitrite and nitrate average values, due to natural variability. Irrespectively of the ripening time and the concentration ranges of both compounds in the SDDCH samples, a SDDCH could be identified as PDO when the nitrite and nitrate concentrations do not exceed 4 mg/kg and 22 mg/kg, respectively (Table 7). This table shows the nitrite and nitrate threshold values proposed for raw meat, *sugna*, and salt, allowing producers to test and accept ingredients for SDDCH PDO production.

4. DISCUSSION

Dry Cured Ham is one of the main meat products obtained with a wide variety of ingredients and technologies, which influence texture and aroma. Nitrites and nitrates represent the main preservatives of meat products, but for SDDCH production their use is forbidden. However, their presence has often been highlighted at levels of 60-70 mg/kg in several dry cured hams labelled "SDDCH PDO”. The aim of the work was thus to verify the natural concentration of such substances in raw materials (meat, salt), technological adjuvants (*sugna*) used to produce SDDCH and in SDDCH ripened over 13 months.

During the dehydration and ripening phases, a slow but progressive loss of moisture, Aw reduction and increase of the salt concentration are observed, which stabilize the ham (Comi & Iacumin, 2012) and influence the enzymatic activity and consequently the sensorial characteristics of the final product (Jiménez-Colmenero *et al.*, 2011). A wide Aw and moisture variability is observed in the tested SDDCH, not dependent on the ripening times. In fact, various hams with different drying times, ranging from 14 to 19 months, have the same Aw and moisture. This is due to differences in the raw meat and in the processing conditions applied, which may vary between one production facility site and another, even if the same protocol is applied. However, this large variability does not affect the wholesomeness and the stability of the hams (Cviková *et al.*, 2016; Kunová *et al.*, 2015; Parolari *et al.*, 2009). In particular, regardless of the ripening time, the Aw of the tested hams was less than 0.92. For this reason, they should be considered as being healthy, edible and complying with the SDDCH Consortium rules (Comi & Iacumin, 2012; Comi & Cattaneo, 2007).

Salt is the key ingredient for the production of hams. In fact, salting is the first step in ham production (Martínez-Onandi *et al.*, 2016) and in particular in SDDCH. Sea salt, medium-grain wet, is used without the addition of other ingredients such as nitrite and nitrate. The production specifications of the PDO only include salt without nitrite. Salt inhibits the development of spoilage and/or pathogenic microorganisms and solubilizes the soluble salt proteins, which are then degraded by the tissue enzymes responsible for ripening. In fact, salt activates these enzymes and especially cathepsins D (Toldrá, 2007).

However, salt can contain nitrite and nitrate as an impurity, but our data showed that the presence of nitrite and nitrate impurities in salt does not affect their concentration in the final product. In fact, considering the levels of the two compounds and the percentage of salt in the SDDCH (up to 6-7%), salt appears to increase the nitrite and nitrate concentration of about 0.1 mg/kg and 0.4 mg/kg, respectively. This increase is not significant respect to the natural levels of nitrite and nitrate of pork meat. However, it was important to find a threshold value for the concentration of nitrites and nitrates for salt as well. This value is 6 mg/kg and 24 mg/kg, respectively.

A threshold is also needed for *sugna*. Our results revealed in *sugna* a low content of both compounds, but higher than in salt. However, the contribution of *sugna* is less than that of salt because the contact between *sugna* and the ham is only on the muscular area not covered by rind and occurs in a phase where Aw is low and diffusion is slow. For *sugna*, we established a threshold of 10 ppm for nitrite, and 28 ppm for nitrate.

Fresh meat may contain natural nitrites and nitrates (Iammarino *et al.*, 2013; Iammarino & Di Taranto, 2012), as well as meat products treated only with salt and sugar and organic meat products not treated with nitrate substitutes (Sebranek & Bacus, 2007.) Data found about the presence of both the additives in the investigated fresh meats were similar to those obtained by Sebranek & Bacus (2007) and very lower compared to those found in meat products treated with nitrates and nitrites by various authors **(**Armenteros *et al.* 2012; Cantoni & Bianchi Paleari, 1980).

The natural origin of nitrites and nitrates in meat is due to the nitrogen metabolism of the animal and the feed. In mammals, nitric oxide derives from the degradation of arginine through the action of the enzyme NO-synthase (Hibbs *et al.*, 1992). In the presence of oxidized haemoglobin or the enzyme superoxide dismutase (Benjamin & Collins, 2003) the nitrogen oxide is then oxidized at cellular level to nitrite or nitrate, and these are eliminated via urine or faeces and/or partly retained in the body. In fact, humans and animals eliminate more nitrate than they actually ingest (Mitchell *et al.*, 1916). Nitrites can also derive from vegetables, used for feeding pigs once ingested, the nitrates are reduced by bacteria, saliva or by endogenous nitrate reductase into nitrite (Li *et al.*, 1997).

Nitrite and nitrate are also ingested directly in this form with feed and food. During the vegetables preservation, bacteria, such as Staphylococci, Micrococci and Streptococci, grow and reduce nitrate to nitrite (Benjamin & Collins, 2003; Li *et al.*,1997). This explains why nitrite and nitrate were found in fresh meat used for SDDCH production. Thus, according to our data, it was possible to suggest a threshold limit for nitrite and nitrate in pork meat suitable for SDDCH production; consequently, the PDO SDDCH producers can accept fresh meat, when the nitrite concentration is below the threshold values of 14 mg/kg and nitrates below 42 mg/kg. In this case, the threshold values expressed on the dry weight (44 mg/kg for nitrites and 166 mg/kg for nitrates) may also be useful to avoid disputes related to the degree of meat moisture. In fact, moisture can vary due to fat content and pig genetics, slaughtering techniques, ageing, time and temperature of meat storage before salting. A similar admissible threshold value of about 30 mg/kg of nitrates in fresh pork or bovine meat and of 40 mg/kg in fresh horse meat were also suggested by other Italian researchers (Iammarino et al., 2013; Iammarino and Di Taranto, 2012).

The most important part of our work concerned the evaluation of the "natural" concentration of nitrites and nitrates in SDDCH. The aim was to discover threshold levels of their presence in order to prevent any illegal additions. In this regard, to avoid discussions concerning the correlation of the concentration of such compounds and the level of ripening of the ham, we formulated a threshold value. Beyond this value, the determined levels can be considered as not having a natural origin, but rather the result of an intentional addition or, in the case of salt and *sugna*, not suitable for use in this production.

Our proposal is in line with several authors who have extensively studied the evolution of nitrites and nitrates in cured meat during its ripening (Sebranek & Bacus, 2007). It has been reported that after an addition of nitrate in concentration of 150 mg/kg to pork and/or bovine meat, a clear decrease of the nitrate is observed by its reduction to nitrite. In any case, the average residual concentration of both is higher than the "natural" concentration. In particular, after their addition, the values found are always higher than 40 mg/kg (nitrite) and 26 mg/kg (nitrate) (Cantoni & Paleari, 1980). This residual concentration can increase when the initial addition is higher than 150 mg/kg of nitrate or equal to 250 mg/kg of a mixture of nitrite and nitrate (Hospital *et al.*, 2017; Armenteros *et al.*, 2012; Comi *et al.*, 2005).

Finally, the method used for the determination of nitrite and nitrate was proven to be valid, efficient, inexpensive, simple and easy to apply and it is recommended by various International Standard Method Organization (EPA, 1993; ISO, 2006; AOAC, 1995).

5. Conclusions

The data obtained highlight that the concentration of nitrite and nitrate in SDDCH PDO must be considered natural when it is, respectively, less than 4 and 22 mg/kg of the ripened product, irrespectively of the ripening time (14-19 months).

The threshold values can be used to determine when SDDCH can legitimately receive the PDO mark or alternatively be designated as Dry Cured Ham (national, foreign ham, etc.).

In our opinion, there is in any case no need to add nitrite or nitrate salts in SDDCH production, because salting at refrigeration temperature, dehydration and subsequent ripening already provide a stable product characterized by low Aw (≤ 0.92) and a uniform and widely acceptable color.

We also suggest to accept raw meat with nitrite and nitrate concentrations below the threshold values of 14 mg/kg and below 42 mg/kg, respectively. The threshold values expressed on dry weight (44 mg/kg for nitrite and 166 mg/kg for nitrate) may also be useful to avoid disputes related to the degree of moisture of the meat.

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Conflict of interest

The authors declare that they have no conflict of interest.

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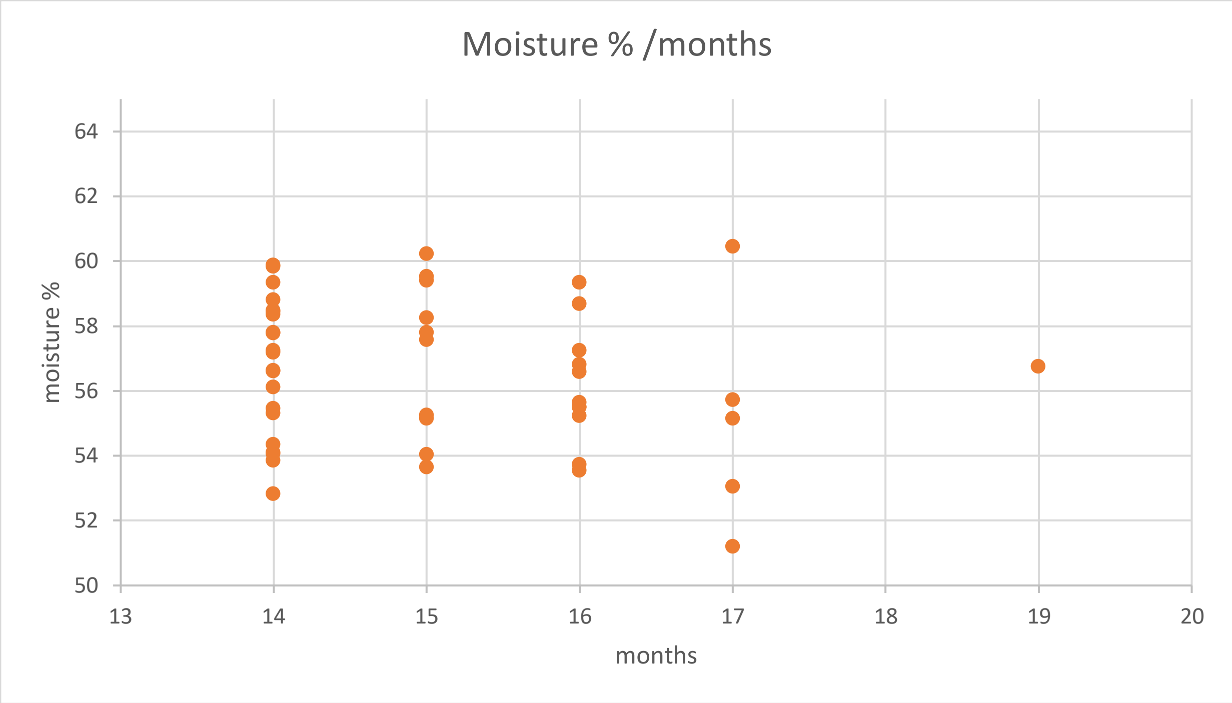

Figure 1: Correlation between moisture and ripening time of dry cured ham

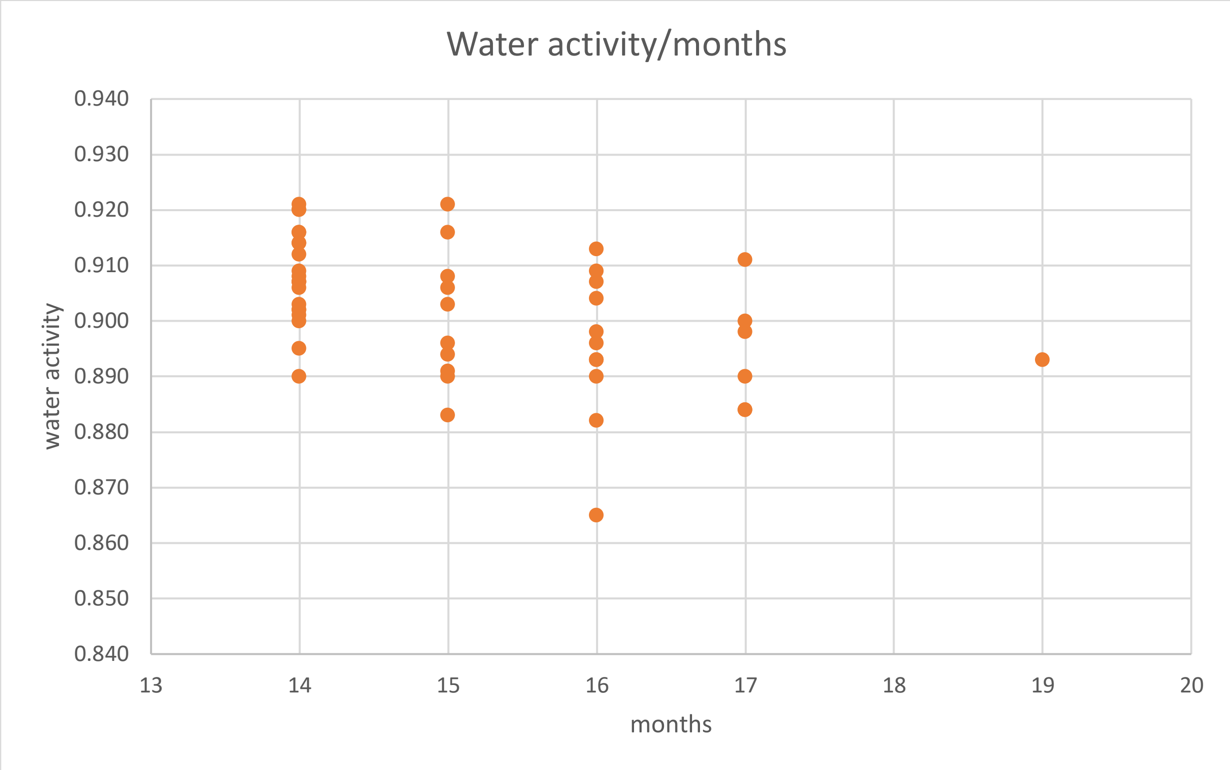

Figure 2: Correlation between water activity and ripening times of dry cured ham

Table 1: International standard methods for nitrites and nitrate detection based on Griess reaction using a cadmium reduction column (LO Man-fung, 2008, modified, https://www.govtlab.gov.hk/g/texchange/sudan.pdf.)

Standard	Samples NO_2/NO_3
ISO 2918/1975 (E) – 3091/1975 (E) – Determination of nitrates, reference method	Meat and meat products
ISO (2003/2006)	Milk and milk products
EPA, (1993)	Various food products
AOAC, (1990	Cheese
AOAC (1995)	Meat – Food for Infants

Table 2: Mean, range and standard deviation of moisture in meat, dry cured ham and sugna (%)

Moisture %	**Meat**	**Dry Cured Ham**	**Sugna**
Mean	73.0 ± 2.1	56.4 ± 2.4	4.3 ± 0.5
Range	66,2-84.2	49.8 – 60.4	4.0 – 4,9
Sample number	50	50	3

Legend: Mean ± standard deviation

Table 3: Nitrites and nitrates in sugna (mg/kg)

Value	**Nitrites WW**	**Nitrites DW**	**Nitrates WW**	**Nitrates DW**
Mean	5 ± 0.3	5 ± 0.3	8 ± 5.2	9 ± 5.4
Range	4-5	4-5	4-14	4-14
Sample number	3	3	3	3

Legend: Mean ± standard deviation; Wet Weight (WW), Dry Weight (DW)

Table 4: Nitrites and nitrates in Salt (mg/kg)

Value	**Nitrites**	**Nitrates**
Mean	1 ± 0.8	6 ± 2.9
Range	< 1-3	2-12
Sample number	10	10

Legend: Mean ± standard deviation

Table 5: Nitrites and Nitrates in fresh meat (mg/kg)

Value	**Nitrites WW**	**Nitrites DW**	**Nitrates WW**	**Nitrates DW**
Mean	2 ± 0.9	7 ± 3.2	8 ± 3.4	32 ± 15.1
Range	<1-7	1-22	1-21	3-83
Median	2	7	8	31
Sample number	50	50	50	50
95% confidence interval	2 ± 0.3	7 ± 0.9	8 ± 1.0	32 ± 4.2
99% confidence interval	2 ± 0.3	7 ± 1.2	8 ± 1.2	32 ± 5.5

Legend: Mean ± standard deviation; WW - Wet Weight; DW - Dry Weight DW); 95% and 99% confidence intervals of the means.

Table 6: Nitrites and Nitrates in San Daniele Dry Cured Ham (mg/kg)

Values	**nitrites WW**	**nitrites DW**	**nitrates WW**	**nitrates DW**
Mean	1 ± 0.5	2 ± 1.3	4 ± 3.1	9 ± 7.1
Range	<1-2	< 1-5	<1-11	<1-26
Median	1	2	4	9
Samples number	50	50	50	50
95% confidence interval	1 ± 0.2	2 ± 0.4	4 ± 0.9	9 ± 2.0
99% confidence interval	1 ± 0.2	2 ± 0.5	4 ± 1.2	9 ± 2.6

Legend: Mean ± standard deviation; WW - Wet Weight; DW - Dry Weight DW); 95% and 99% confidence intervals of the means.

Table 7: Nitrites and Nitrates threshold value (mg/kg)

Product	**nitrites WW**	**nitrites DW**	**nitrates WW**	**nitrates DW**
Fresh meat	14	44	42	166
Dry cured ham	**4**	**10**	**22**	**52**
Salt	6	6	24	24
Sugna	10	10	28	28

Legend: WW - Wet Weight; DW - Dry Weight DW