Evaluation of water activity (a_w) as possible parameter for the preservation of dried natural casings

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Abstract

A_w as possible parameter for the preservation of dried natural casings was studied by investigating the survival of *Escherichia coli*, *Bacillus cereus* and *Listeria monocytogenes* during the drying process of the casings.

Individual dried beef casings were rehydrated and subsequently inoculated with *Escherichia coli*, *Bacillus cereus* and *Listeria monocytogenes*. On day 30 post inoculation, the presence of *E. coli* and *B. cereus* could no longer be confirmed, while over 4 log cfu / g casing *L. monocytogenes* remained.

This study indicates that the drying period of 30 days at low a_w levels is not sufficiently long to eliminate all bacteria present on the natural casings. A_w solely can therefore not serve as a useful parameter for the correct preservation of dried casings. It is useful to link other parameters such as microbial growth.

Keywords: Dried natural casings; Water activity; Preservation; *Listeria monocytogenes*

1 Introduction

Intestines of pigs, sheep and cattle have already been used for thousands of years as casing of a sausage. The technique for cleaning casings the same (Ockerman and Hansen, 2000). There is no significant difference in efficiency between manually or mechanically cleaned sheep casings (Koolmees et al., 2004). In general, the submucosa layer is used as a natural casing and, dependent on animal species, used as a casing for various types of sausages (Houben, 2005). Natural casings are mostly preserved by salting, curing and/or drying (Fischer and Schweflinghaus, 1988). Natural casings preserved by salting at a water activity (a_w) level of 0,85 or lower for at least 30 days is, sufficient for reducing bacterial contamination, except for *Clostridium* spores (Wijnker et al., 2006). Another method for preservation is drying natural casings (Schmidt, 1968; Beutling, 2004; Wijnker, 2009). With this method, little is known about possible food safety risks.

More is known about drying meat related in relation to microbiological safety for human health. Drying is probably the oldest form of food preservation (Gailani, 1985). In the earliest civilizations, meat was already dried to preserve food. After meat was stripped or pulled, it was dried using wind, sun, or fire. Native Americans would dry venison, buffalo, and moose meat as a mobile and nourishing food. This meat was described as Jerky, 'charqui' in Spanish, by American pioneers. The easiest way to make this is to slice meat into strips and dry it. Half a kilo of fowl or meat is dried to approximately 113 g after being made into Jerky. The popularity of Jerky for home food preservation is mainly thankful to its comfort and flavour, as it enables people to use different recipes and processes to make it (Nummer et al., 2004).
Biltong, defined as dried and uncooked meat which is mainly utilized in South Africa, is another example of dried meat. According to an old historical reference from 1852, "Biltong" thanks its name to the Dutch "bil" (posterior thigh, rump) and "tong" (strip, tongue-shaped) (van der Heever, 1970). The origin of Biltong lies in the Netherlands, as the Dutch, escaping from the British regulation in South Africa about 200 years ago, added vinegar and spices to meat in order for it to be preserved and hung it on bullock vehicles over 3 to 4 days where it could dry (Burfoot et al., 2010).

The production of Jerky by home drying of meat is regarded to be of no danger. However, diseases caused by such food products have caused people to be skeptical regarding the stated safety (Nummer et al., 2004). There even is evidence of outbreaks by commercially produced dry meat. The major source of comprehensive outbreaks are the enteric bacteria. These bacteria can be reckoned as "natural contaminants" of the raw meat used to produce Biltong and Jerky, they may be present in raw and untreated spices used to season the meat, or they might have infected the food through cross contamination as a result of minimal control in the production environments.
While producing traditional Biltong, only low temperature heating processes are used and any enteric pathogens found on the raw meat will only be destroyed either by acidic coatings or marinades or by very low a_w values. However, a significantly low a_w can very effectively „preserve" bacteria in a feasible, but non-growing state (Burfoot et al., 2010).

A_w is usually used to measure the availability of water in a food environment is (Capozzi et al., 2009), being a well-known parameter in food for the presence of unbounded water (Stekelenburg, 1991). As free water is crucial for the development of micro-organisms (Capozzi et al., 2009), a_w can be an assessment on the microbiological stability or sustainability of a foodstuff (Stekelenburg, 1991). The quantity of water availability in a material for microbial growth can therefore be indicated (Capozzi et al., 2009). Micro-organisms have a constraining a_w level for growth (Scott, 1957). Most bacteria grow rapidly at a high a_w (0.99), however, microbial growth does not occur at a_w below 0.6 (Corry, 1973).

A_w is defined as the relationship between the water vapour pressure of a food and the similar pressure of pure water, tested under the same conditions (Scott, 1957). The a_w value can vary between a minimum of 0.0 for an entirely dry product and a maximum of 1.0 for pure water (Stekelenburg, 1991). The relative level of moisture of the atmosphere in equilibrium with the food is calculated by multiplying the a_w value by 100. As micro-organisms are only able to use the available water, a_w is a better ratio for the actual microbial growth than the simple water content (Scott, 1957).

The a_w of food can be decreased by various methods: by the addition of solutes or hydrophilic colloids, cooking, drying and dehydration: (e.g., egg powder, pasta), or by concentration (e.g. condensed milk) which limit microbial growth to make the food microbiologically stable and free of dangers (Dilbaghi and Sharma, 2007).

The inhibitory effect of a low a_w level (Gutierrez et al., 1995) is linked to the fact that the turgor pressure in a cell is caused by the a_w of the internal environment and the a_w in the external medium. Immediate outflow of water caused by hyperosmotic shocks, as well as a decrease in the volume of the cytoplasm, is described by plasmolysis (Csonka, 1989). A general reaction to the temporary reduction of turgor is the cytoplasmatic accumulation of so-called “compatible solutes” (Sperber, 1982; Beals, 2004).
This reaction of the reduction of a_w can prevent cell-death, but this highly depends on complicated circumstances, both intrinsic and extrinsic, that vary within food types, systems and techniques, and types of flora included (Lenovich, 1987). Research done by Park and Beuchat (2000) on the survival of *Escherichia coli* O157:H7 in potato starch absolutely indicate that the bacterial activity at a lower a_w level is mainly a result of the composition of foods. Therefore, the viability of certain pathogens at a reduced a_w level can only be analysed by natural casings themselves (Wijnker et al., 2006). The extent to which the survival of bacteria in preserved natural casings by drying is affected by a reduced a_w level has never been researched, except for a study done by Gabis and Silliker (1974) on the survival of Salmonella spp. and an investigation on the presence of certain pathogens in dry-salted casings (Houben, 2005).

There are no microbiological criteria described for dried natural casings in existing legislation. Even the survival of micro-organisms by drying of natural casings has never been investigated. Therefore the purpose of this study was to examine the antimicrobial properties of drying after adding relevant pathogens to natural casings. In addition, a_w was evaluated as a suitable parameter with possible predictive qualities for the preservation after drying.

2 Materials and methods

2.1 General

The materials and methods used in this study are based on those described by Wijnker et al. (2006).

2.2 Bacteria

The following bacterial species have been selected:

- *Listeria monocytogenes* (ATCC 7644);
- *Escherichia coli* (ATCC 10536);
- *Bacillus cereus* (ATCC 9139).

All these bacteria were cultured in 10 mL Tryptone Soy Broth (TSB) for 24 hours at 37 ^{o}C. Subsequently, the bacteria were diluted and counted. The final inocula was made of 1,84 * 10^{9} colony-forming units (cfu) mL^{-1} of *L .monocytogenes*, or 8,8 * 10^{8} cfu mL^{-1} of *E. coli* or 2,0 * 10^{7} cfu mL^{-1} of *B. cereus*.

2.3 Sample preparation

Dried beef casings were obtained via the European Natural Sausage Casings Association (ENSCA). Dried beef casings, which were rehydrated with sterilized water, were cut in pieces of 10 cm length. The pieces of casing were weighed. Then the pieces of casing were inoculated with 1 mL. inoculum from or *E. coli*, or *L. monocytogenes* or *B. cereus*, for 8 measuring moments in duplo. For every sample moment (total 8 times) there were always 3 samples inoculated. In addition there was for every sample moment a negative control. Samples were placed in an incubator set at a temperature of 25 ^{o}C. An open container of water with sufficient volume for the experiment was placed in the incubator. In practice, the casings are dried in open air, possibly exposed to the sun. The water in the container ensures maintenance of an appropriate humidity relative to the set temperature.

2.4 Preservation by drying

The a_w starting values of the dried beef casings were measured. To investigate necessary time from rehydration to original dry a_w value, the casings were put into an incubator (Heraeus, type B5050 and B5060, Germany) set at a temperature of 25 ^{o}C for 1 day. The temperature and the relative humidity have been measured constantly by data loggers (Tinitag, type view 2, UK). The a_w was measured using a a_w-meter (Novasina, type LabSwift-a_w, Switzerland).

2.5 Inoculation procedure

Dried beef casings were cut in pieces of 10 cm length; these were rehydrated with sterilized water. After rehydration the casings were ligated with suture and inoculated with or *E. coli*, or *L. monocytogenes* or *B. cereus*, for 8 measuring moments in duplo (duplo comes from stomacher bag). For every sample moment (total 8 times) there were also 3 samples inoculated with *E. coli*, 3 with *L. monocytogenes* and 3 with *B. cereus* (table 1). In addition there was for every sample moment a negative control. In total per sampling moment 10 pieces of casing were used, making a grand total of 80 samples.

Table 1, primary contamination

Species	Amount in 1 mL inoculums in cfu in log count
L. monocytogenes	9.26 $\log_{10}$ cfu mL^{-1}
E. coli	8.94 $\log_{10}$ cfu mL^{-1}
B. cereus	7.30 $\log_{10}$ cfu mL^{-1}

2.6 Micro-biological analysis

To check the initial contamination prior to the experimental inoculation, beef casings were tested on the presence of the same bacteria used. The casings were tested at 8 different moments, comparable to the investigation of Wijnker et al. (2006). Every measuring moment, samples were tested in threefold. Samples were weighed and added with buffered pepton water (BPW) to a stomacher bag. The volume ratio of sample : BPW was 1 : 99. This dilution was mixed with a stomacher (Colworth, type 400, UK). Out of the stomacher every sample has been tested in duplo producing 6 analysis results per measuring moment. Decimal dilutions were made to create countable dilutions. Next, three dilutions were applied to agar plates with a specific growing medium. The agar plates (table 2) that were used for the bacterial research were based on department protocols, an article of Mossel et al. (1967), and on ISO standards.

Table 2, ISO methods and media used for plating and enrichment

Species	Plating medium	ISO	Enrichment
E. coli	MacConkey Agar (MCA) (24h, 37 °C)	7251	Buffered Pepton Water (BPW) (24h, 37 °C)
L. monocytogenes	Compass Listeria (24h, 37 °C)	11290	Fräser broth (24h, 37 °C)
B. cereus	Mannitol egg Yolk Polymyxin Agar (MYP) (24h, 35 °C)	7932	

2.7 Statistical analysis

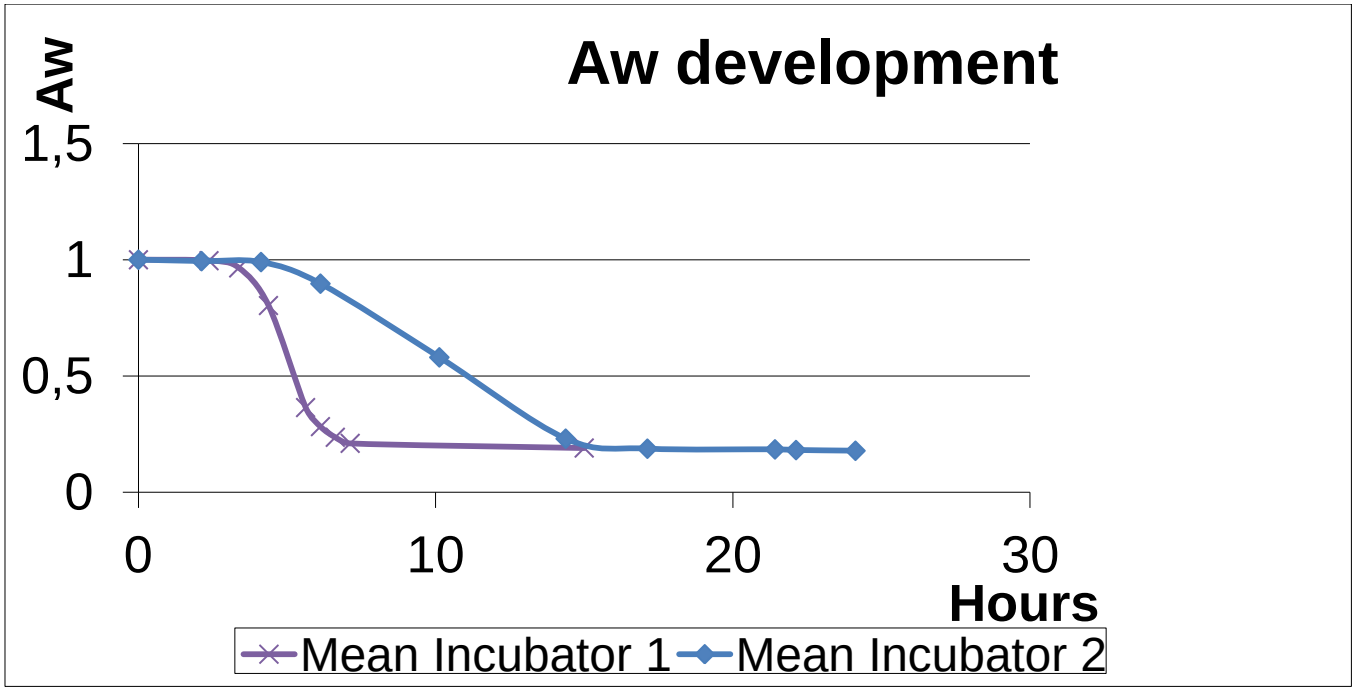
Results are presented in figures one and two. Only plate counts between 25 and 250 cfu each plate were, based on department protocol, included in the interpretation. In case of more values for one measurement, these values were averaged.

3 Results and discussion

Starting a_w value

The starting a_w value of dried casings is approximately 0,3. According to figure 1, it takes between 6 and 12 hours to get to the starting a_w value when drying rehydrated casings.

Figure 1



Contamination of the casings before inoculation

To reduce the influence of the presence of possible bacteria, dried casings have been analysed microbiologically before the inoculation. The presence of *E. coli, B. cereus* and *L. monocytogenes* were analysed. Presence of any *Bacillus* specie, not being *cereus* (nor *antracis*), was confirmed. The presence of bacteria in natural casings was confirmed in several studies (Wijnker, 2009; Koolmees et al. 1997). In addition, it is well-known that Muscoid flies which may come into contact with air-dried beef casings are associated with transferring pathogens (Greenberg, 1973; Banjo et al., 2005). This means that there are factors during the drying process which could lead to cross-contamination with certain micro-organisms.

Bacteriological analysis

In table 1, the results of the bacterial analysis are presented. The number of bacteria is given in cfu in log count / g casing.

Table 1

Day	*E. coli*	*L. monocytogenes*	*B. cereus*
0	8,94	9,26	7,30
1	7,20	6,27	6,94
3	5,76	4,61	4,61
6	7,40	5,90	4,42
8	4,87	4,59	3,59
13	7,10	6,56	7,08
20	7,48	5,83	4,45
27	0	4,64	2,95
30	0	4,55	0

The reduction in the number of bacteria is presented in figure 2 and the gradient of the relative humidity (RH) and the temperature in figure 3.

Figure 2

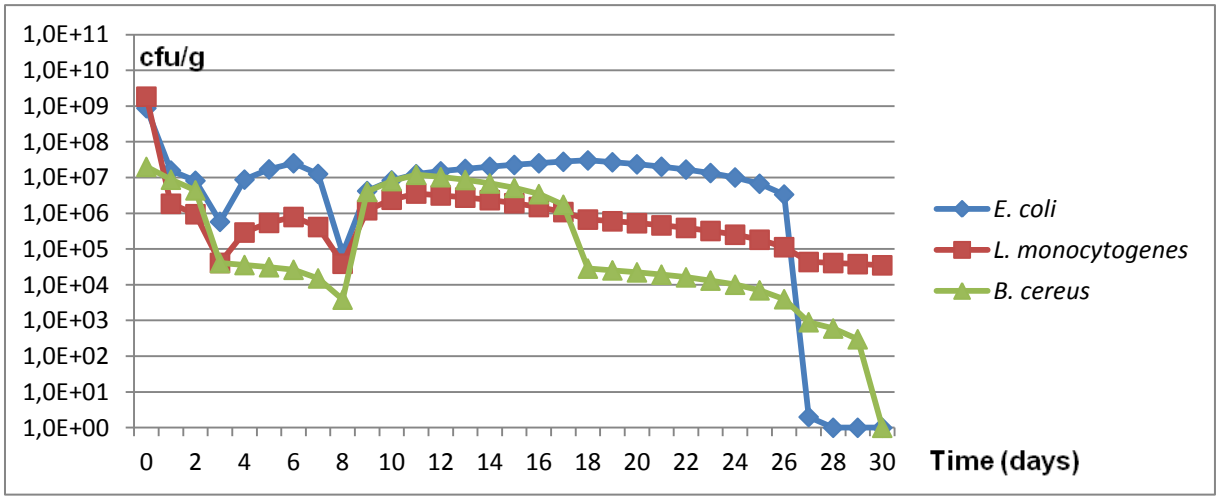
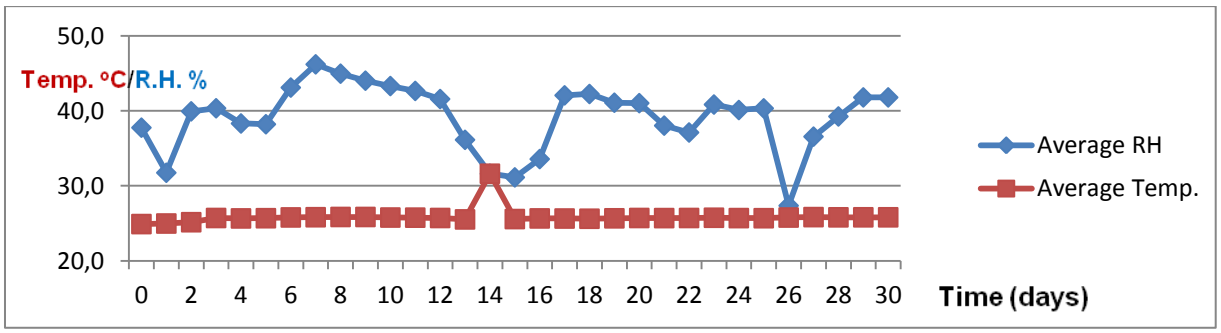


Figure 3



Osmotic stress

According to the so-called turgor pressure, the internal osmotic pressure of a bacterial cell is normally higher than the external osmotic pressure (Gutierrez et al., 1995; Abee and Wouters, 1999; Wood et al. 2001). As a result of drying, available moisture is reduced and cells are exposed to osmotic stress, requiring the micro-organisms to actively maintain their turgor pressure using various mechanisms (Csonka, 1989). As a fact, several osmosensors are involved with osmotic stress that induce responses (Poolman et al., 2004). A bacteria maintains a higher concentration of solutes in the bacterial cytoplasm than in external environment. A decreasing a_w value leads to a direct osmotic up-shift, which causes bacterial cells to activate transporters creating a higher concentration of solutes in the cell by synthesis or the taking up of organic ions (Poolman et al., 2002). The response of micro-organisms to osmotic pressures concerns both controlled accumulation of compatible solutes and various gene expression of systems for the uptake of compatible solutes. Significant knowledge and information about these factors is necessary for the preservation of foods (Gutierrez et al., 1995).
Water can be easily transported through cell membranes, whereas solutes cannot. Water will be extracted from a cell when the osmolarity in the external environment is increased, and similarly, water will be transported into a cell when the osmolarity in the external environment is decreased. Regulation of the number of different solutes is possible by synthesis or transport mechanisms (Record et al., 1998).

Overall, it can be said that the Gram-positive bacteria *L. monocytogenes* and *B. cereus* handle osmotic stress better than the Gram-negative bacterium *E. coli*. According to Burfoot (2010), drying processes of meat lead to reductions of *E. coli* up to 3 log and reductions of *L. monocytogenes* to 4.5 log. In this investigation *L. monocytogenes* shows also a reduction of 4.5 log. Drying processes make it possible for a pathogen level to reduce increasingly as a_w is decreased. As a result of the mentioned adaptation possibilities, it is possible that the bacteria show signs of growth in the curve.

In the current study the presence of *L. monocytogenes* could be confirmed after storage for 30 days at a a_w value of approximately 0.30. These findings are in line with a study done by Ingham et al. (2004) with salted pork rind, dried to an a_w value of 0.28 and stored for 5 weeks at 21 ^{o}C which still contained viable *L. monocytogenes*. This shows that a desiccated condition lowers the metabolism of a bacteria so that there is no more growth possible, allowing vegetative cells and spores, to survive months or even years (Beuchat et al., 2013).

A_w

The concept of aw comes from thermodynamic laws and is only used at equilibrium. According to evidence, a_w is useful to link to other parameters such as microbial growth, shelf life and texture (Franks, 1991).

Utilization of a_w on food preservation is based upon lowering the a_w of the food to below the minimum for growth of microorganisms that are expected to proliferate under the storage conditions of that specific food. Organisms able to grow in extreme dry environments have received proportionality more attention (Gailani, 1985).

A_w can serve as a useful parameter for the preservation of dried natural casings. A study of Franks (1991) shows this too. Conclusion of this study is that measured relative vapour pressure, is often not similar to a_w. Measured relative vapour pressure can serve as a useful, but not the only parameter for microbial shelf life and microbial state (Franks, 1991).

4 Conclusions

Because there is a lack of scientific evidence on the efficacy of drying for pathogen inactivation in natural casings (EFSA, 2012), this study has been performed.

Salting with NaCl for a 30 day period is a standard operating procedure (SOP) in the casings industry. It has been the standard animal health risk mitigation treatment prescribed in EU legislation for years (EFSA, 2012).

Currently there are no specific requirements for drying natural casings. In this study a drying period of 30 days at ambient temperatures was chosen, similar to the preservation technique using salt as standard industry practice. This study indicates that the drying period of 30 days at low a_w levels is not sufficiently long to eliminate all bacteria present on the natural casings.

A_w solely can therefore not serve as a useful parameter for the correct preservation of dried casings as some bacteria, like *L. monocytogenes*, can apparently survive an a_w level of 0.3 even after 30 days. It is useful to link other parameters such as microbial growth. Additional studies should therefore be proposed to determine when dried casings can be considered properly preserved.

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