Extension of Meat Shelf-life by High Level Carbon-Monoxide Modified Atmosphere

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Background

The growing demand for fresh (non-frozen) transatlantic meat bulk shipments, as well as individual pallet loads, is constantly driving the market to seek methods that will allow further extension of fresh meat shelf life, such as the use of modified atmosphere for meat packing (MAP). Grossly, two factors contribute to meat spoilage: oxidative rancidity and growth of microorganisms, both resulting from the inability of dead tissue to protect itself. Oxidative rancidity is directly or indirectly the outcome of the presence of atmospheric oxygen. Muscles as high reactivity tissues are abundant with oxygen kept in hemoglobin- (Hb) and myoglobin- (Mb) bound form (1). Hb/Mb have high affinity for oxygen, but the oxy- forms of Hb/Mb tend to undergo autooxidation, a process leading to formation of Met- Hb/Mb and superoxide radical, which itself converts to H_2O_2 (2) [equation 1: globin-Fe^{+2}-O_2 $\square$ globin-Fe^{+3} + O_2^- $\square$ H_2O_2]. The main method currently in use to avoid oxygen hazards for shelf-life extension of wholesale as well as retail meat (both fresh and frozen) is vacuum packing. The pitfalls of this method are undesired color - of the deoxy- heme forms of Hb/Mb - and rancidity - occasionally occurring when air perfuse through small leaks which might form in the package - leading to peroxidative tissue spoilage (6). MAP (various mixtures of gasses, usually O_2, CO_2 and N_2) is an alternative design to extend meat shelf-life by preventing growth of aerobic microorganisms and oxidative damage. However, even in MAPs excluding oxygen from the atmosphere, Hb/Mb remain in oxygen-bound form due to low residual oxygen. Therefore, these MAPs cannot prevent Hb/Mb autooxidation (eq. 1) and the ensuing rancidity. Carbon monoxide is a ligand that binds with much higher affinity (X200) than oxygen to Hb/Mb divalent heme iron. Therefore, inclusion of this gas in MAP allows replacement of Hb/Mb bound oxygen by CO [equation 2: globin-Fe^{+2}-O_2 + CO $\square$ globin-Fe^{+2}-CO+ O_2]. The heme iron in the stable carbon monoxide complex is devoid of oxidative activity.

Objectives

To extend meat shelf-life by use of CO atmosphere to prevent oxidative rancidity.

Methods

Meat sources: Fresh meat sources (main beef and some poultry) were from local Israeli slaughter houses (Marbek Ltd. for beef and Miluot Ltd. for poultry); **Bacterial count:** the procedure entailed treatment of a 25 cm^2 surface area sample of meat with 25 ml (according to health regulation standards). The meat was introduced into the solution and agitated by stomacher apparatus (Seward Lb U.K). The suspension was diluted in a ten fold series in 0.1M phosphate buffer, pH 7.0. One ml of each dilution was applied to three types of 60 mm diameter growing plates, the first containing Plate Count Agar (PCA, Difco) incubated at 33° C for 48 hours to enable total viable count, the second containing SPS agar (Difco) allowing Clostridium growth, and the third as the first type, but allowing microaerophile growth. The second and third types of plates were confined within sealed anaerobic jars supplied with gas containing kits (Oxoid U.K) and incubated 24 hours at 35° C.

Quantitative evaluation of iron oxidation state in meat: To distinguish between oxy-, carbomonoxy- and oxidized Hb. To assure that differences can be seen in meat we divided a fresh beef meat into 3 groups in each 5 pieces of ~100 gram each. The first group was treated immediately, while second and third groups were kept refrigerated for three weeks under air or CO, respectively, and then treated as follows: each meat piece was crushed three times in a blender for 5 min. and chilled on ice for 10 min. between blends. The slurry was centrifuged for 30 min. in the cold at 40,000xg and the supernatant separated. In blood samples the red cells were separated by 10 min centrifugation at 6,000xg and immediately lysed by dilution into X10 hypotonic 5 mM pH 7.4 buffer. The absorption spectra in the 400-700 nm range were measured in the range of 350-700 nm. The absorption spectrum of fresh meat matched with that of oxy-Mb-Hb from the literature. The CO-saturated meat matched with that of carbomonoxy-Mb-Hb, whereas meat kept aged on air had a typical spectrum of Met- Mb-Hb and hemichrome.

Results and discussions

Safety: Subacute toxicity of CO-associated meat consumed through the gastric system: 12 cats and 4 dogs were divided into control and experimental groups. All animals were fed a diet of ground beef meat only, three times a day for one month. Each meal contained 30-40 grams meat per cat and 150-200 grams per dog. The meat was fresh (1-2 days after slaughter) and kept refrigerated for 2-24 hours and sealed under a MAP of 100% CO or air (control). Bags were opened in cages and the meat was immediately consumed by the animals. Each week, blood samples were drawn from the animals. Typical spectra of hemoglobin from the control (fed on air packed meat) and experimental (fed on CO packed meat) cats indicate that no difference could be observed between the two spectra of blood form control animals and blood from animals fed CO-associated meat, both typical of oxy-hemoglobin;

Toxicity due to release of CO from cooked CO-associated meat: in this set of experiments the amount of meat used was that of a family size, 0.2-2.0 Kg. Beef meat (ground or chunks) was treated with CO to full saturation and kept refrigerated for the time period regularly kept at a home (up to two weeks). The meat was opened in a closed ~10 m^3 kitchen size room. CO released from the package by opening did not exceed 5 ppm even if the room was hermetically closed. The CO-associated meat was cooked for 60–140 minutes in a sealed pot (pressure pot), cooled to room temperature and the CO level in the pot was measured. The CO level was expressed as ppm CO in atmosphere, should the CO from the pot diffuse into the sealed room. The meat average CO level from 20 experiments performed was 0.056±0.026 ppm. Strict regulation calls for an upper CO threshold (constant exposure for 8 hours) of 25 ppm as a toxic level for inhalation of this extremely toxic gas.

Penetration of CO from MAP : To avoid changes in texture of the fresh muscle tissue, the gas pressure employed was only 1.1- 1.4 atmospheres. The rate of CO diffusion should depend on its partial pressure (per cent) and the meat resistance (at chilled temperature), a parameter that varies with meat source and size. The depth of gas penetration from surface to core could be followed taking advantage of visually observable differences in the red nuance of oxy- and carbonmonoxy- Hb/Mb. a) Time-course of penetration of CO from essentially all CO MAP: at time intervals (of days), sample were cut transversely. Fresh chunks of 0.5-1.5 Kg were turned into CO-saturated by packing under 100% CO MAP. The meat was kept refrigerated at 2-8°C. Control chunks from the same source were treated identically but with air instead of CO. Color changes, which were brown in the air-treated samples and wine-red in the CO-treated samples, propagated with time from the surface towards the center of the chunks. The surface color of the air-treated meat became brown after 3 days and completely dark brown after 18 days. In the CO-treated meat, the depth of color changes were 3-5 mm from the surface after 1.5 hours, 2 cm after 12 hours and 95% complete after 3 days. After seven days the color change was complete; b) Penetration of CO into meat preserved under high and low CO MAPs: To allow comparison of both small (retail) and wholesale meat chunks, we measured CO diffusion of medium-size fresh beef chunks of ~ 5Kg. Fresh meat chunks were packed under three MAPs: 100 % CO, 10% CO/90% N_2 and 1% CO/99% N_2. Full penetration of CO occurred after 10 days in the case of 100 % CO. However, even after 3 weeks under 1% MAP CO was bound at the surface only, and under 10% MAP - at 1-2 cm from the surface, while the rest of the meat became brown at this stage. Additionally, following exposure of the CO-saturated meat to air it retained its red color for additional two weeks in the cold.

Edibility: a) Microbiology: the shelf life of a meat in a non-contaminated form ("preservation duration") was determined by the duration until $1X10^7/cm^2$ for total viable aerobic and $1X10^4/ cm^2$ of microaerophiles, were reached (international standard for edible meat). Twenty experiments were carried out as follows: fresh meat beef small cuts weighing 25-35 grams were kept either refrigerated at (2-8° C) or at room temperature (22-30° C). Bacterial counts were carried out at intervals of 1-4 days (according to growth rate) for experiments in which the meat was kept refrigerated and at 2 hours intervals for meat kept at room temperature. For refrigerated meat experiments the length of microaerophiles "preservation duration" was 8.7±2.1 days (expressed as mean ± S.E.) and for CO-associated meat it was 18.9± 3.3 days. The length of total count in refrigerated meat experiments "preservation duration" was 11.1±2.1 days for control (air-packed meat) and 23.1±2.8 days for CO-associated meat. For experiments in which meat was kept at room temperature the "preservation duration" of total counts was measured as 13±1 hours for control and 30±1 hours for CO-associated meat. Clostridium was not found in any of the meats; Comparison of bacterial growth in meat packed under N_2 and CO: fresh beef meat was brought to the laboratory under cold conditions within 5-8 hours post slaughter. Samples weighing 4.00±0.18 grams were packed under Air, 100% N_2 or essentially-all CO. The bags were stored for 24 hours at room temperature (12-20° C) and total gram negative bacterial groth counted.. The average results of 20 experiments mean ± S.E) were: Air - 2.7 ±1.1 $X10^6$: N_2-4.6 ±2.5 $X10^5$: CO-2.0 ±1.0 $X10^4$. The reduced growth on CO as compared to N_2 suggests inhibition of bacterial growth by CO beyond oxygen expel. **b) Off-Odors:** Although odor may sometimes be less sensitive than bacterial counts, it is a very useful indicator to consumer, because it does not necessitate laboratory tests. All meats preserved under 100% CO retained a pleasant fresh meat odor for at least 18 days in the cold, whereas air-treated meat developed bad off-odors. At higher temperatures off-odors developed faster, but still under 100% CO meat was preserved as fresh much longer (3 days) than air-treated meat (Table 2). Additionally, following exposure of the CO-saturated meat to air it retained its red appealing color and was devoid of any off-odors for additional two weeks in the cold.

Table1: Preservation Duration of fresh-meat odors

	Size	Temperature Range ,C^0	Preservation of fresh odor, days
Beef	Slices[a]	2-9	18
Beef	Slices	22-30	3
Beef	Chunks[b]	2-9	18
Beef	Chunks	14-17	7
Veal	Slices	22-30	3
Turkey	Slices	22-30	3

*Under similar conditions air-packed meat developed bad odors;
a) 25-35 grams; (b) 300-900 grams

Conclusions

The results of the current study confirm the working hypothesis that CO-associated meat consumed even raw, and more so after cooking when it is practicably no longer part of the meat, should be of no harm. This is reasonable on the basis of the fact that by heme-oxygenase catabolism one molecule of CO is produced of each heme any way. Thus, the intake of CO-associated meat through the gastric system can at most (saturated and uncooked) produce two CO molecules endogenously, a situation equivalent of eating double amount of meat.