MORTADELLA SAUSAGE FORMULATIONS WITH MECHANICALLY SEPARATED LAYER HEN MEAT PREBLENDED WITH ANTIOXIDANTS

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ABSTRACT: At the end of the laying cycle, layer hens can be used for production of mechanically separated meat (MSML) for sausage manufacture. However, mechanically separated meats are susceptible to lipid and pigment oxidation during storage, problems that could be prevented by the use of antioxidants present in curing additives traditionally used in meat processing. The objective of this work was to evaluate the quality and stability of Mortadella produced with MSML added of sodium erythorbate (1000 ppm) and sodium nitrite (150 ppm) through preblending or during chopper processing (control treatment), after long term frozen storage of raw meat ingredient. The products were stored for 40 days at 7ºC and evaluated in regard to the oxidative stability of lipids (TBARS values), objective color, and microbiological and sensory attributes. Preblending MSML lessened rancidity of the final product in comparison to the control (0.2 and 2.2 mg malonaldehyde per kg, respectively), and elicited better color (a* = 7.5 and 10.5, respectively). Total counts of psychrotrophic microorganisms were similar for both treatments (2 log CFU g^{-1}). Previous blending of nitrite (150 ppm) and erythorbate (1000 ppm) in mechanically separated meat soon after the extraction is recommended when the raw material will eventually go through prolonged frozen storage before its use in Mortadella sausage processing.
Key words: poultry meat, nitrite, erythorbate, oxidative stability, sensory evaluation

MORTADELA ELABORADA COM CMS DE GALINHAS POEDEIRAS PRÉ-MISTURADA COM ANTIOXIDANTES

RESUMO: Uma das formas de utilização das galinhas poedeiras ao final do ciclo de postura é a obtenção de carne mecanicamente separada (MSML) para a elaboração de embutidos. Entretanto, carnes mecanicamente separadas apresentam alta susceptibilidade à oxidação lipídica e dos pigmentos. O uso de antioxidantes tradicionalmente utilizados na cura de carne para incorporação prévia à matéria prima poderia evitar estes problemas. Assim, o objetivo deste trabalho foi avaliar a qualidade e a estabilidade de mortadelas elaboradas com MSML com adição de eritorbato (1000 ppm) e nitrito (150 ppm), como pré-cura antes da estocagem congelada, ou durante o processamento no homogeneizador (cutter) de bacia rotativa (controle). Os embutidos foram avaliados durante 40 dias (7ºC) quanto à estabilidade oxidativa dos lipídios (índice de TBARS) e da cor objetiva, assim como a estabilidade microbiológica e sensorial. A pré-cura da MSML garantiu um produto final com menor rancidez quando comparado com o controle (0,2 e 2,2 mg malonaldeído/kg, respectivamente) e melhor coloração (valores de a* entre 7,5 e 10,5, respectivamente) ao longo de todo o tempo de estocagem. As contagens totais de microrganismos psicrotróficos não diferiram entre os tratamentos (2 log UFC g^{-1}). A mistura prévia de nitrito (150 ppm) e eritorbato (1000 ppm) em carne mecanicamente separada que possa vir a sofrer estocagem congelada prolongada antes de ser utilizada na elaboração de embutidos é recomendável.
Palavras-chave: carne de aves, nitrito, eritorbato, estabilidade, análise sensorial

INTRODUCTION

The number of layer hens housed in 2004 in Brazil was approximately 62.8 million (UBA, 2005). Volumes of biological wastes, labor and transportation costs associated with the disposal of layer hens, are some of the main economical and environmental prob- lems of the poultry industry (Lyons, 2001). Spent hens are normally slaughtered and made available for other uses, such as feed production, concentrated stock preparation, or domestic consumption as soups and stews (Ajuyha et al., 1992; Voller-Reasonover et al., 1997). Another end use for spent hens is the production of mechanically separated layer hen meat (MSML)

for the sausage industry (Lee et al., 1997; Trindade et al., 2004; 2005).

Chemical and structural alterations during mechanical separation, the development of undesirable aromas (rancidity) and the loss of its characteristic red color, because of lipid and pigments oxidation, respectively, make mechanically separated meats having low stability during storage. Quality loss can also result from the redistribution of the higher initial microbial load from the more exposed areas and larger availability of nutrients, originated from the breakage of the cells, which favor the growth of microorganisms.

Because of its curing activity nitrite is an important ingredient in meat preblending, it functions also as anti-oxidant and in the production of the typical aroma of cured meats. Nitrite also has important anti-microbial effects, avoiding the production of toxins by *Clostridium botulinum* (Hasiak et al., 1984; Kolodziejska et al., 1990). A maximum of 150 ppm of nitrite is allowed by the Brazilian food safety legislation in meat products (Brasil, 1998).

Sodium erythorbate is used in meat products to speed up the formation and stabilize the characteristic pink color of nitrite-cured meats. The utilization of antioxidants can minimize functional deteriorations in muscle proteins during storage (Xiong & Decker, 1995). Erythorbate is an antioxidant, so this effect could also be expected. The Brazilian legislation does not limit the use of sodium erythorbate in sausages (Brasil, 1998).

This project was carried out to evaluate the quality and stability of Mortadella produced with MSML preblended or not with sodium erythorbate and sodium nitrite, and kept under frozen storage for 90 days before processing.

MATERIAL AND METHODS

Raw Material

Carcasses from 18 month-old White Leghorn hens were obtained from a local poultry slaughterhouse. Fifty carcasses were processed into MSML using a POSS extractor model PDE 1000. The average proximate composition for the MSML obtained was 15.15, 16.89, 63.94, and 2.06% for protein, fat, moisture and ash, respectively. The MSML was separated in portions of 9.5 kg, which were mixed for three minutes with either 0.5 kg of pure, distilled water (control treatment), or distilled water with dissolved additives (preblending treatment: 1.5 g of sodium nitrite and 10.0 g of sodium erythorbate, equivalent to 150 ppm and 1000 ppm, respectively). The raw material was then separately packed in polyethylene bags, and stored at −18ºC (±1ºC) for 90 days.

Mortadella Formulation and Processing

Table 1 shows the formulations (approx. 10 kg batches) used in both treatments. The MSML were chopped in a block cutter and then weighed; all ingredients and the ice were also separately weighed. The comminution was initially carried out in a cutter up to temperatures around 10ºC. The refinement of the masses was finished in a colloidal mill. The temperature of the masses was around 14ºC after this process. Then, the emulsions were stuffed (around 500 g per unit) into 90 mm-wide, impermeable plastic casings. They were cooked in a smokehouse until the internal temperature reached 72ºC (approximately 120 min.); the products were then cooled in a shower until the internal temperature dropped to 40ºC, and stored at 7ºC.

Technological characterization

Stability of the emulsions - Stability was determined using the method of Parks & Carpenter (1987) with 10 replicates for each sample. Approximately 50-g samples of emulsion were poured into plastic bags (PA/PEBD), which were sealed and cooked in a water bath at 70°C for 1h. The exudate was then weighed to determine the percentage of cooking loss.

pH - The pH was measured in a pHmeter with the electrode directly placed into the sample. Three measurements were made in products from each treatment.

Shear Force Value - Shear strength was measured using a TA – TX 2i texture analyzer equipped with a Warner-Bratzler device at a test speed of 20 cm min^{-1}. Ten samples were analyzed for each treatment. The samples were cut into 20 mm high and 13 mm diameter cylinders, compressed on their round surfaces

Table 1- Formulations used in Mortadella processing.

	Preblending	Control
Raw Material		
MSML	8.00 kg	8.00 kg
Ingredients		
Salt	200.0 g	200.0 g
Commercial condiment	100.0 g	100.0 g
Soy protein	100.0 g	100.0 g
Cassava starch	500.0 g	500.0 g
Mashed garlic	100.0 g	100.0 g
Sodium tripolyphosphate	30.0 g	30.0 g
Sodium nitrite	1.2 g*	1.2 g
Sodium erythorbate	8.0 g*	8.0 g
Ice	1.10 kg	1.10 kg

*Sodium nitrite and sodium erythorbate were added to the MSML before the storage, as described above.
MSML: mechanically separated layer hen meat.

Evaluation of storage stability

Stability tests were carried out every seven days, from day 5 through day 40 after processing. In each point, 2 units (500 g each) were evaluated.

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was determined through TBARS, using the distillation method of Tarladgis et al. (1960). The modification to this procedure was the addition of butylated hydroxytoluene before blending, to prevent auto-oxidation (Pikul et al., 1983). The TBARS test, when performed with the distillation procedure, is considered to be more sensitive and also more suitable for high-fat samples (>10%) where turbidity may occur in the extracted samples (Shahidi, 1998). The TBARS value was calculated from a standard curve, and expressed as mg Malonaldehyde kg^{-1} of the product. Three samples (10 g each) were analyzed for each treatment.

Color measurements

Objective color was measured using a portable Minolta spectrophotometer, model CM508d, with illuminant C and a 2º angle. The readings for calibration and evaluation were performed using the CIE L*a*b* scheme (L* = lightness, a* = redness, b* = yellowness). Measurements were made on six slices from each treatment.

Microbiological analysis

On the first and last days of the stability evaluation, the treatments were analyzed for *Salmonella*, faecal coliforms, sulfite-reducing clostridia, *Staphylococcus aureus* and enterobacteria. The total count of psychrotrophics was performed on all 6 sampling points. Microbial analysis was carried out according to the methodology described by Vanderzant & Splittstoesser (1992). Detection limits for psychrotrophic aerobes, sulfite-reducing clostridia, *Staphylococcus aureus* and enterobacteria was 10 CFU, and <3 MPN g^{-1} for fecal coliforms.

Descriptive sensory evaluation

Sensory analysis of the products during the shelf life was performed by odor and appearance evaluations, using intensity scales and sixteen experienced panelists. Attributes of pink color, rancid aroma, and fermented aroma were evaluated in non-structured scales of 10 cm, representing "absent" at the beginning and "intense" at the end. Color evaluations were performed under regular white light, whereas odor evaluations were carried out under a green light, with the objective of disguising color differences among the different treatments.

Statistical analysis

Trials were set up in a completely randomized design with 2 treatments (n = 2); measurements were made at days 5, 12, 19, 26, 33 and 40. Data were analyzed by ANOVA to compare results among the treatments and regression to study trends during the evaluation period. The means were compared using the Tukey's test. The SAS® (Copyright© 2004, SAS Institute Inc.) software with its proc mixed tool was used. A significance level $P = 0.05$ was adopted.

RESULTS AND DISCUSSION

Emulsions stability

Preblending mechanically separated meat of white layer hens (MSML) did not affect stability of emulsions ($P > 0.05$) using this raw material as the only meat ingredient. The average values obtained in the emulsion stability tests were 2.59% (SEM = 0.13) of released juice in the CONT treatment (Mortadella made with MSML stored for 90 days with no previous addition of additives), and 2.70% (SEM = 0.09) of released juice in the PRE treatment (Mortadella made with MSML stored for 90 days preblended with 150 ppm of sodium nitrite and 1000 ppm of sodium erythorbate).

pH

Addition of nitrite and erythorbate before storaging the MSML did not affect the final pH of the Mortadella. The elevated pH values of 6.68 for both treatments can be explained by the high content of mechanically separated meat (100% MSML) used in the products. Higher pH values in emulsified meat products improve the emulsification capacity of the myofibrillar proteins and fluid retention (Field, 1988).

Shear force value

The values of the peak shear force registered were 0.34 kg (SEM = 0.03) for PRE, and 0.41 kg (SEM = 0.02) for CONT; there were no differences ($P > 0.05$) between treatments. Data are in accordance with those reported by Smith (1987), who registered that texture of gels prepared from turkey mechanically separated meat (MSM), stored at -20ºC for 26 weeks, with or without antioxidants, was similar. On the other hand, minced chevon preblended either without additives (control) or with 2% ginger extract (GE) was used to prepare cooked chevon patties. The control patties had significantly lower shear force values than the GE patties (Reema-Raj et al., 2005).

Evaluation of storage stability

TBARS

The average TBARS values for CONT were higher ($P < 0.05$) than those of PRE in every interval (Table 2). The levels of additives used (150 ppm of nitrite and 1000 ppm of erythorbate) were enough to inhibit lipid oxidation during the storage of Mortadella. Sausages made with more than 15% turkey MSM stored for 90 days presented high indices of TBARS, resulting in product with a poorer quality than that of sausages made with fresh MSM (Froning et al., 1971). Frankfurters made with 20% chicken MSM preblended with nitrite, ascorbate, and polyphosphate and stored for one, three, and six months, presented lower TBARS values than sausages made with control MSM (without preblending), when MSMs stored for up to six months were used (Pollonio, 1994). Chicken mince (CM) preblended with 5, 7.5, 10, or 12.5 ppm of alpha-tocopherol acetate (TA) had lower TBARS values and metmyoglobin percentages in comparison to the control sample (without preblending) in refrigerated storage, indicating better oxidative stability of the antioxidant-treated CM (Sahoo et al., 2004).

Objective color

CONT presented a lower average index for the red color ($P < 0.05$) than PRE throughout the entire storage period (Table 3). Preblending nitrite and erythorbate protected the pigments of the MSML against oxidation during frozen storage, keeping them available for the pink color development during the cooking process. A more reddish coloration was obtained in Mortadella made with preblended MSML in comparison to the non-preblended MSML product. These data are in accordance with results reported by Pollonio (1994), who found significantly lower a* values for sausages made with 20% chicken MSM stored for six months without additives, when compared to sausages made with MSM preblended with a combination of nitrite, ascorbate and polyphosphate, stored for the same period (a* = 4.1 and 6.9, respectively).

Microbiological Analyses

No difference ($P > 0.05$) was detected between the average psychrotrophic microorganisms counts for any treatment or between the time intervals (Table 4). Counts were done weekly along the refrigerated storage. Several analyses were also carried out to elicit a

Table 2 - Mean values of the TBARS analyses in Mortadella made with preblended and non-preblended MSML during refrigerated storage period.

		Storage time (days) malonaldehyde					
		5	12	19	26	33	40
		-- mg kg⁻¹ --					
CONT	Average	2.26 a[z]	2.25 a	2.33 a	2.27 a	2.36 a	2.27 a
	SEM	0.01	0.02	0.04	0.01	0.02	0.01
PRE	Average	0.21 b	0.20 b	0.22 b	0.21 b	0.23 b	0.24 b
	SEM	0.01	0.01	0.00	0.01	0.01	0.01

[z]Means followed by different letters in the same column differ by Tukey's test ($\alpha = 0.05$). TBARS: thiobarbituric acid reactive substances. MSML: mechanically separated layer hen meat. CONT: mortadella made with MSML stored for 90 days at -18°C without additives. PRE: mortadella made with MSML preblended with 150 ppm of sodium nitrite and 1000 ppm of sodium erythorbate, previously stored for 90 days at -18°C. SEM: standard error of mean.

Table 3 - Means and standard errors of means of the a* values in preblended and non-preblended MSML Mortadella during refrigerated storage period.

		Storage time (days)					
		5	12	19	26	33	40
CONT	Average	7.53 a	7.22 a	7.39 a	7.37 a	7.28 a	7.25 a
	SEM	0.60	0.01	0.02	0.01	0.04	0.21
PRE	Average	10.6 b	10.33 b	10.36 b	10.11 b	10.46 b	10.32 b
	SEM	0.07	0.08	0.22	0.25	0.12	0.00

Means followed by different letters in the same column differ by Tukey's test ($\alpha = 0.05$). MSML: mechanically separated layer hen meat. CONT: mortadella made with MSML stored for 90 days at -18°C without additives. PRE: mortadella made with MSML preblended with 150 ppm of sodium nitrite and 1000 ppm of sodium erythorbate, previously stored for 90 days at -18°C. SEM: standard error of mean.

more detailed microbiological characterization of the products. The analyses were done at the beginning (5th day) and at the end (40th day) of the storage. Analyses were performed for the detection of microorganisms which are limited in Mortadella by the Brazilian legislation (ANVISA, 2001): Salmonella, faecal coliforms, sulphite reductant clostridia, and *Staphylococcus aureus*. Analyses were also performed for enterobacteria. None of these microorganisms were detected neither in CONT or PRE.

Sensory Evaluation

Panelists detected a more intense pink coloration ($P < 0.05$) shade in PRE than in CONT samples (Table 5). Considering that the redder the better the visual quality of the Mortadella, the product made with 100% preblended MSML was significantly better during the refrigerated storage. Results were similar to those obtained in the objective analysis of the red color (value a*). PRE samples presented average a* values significantly higher than those obtained for CONT at all evaluated intervals during the refrigerated storage.

Attribute fermented aroma was used by the panelists to evaluate if the product presented a characteristic aroma of microbiological deterioration. The panel's averages did not differ ($P < 0.05$) between CONT and PRE, with values under one (0 = absent; 10 = intense) along the refrigerated storage period (Table 5). Data obtained in the sensory evaluation of the fermented aroma match those registered for the microbiological (total count of psychrotrophic microorganisms), which were very low for both treatments along the entire storage period.

The rancid odor is directly related to the lipid oxidation in foods. In this sensory attribute, the results of the panel showed the presence of a higher ($P < 0.05$) rancid odor in CONT than in PRE Mortadella along the storage period (Table 5). Products elaborated with MSML stored without the addition of additives were thought to be more oxidized than the ones made with preblended MSML by the panelists. These results obtained in the sensory evaluation are also in accordance with the objective analyses, for the average of the TBARS values observed in the CONT samples were

Table 4 - Total count of psychrotrophic microorganisms in CONT and PRE Mortadella during refrigerated storage.

	Storage period (days)					
	5	12	19	26	33	40
	-- log CFU g sample[-1] --					
CONT	2.2 a[z]	1.9 a	2.1 a	2.0 a	2.0 a	2.0 a
PRE	2.2 a	1.7 a	1.9 a	1.9 a	1.8 a	1.9 a

[z]Means followed by the same letter in the same column do not differ by Tukey's test ($\alpha = 0.05$). CONT: mortadella made with mechanically separated layer hen meat (MSML) stored for 90 days at -18ºC without additives. PRE: mortadella made with MSML preblended with 150 ppm of sodium nitrite and 1000 ppm of sodium erythorbate, previously stored for 90 days at –18ºC.

Table 5 - Descriptive sensory analysis results of the CONT and PRE Mortadella during refrigerated storage (7ºC).

Attribute		Storage period (days)					
		5	12	19	26	33	40
Pink color	CONT	1.66 a[z]	1.82 a	1.88 a	1.94 a	2.20 a	2.00 a
	SEM	0.06	0.16	0.05	0.16	0.04	0.11
	PRE	3.65 b	4.42 b	4.30 b	4.72 b	4.24 b	4.44 b
	SEM	0.27	0.40	0.44	0.09	0.11	0.66
Fermented	CONT	0.83 a	0.49 a	0.68 a	0.82 a	0.56 a	1.10 a
Aroma	SEM	0.15	0.01	0.07	0.06	0.10	0.06
	PRE	0.35 a	0.38 a	0.50 a	0.53 a	0.38 a	0.72 a
	SEM	0.14	0.03	0.05	0.17	0.02	0.04
Rancid	CONT	4.40 a	4.42 a	4.72 a	4.06 a	4.17 a	4.58 a
Aroma	SEM	0.09	0.20	0.14	0.12	0.48	0.21
	PRE	1.76 b	1.56 b	1.84 b	1.57 b	1.60 b	2.76 b
	SEM	0.43	0.52	0.05	0.61	0.30	0.29

[z]Means for the same attribute followed by different letters in the same column differ by Tukey's test ($\alpha = 0.05$). CONT: mortadella made with mechanically separated layer hen meat (MSML) stored for 90 days at -18ºC without additives. PRE: mortadella made with MSML preblended with 150 ppm of sodium nitrite and 1000 ppm of sodium erythorbate, previously stored for 90 days at -18ºC. SEM: standard error of mean.

higher ($P < 0.05$) than the ones observed for the PRE samples along the refrigerated storage, showing more lipid oxidation in CONT samples.

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