

Research Article

Effects of Beeswax Coating on the Oxidative Stability of Long-Ripened Italian Salami

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Beeswax coating of foods put a barrier to oxygen, light, and vapour that can help to prevent oxidation of fat and pigments and water loss. The amounts of 2-thiobarbituric acid reactive substances (TBARS) and water activity (A_w) were assessed in Italian salami coated with beeswax at 55 days of ripening and compared with controls at 5, 6, and 7 months of shelf life. The results were correlated with sensory quality. TBARS levels were below 0.8 mg kg^{-1} in the beeswax-coated salami until 6 months of aging (median 0.697, max 0.795) and significantly higher in the uncoated salami (median 1.176, max 1.227). A slight correlation between the amount of TBARS and A_w was observed in beeswax-coated salamis, whereas this effect was masked in controls by the large A_w variability observed at 7 months. Beeswax coating prevents case hardening and facilitated the peeling.

1. Introduction

Beeswax is natural glazing agent that can be used in food to prevent water loss and provide protection during storage. It is often used to prevent water loss and retard shrinkage and spoilage in fruit and cheese. Refined beeswax coating is a natural alternative to plastic envelopes that does not harm the environment and meets the criteria for GRAS status defined by the FDA for food packaging materials [1]. It is approved for food use in most countries and in the European Union under the E number “E901” [2]. Colour changes occur due to oxidation phenomena involving myoglobin during ripening of salami. Moreover, shrinkage due to dehydration results in aspect modifications, mainly ascribable to fat aggregation [3]. Even after salami has reached water activity (A_w) and pH values that make the product shelf stable without refrigeration, environmental conditions for storage (relative humidity, relative air speed, temperature, and light) need to be controlled to prevent excessive water loss, product shrinkage and too hard consistency, oxidative changes, and excessive growth of moulds. During distribution salami is often wrapped in plastic film with reduced oxygen and water vapour permeability to prevent

contamination by dirt and off-odour and protect the products from oxygen and loss or uptake of moisture. Oxygen moisture barrier properties of beeswax are intermediate between low and high density polyethylene [4]. It is also a barrier to photooxidation. Beeswax is also one of the most effective materials employed to decrease water vapour permeability of edible films due to its high hydrophobicity and solid state at room temperature [5, 6]. These properties have been used to preserve and improve the sensorial quality of salami during aging. Local producers in the area of Bologna (Italy) used to coat salami with beeswax, after the products are shelf stable (i.e., water activity has decreased to values below 0.92). They use the natural beeswax to limit an excessive water loss during storage. Wax coating also prevents case hardening and mould development and made the peelability easy. This study was aimed at assessing the oxidative stability and sensorial characteristics of salami after beeswax coating.

2. Materials and Methods

2.1. Reagents. The reagents were as follows: trichloroacetic acid (TCA) 99%; 1,1,3,3-tetramethoxypropane (TMP) 99%;

2-thiobarbituric acid (TBA) 98% (Sigma-Aldrich, Italy); ethylenediaminetetraacetic acid disodium salt (EDTA) (AnalR® VWR); propyl gallate (Fluka); hydrochloridric acid 37% (Merck); sodium hydroxide 20% water solution (Carlo Erba, Italy); TCA 100% p/v (100 g TCA 99%, water up to 100 mL); TMP stock solution (1000 mg kg⁻¹); extracting solution (75 mL TCA 100%, 20.8 mL HCl 0.25 M, 1 g EDTA, 1 g propyl gallate, and water up to 1000 mL); TBA reagent (TBA 80 mM in in NaOH 1 M, pH corrected at 4.0–4.2 with HCl 1 M); TMP (5 mg kg⁻¹) working solution (250 µL TMP stock solution, 3.75 mL TCA 100%, and HCl 0.25 M up to 50 mL).

2.2. Samples. Twelve salamis “Felino IGP” were provided by a local producer at approximately 55 days of ripening. The salami had the typical characteristics (length 40–45 cm, diameter approximately 6 cm, weight 1.0–1.2 Kg, weight loss 35–37.5%, and water activity 0.90–0.92). The salamis derived from a single lot were divided into two groups. Six were used as control. The remaining were brushed, washed, and put back to dry in ventilated cells. Then they were wrapped in a cotton gauze and tied and covered with beeswax (yellow organic beeswax melted and held at 62–64°C) for 3–5 seconds. The resulting wax layer had a thickness of 2–3 mm. Therefore, all salamis were hang up to dry in well-aired cellars up to 5–7 months, packed in cartoons, and stored in a dark room at 5°C ± 1°C (relative humidity 85–90%) until analyses. Comparative assessments were made on two salamis for each treatment group (wax coated and uncoated) at 5, 6, and 7 months.

2.3. Quantification of TBARS. Three slices (5 mm thick) were taken from centre and intermediate parts from each salami. These samples were minced for 10 seconds with a Moulinette® and 2.5 g of the homogenate was analysed for 2-Thiobarbituric Acid Reactive Substances (TBARS) using the method developed by Wang et al. [7] for meat and meat products. The entire protocol (sampling and analyses) was repeated two times (i.e., after 2–3 days) using other portions of each salami. A total of eight measurements (2 salamis, 2 replicates, and 2 repetitions) for each treatment group and storage time were obtained. Sample homogenates were put in vials in an ice bath, mixed with 20 mL of chilled extracting solution, and homogenised at 16,000 rpm for 2 minutes with Ultra-turrax® (model T25 basic IKA, Labortechnik, Italy). Additional 5 mL of extracting solution was used to wash the blades of the Ultra-turrax (final dilution 1:10); then the extracted samples were filtered (Whatman® paper filter n.4). Samples were continuously maintained in a chilled bath until the filtration. Two mL of the filtrates was mixed with 2 mL of the TBA reagent and incubated at 40°C in a water bath for 90 minutes and then chilled in fridge at 6–8°C for 30 minutes. Therefore the absorbance at 532 nm was read (5 replicates) on a spectrophotometer (Perkin-Elmer, model Lambda 1). A calibration curve was designed using standards at concentration in the range of 0.025 to 0.7 mg mL⁻¹ of TMP (5 mg kg⁻¹) working solution. By using the above-mentioned TMP solutions, malondialdehyde (MDA) standards in the

range of 0.15 to 4.26 nmol mL⁻¹ were prepared. Nine TMP standards (from 20 to 560 µL of the TMP working solution) were mixed with 2 mL of TBA reagent, 300 µL of TCA 100%, and HCl 0.25 M up to 4 mL. A blank solution was made as described before, but without TMP. Vials with the TMP standards and blank were incubated as described for the samples and the absorbance values at 532 nm (Abs₅₃₂) were read (average of 5 replicates). Ten-point standard calibration curves were designed. The coefficient R² must be between 0.995 and 1 in order to accept the curves for TBARS quantification. The samples’ TBARS concentration was calculated by interpolation of their measured absorbance values (Abs). The resulting value was multiplied by the dilution factor of the sample (2.5 g in 25 mL) and extract (1:2) to correct for the final concentration. Results are expressed as mg kg⁻¹ of MDA equivalents TBARS.

2.4. Water Activity. The water activity (Aw) was assessed with a dew point water activity meter (Aqualab Series 3, Decagon, US) using the procedures recommended by the producer.

2.5. Sensory Test. A hedonic test was conducted with eight untrained assessors who scored the acceptability of 3 attributes (texture, taste, and flavour) using the following 1–10 point scale: texture (1 = hard; 10 = soft); presence of acid taste (1 = sharp burning; 10 = mild acidic); rancid off-flavour (1 = none; 3 = slightly perceived; 10 = strong). With this aim, the salamis analysed for the TBARS at 6 and 7 months of storage were cut in slices and the panel was asked to comparatively assess the quality of the salami (blind test between beeswax-coated or noncoated salami).

2.6. Statistical Analysis. TBARS values recorded for different categories (wax or not coating) and periods (0, 1, and 2 months of storage) were summarized graphically as box and whiskers plots. Statistical analyses were performed using the R package “stats” (version 2.15.3) [8]. Data relative to samples from different categories and period were analysed with Bartlett’s test to assess homoscedasticity (homogeneity of variance). When departures from normality of data were observed nonparametric alternatives to the analysis of variance (ANOVA) were used. In particular, the Wilcoxon signed-rank test was used for comparing the MDA equivalent TBARS values observed in salami packaged with or without wax. Differences among samples taken at 5, 6, and 7 months were analysed with the Kruskal-Wallis rank sum test. When differences were considered to be significant at $p \leq 0.01$ the significance of individual pair differences (aging periods) was tested for inequality using the multiple comparison test after Kruskal-Wallis using the R package “pgrmness.”

3. Results and Discussion

3.1. Effect of the Beeswax Coating on the TBARS Concentration. Slower drying and ripening at low temperatures of the beeswax-coated salami resulted in less lipid oxidative changes. The concentration of TBARS (MDA equivalents) is reported in Table 1. Values were below 0.8 mg kg⁻¹ in

TABLE 1: TBARS concentrations in salami according to aging period and beeswax coating.

Ageing (months)	Coating	TBARS (MDA mg kg ⁻¹)			Wilcoxon ¹ signed-rank test
		Mean ²	Median	Range	
5	Beeswax	0.693 ^a (±0.017)	0.696	0.653–0.713	<i>p</i> = 0.0039
	Control	1.016 ^b (±0.045)	1.029	0.938–1.059	
6	Beeswax	0.678 ^a (±0.075)	0.697	0.561–0.795	<i>p</i> = 0.0039
	Control	1.166 ^c (±0.045)	1.176	1.104–1.227	
7	Beeswax	1.106 ^b (±0.039)	1.098	1.048–1.173	<i>p</i> = 0.0039
	Control	1.869 ^d (±0.023)	1.872	1.819–1.897	

Note: mean, median, and range calculated from $n = 8$ measurements (i.e., 2 samples, 2 replicates, and 2 repetitions for each treatment group). Samples with significant differences in their malondialdehyde level are indicated by different letters; ¹significant differences detected between samples at the same aging period (Wilcoxon test).

²Significant differences detected between samples at different aging period (Kruskal-Wallis test) chi-square = 15.3934; *p* value = 0.0004543.

the beeswax-coated salami until 6 months of aging (median 0.697, max 0.795) and significantly higher in the uncoated salami (median 1.176, max 1.227). At 7 months the MDA median values were equal to 1.098 and 1.872 mg kg⁻¹ in wax coated and uncoated salami, respectively. Limits for TBARS (MDA equivalents) have been suggested at 0.5 mg kg⁻¹ of meat for threshold of consumer detection of rancidity [9–11] and 1.0 mg kg⁻¹ for sausage products [12]. However, detection limits have not been set for salami. The lipid peroxidation in raw ripened sausages (salami) involves transformation of primary products of lipid degradation (alkyl free radical with a group of conjugated bonds) into secondary products, including MDA [13–15], and this is correlated to the increase of TBARS concentration. The presence of high TBARS values in sausages after ripening can be explained by availability of oxygen consequently to the mechanical process [16]. However, the use of vacuum stuffing, antioxidants, the protective effect of some starters [17], and also the storage of ripening sausage in vacuum or modified atmosphere without oxygen [18] can significantly contribute to an increased oxidative stability. The latter condition can occur with the use of beeswax coating. In a study of Novelli et al. [19] concerning “Milano” salami with similar fat content (approximately 30%), the TBARS values were equal to 1.39 ± 1.08 mg MDA kg⁻¹ at >3 months of aging, which is a value higher than those of the beeswax-coated salami at 7 months of aging.

Even if the consumers do not perceive any flavour deterioration, lipids oxidation involves loss of unsaturated fatty acids (nutrient loss) and the end products of lipid oxidation may be mutagenic and carcinogenic. Malondialdehyde (MDA) can react with DNA and form MDA adducts [20, 21]. Storage of ripening sausage in vacuum or modified atmosphere has resulted in increased oxidative stability of raw sausage after 2 and 5 months of storage [18]. Beeswax coating appears to give similar protection.

3.2. Correlation between TBARS Concentration, Aw, and Sensory Evaluations. The beeswax-coated salami had significantly higher mean Aw values, from 0.903 to 0.888, between 5 and 7 months of aging. The uncoated salami had Aw values in a range of 0.812 to 0.821 (Table 2). The sensory test did not reveal relevant differences in the taste and flavour of different

TABLE 2: Changes in the water activity values (Aw) in salamis with or without beeswax coating.

Ageing period	Control	Beeswax coating
5 months	0.821 ± 0.001 ^a	0.903 ± 0.002 ^c
6 months	0.812 ± 0.002 ^a	0.899 ± 0.002 ^d
7 months	0.820 ± 0.011 ^a	0.888 ± 0.004 ^e

Aging period at the beginning of the shelf life = 5 months.

Differences related to coating: F calculated = 1192.81; F critical = 4.75; *p* value = 2.21 × 10⁻¹³.

Differences related to aging (in salami with beeswax coating): F calculated = 23.03; F critical = 5.14; *p* value = 0.0015.

Means with different letters are significantly different.

treatment groups, whereas texture was softer in the beeswax-coated salami (Figure 1). The softer texture was related to the lower loss of water. There is a statistically significant relationship between TBARS and Aw values, but only for the beeswax-coated salami (Spearman's rank correlation rho = -0.623; *p* value = 0.0011). Within this group the concentration of TBARS slightly increased in consequence of the water loss, but this effect was masked in controls by the large Aw variability observed at 7 months (Aw = 0.820 ± 0.011; range 0.814–0.832) (Table 2 and Figure 2). Any difference was perceived during the sensory test with regard to sharp burning taste (Wilcoxon paired sign test *p* value > 0.05). Also the presence of rancid off-flavour was not perceived and only two panel members (out of eight) reported value above the limit of perception in the uncoated salami at 7 months of aging. The panel test probably did not give rise to a negative score (rancid off-flavour) because the TBARS concentration was relatively low (1.819–1.897 mg MDA kg⁻¹) also in these samples. An unpleasant fruity flavour was perceived by some panel members in the salami coated with beeswax at 7 months of aging. This flavour deterioration might be related to spoilage by heterofermentative lactic acid bacteria in consequence of the higher Aw [22, 23]. There are no published studies available that define TBARS values that are associated with the presence of rancid off-flavours in “Felino” salami.

Šojić et al. [24] reported that vacuum and MAP packaging can contribute to better oxidative and sensory stability of

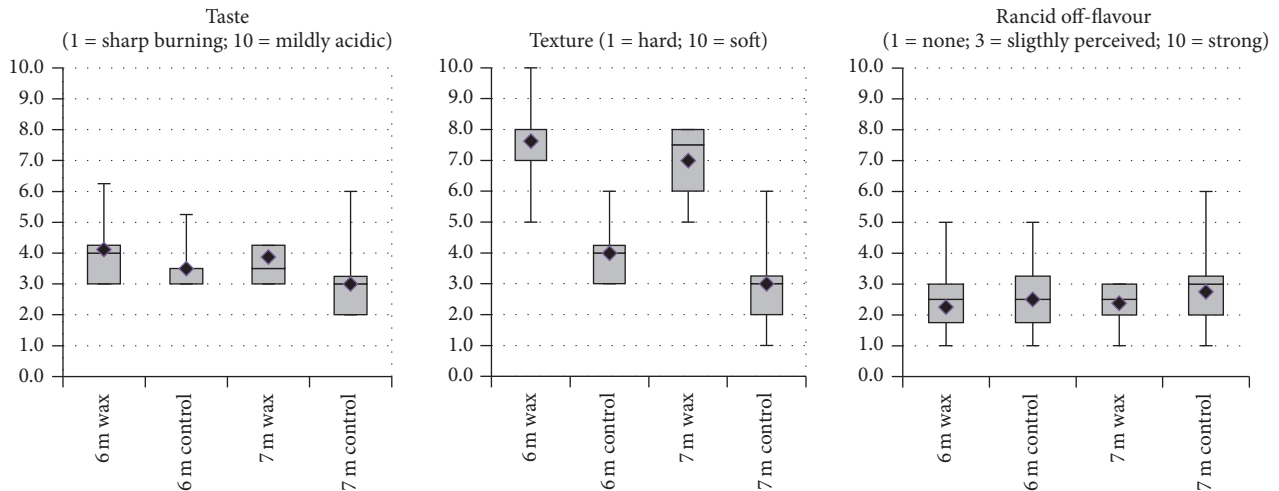


FIGURE 1: Box and whiskers plot for sensory quality of salami at 6 and 7 months of aging. Notes: box indicates quartiles and the central line the median. The lines (“whiskers”) show the largest or the smallest observation. The symbol \blacklozenge indicates average values.

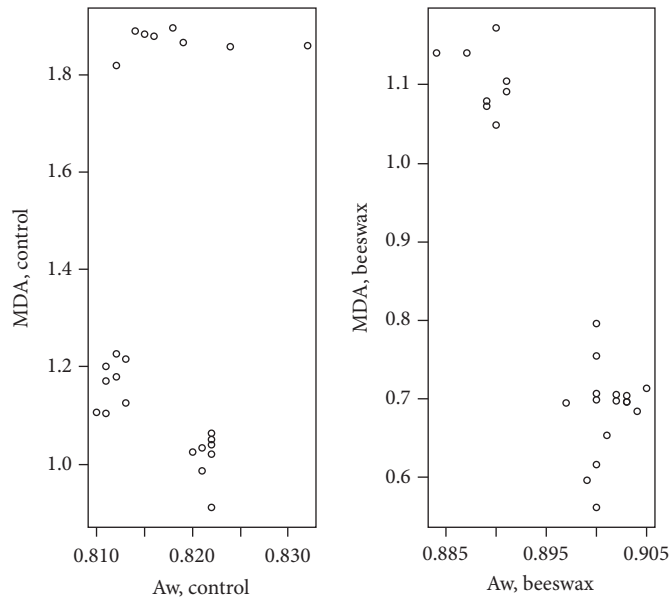


FIGURE 2: Relationship between TBARS and Aw values.

dry fermented sausage and that TBARS values are negatively correlated with odour and taste of salami aged for 7 months. Bañón et al. [25] observed that the main causes of loss of sensory quality of salami limiting their shelf life under the tested retail (aerobic) conditions include flavour deterioration associated with rancidity, bitterness, and mouldy off-flavour, together with hardening and loss of juiciness.

4. Conclusions

The results of this preliminary study indicate that beeswax coating can be a useful alternative to the plastic packaging. This natural coating material can be used to increase the aging period without compromising the texture, which remains soft and is appreciated for its better flavour. Beeswax effectively

reduces the development of lipid peroxidation products in these salamis.

Additional Points

Practical Applications. Beeswax coating can be used to preserve and improve the sensorial quality of salami during aging. It can be a natural packaging material that does not harm the environment and has good oxygen moisture barrier properties.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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