Sensory profile of warmed-over flavour in tenderloin from steers supplemented with alpha-tocopherol

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ABSTRACT - The objective of the present study was to evaluate the occurrence of warmed-over flavour (WOF) in cooked tenderloin and the influence of alpha-tocopherol on its inhibition. A total of 24 animals were confined, 12 of which received 1200 mg/head/day of alpha-tocopherol acetate for 90 days. Longissimus dorsi muscle cuts (tenderloin) were obtained for sensory profile assessment by nine trained tasters. The tasters evaluated the taste of the meat based on four general and 18 specific attributes. The results of the evaluations were analysed with ANOVA, post-hoc tests of the means (Tukey tests), and principal component analysis (PCA). There was no significant difference in the WOF between the cuts of meat from the supplemented and non-supplemented animals. However, as the refrigeration period increased, there was a decrease in the intensity of the umami and sweet taste attributes and the flavour and aroma of the roast meat as well as an increase in the intensity of the oxidised vegetable oil flavour and the aromas of fish, hard-boiled egg, flaxseed oil, and oxidised vegetable oil. The samples that had been stored for one day were characterised by PCA as having sweet and umami tastes and the flavour and aroma of roast meat, whereas after three days, the samples were classified as having sour and bitter tastes, the flavour of chicken and nuts, and the aroma of fish. The typical sensory attributes desirable for roasted meat decreased in intensity during the three days of storage after cooking, whereas the intensity of unpleasant (oxidative) attributes for the consumer increased.

Key Words: antioxidant, cooking, lipid oxidation, rancidity, storage, vitamin E

Introduction

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products. Post-slaughter biochemical changes that are involved in the transformation of muscle into meat, accompanied by the loss of antioxidant cellular defences, facilitate the oxidative processes of meat lipids (Henckel et al., 2000). Cooking enhances oxidation, as it causes tissue rupture, which releases iron, and causes protein denaturation, inactivating antioxidant enzymes (Souza et al., 2007).

The term warmed-over flavour (WOF) is used to define the rapid increase in oxidation in cooked meat products, which is characterised by the rancid flavour developed during storage under refrigeration (Mielche & Bertelsen, 1994). The cooking temperature, time, and final internal temperature of the meat can influence the development of WOF (Broncano et al., 2009). The effects of such cooking parameters are related to differences in the formation of Maillard reaction products (MRP) in the meat, which may include antioxidants suitable for preventing the development of WOF in cooked meat (Bailey, 1998).

The development of WOF can be effectively controlled or delayed by the use of antioxidants, which can be used alone or in combination. Among the most commonly used are chelating agents (phosphates, EDTA, etc.), synthetic phenols (BHT, BHA, etc.), natural antioxidants (flavonoids, alpha-tocopherol, etc.), and nitrates (Ahn et al., 2007).

Alpha-tocopherol has antioxidant effects on cellular and subcellular membranes, which are likely the sites where WOF starts. Additionally, alpha-tocopherol maintains its antioxidant activity in cooked products because it is resistant to heat (Descalzo & Sancho, 2008). Furthermore, diets supplemented with alpha-tocopherol have been shown to reduce the oxidative effects of salt and the development of thiobarbituric acid-reactive substances in chicken and pork (Hasty et al., 2002; Juntachote et al., 2007) and to slow the oxidation of cholesterol after cooking pork (Souza & Silva, 2006). In fresh beef, retardation of lipid oxidation and reduction in the formation of metmyoglobin have also been observed (Luciano et al., 2009).

Previous studies have shown that descriptive sensory analysis is an excellent tool for the evaluation of WOF in meat (Byrne et al., 2001; Bryhni et al., 2003). Therefore,
the present study was developed to evaluate WOF in roast beef and the influence of alpha-tocopherol on its inhibition.

**Material and Methods**

*Longissimus dorsi* muscle samples (tenderloin) were obtained from Nellore bull calves (±24 months) that had been castrated and confined until a 6 mm thickness of subcutaneous fat was achieved. A total of 24 animals were confined, 12 of which were supplemented for 90 days with 1200 mg/head/day of alpha-tocopherol acetate (1200 IU); the 12 other animals received no supplement.

The procedures for meat handling and processing during and after slaughter were similar to those employed in slaughterhouses with federal inspections. The pH of the meat was measured after maturation, and the values were between 5.5 and 5.8.

The muscles were stored for 14 days at 2 °C and then frozen at -27 °C until required for analysis (90 days). Muscles were kept at 5 °C for approximately 12 hours to thaw and subsequently cut into 1.5 cm-thick steaks. These steaks were roasted (in an electric oven, brand Venâncio, model FEP 90 série Itália) until they reached an internal temperature of 70 °C (measured with a metal thermometer, brand TESTO, model 0602.5792). Once this temperature was reached, the edges of the meat were removed, and the steaks were cut into ±1 cm$^3$ cubes, thereby avoiding visible fat and connective tissue. The cubes of the roasted meat were placed together and mixed to randomise the sample; then, groups of three pieces were selected, covered with parchment paper, wrapped in polyethylene bags (20 × 30 cm), and kept refrigerated (±5 °C) until use (1, 2, or 3 days). For sensory evaluation, the meat was reheated in bain-marie at 70 °C for 5-10 minutes and then served to the panel of tasters. Each package of three pieces represented a repetition of each treatment, totalling three packs (repetitions) per treatment for each taster.

The sensory profile of each sample was determined by nine trained tasters following the methodology of quantitative descriptive analysis (QDA) (Stone & Sidel, 1985), which was modified to exclude the appearance and texture attributes.

All of the sensory tests were performed at the Laboratory of Sensory Analysis in the Department of Food and Nutrition, Faculty of Food Engineering (UNICAMP) in individual booths with red lighting for the assessment of aroma and flavour.

The development of descriptive terminology for the roasted meat samples (tenderloin) was conducted using the Network Method (Moskowitz, 1983). An assessment form was prepared using descriptive terms, which consisted of four general and 18 specific attributes (Table 1). To facilitate understanding and consensus of the tasters, the definitions of each descriptive term were established by the sensory team.

Each specific attribute was assessed using an unstructured scale of 9 cm; the extremes of which were defined in terms of the minimum and maximum intensity of each specific attribute. For the specific attribute of roast meat, the intensities of the general attributes of aroma and flavour ranged from “low” to “high”, whereas for all of the other specific attributes, the intensities ranged from “none” to “high”. The design was of completely randomized blocks (Meilgaard et al., 1987).

Tasters were assessed using analysis of variance performed on the results of each taster (sources of variation: samples, replicates). The tasters exhibited good discriminative power ($P_{\text{sample}} \leq 0.50$) and good repeatability of judgements ($P_{\text{replicates}} \leq 0.05$), and there was consensus among all of the tasters. Analysis of variance was also used to evaluate the results of the quantitative descriptive analysis via an assessment of the F values for the taster, the sample, and the taster × sample interaction. In this case, significant F values ($P > 0.05$) for the taster attribute (indicating that the tasters used similar parts of the scale to express the sensation caused by the same sample), insignificant F values ($P < 0.05$) for the sample (indicating that the tasters identified differences between samples), and significant F values ($P > 0.05$) for the interaction (indicating that there was consensus among the tasters) were considered to be ideal.

The sensory profile of the tenderloin samples was determined by the tasters of the sensory descriptive team. The results of the assessments made by these judges were subjected to analysis of variance, post-hoc tests of the means (Tukey tests), and principal component analysis (PCA) using the statistical programme SAS (Statistical Analysis System, version 6.12). The tasters tasted replicates (as described above) of the samples from the two treatments.

### Table 1 - General and specific attributes used for the descriptive evaluation of the samples

| General attributes | Specific attributes                                                                 |
|-------------------|-------------------------------------------------------------------------------------|
| Aroma             | Roast meat, chicken, fish, flaxseed oil, oxidised vegetable oil, nut, hard-boiled egg, cardboard |
| Taste             | Salty, sweet, bitter, sour, umami                                                  |
| Flavour           | Roast meat, chicken, oxidised vegetable oil, nut                                   |
| Aftertaste        | Umami aftertaste                                                                   |

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(meat originating from animals treated with 0 or 1200 mg vitamin E). The sources of variation considered were the samples, the tasters, and the sample × taster interaction.

**Results and Discussion**

There was no significant difference (P>0.05) in the production of WOF in the meat from the animals that did and did not receive the alpha-tocopherol acetate supplement. It is likely that the levels of alpha-tocopherol in the muscles were insufficient to avoid oxidation during cooking and subsequent refrigeration. However, the experimental results were very interesting regarding the production of WOF in cooked meat and its characterisation during three days of storage under refrigeration (5 ºC).

As there was no significant difference (P>0.05) between the two levels of supplementation, the discussion in this paper will focus on the sensory behaviour of the roasted tenderloin samples during the three periods of refrigerated storage.

The performance of each taster was evaluated. All of the tasters had good discriminatory capacity of attributes, judgement repeatability, and consensus with the sensory team for most attributes. A few undesirable values were observed for the discrimination between the samples and repeatability, which were distributed across the tasters and the attributes. The analysis of variance had a significant (P<0.05) F value for the taster × sample interaction, but this interaction was not considered to be important when the sample × intensity interaction was graphically analysed for each attribute evaluated by the judges. The F value of the sample was significant (P<0.05), indicating that the tasters identified differences between at least two of the samples tested, and finally, the F value of the taster was also significant, indicating that the tasters used different parts of the scale to rate the intensities of the attributes, which is not uncommon and is difficult to avoid in sensory analyses.

The analysis of taste (Figure 1a) was based on five specific attributes: sweet, salty, sour, bitter, and umami. The results clearly demonstrate that there is a decrease in the intensity of the sweet and umami tastes as the cooling period of the roasted meat sample increases. It is clear from the results that the development of WOF is inversely related to the sweet and umami tastes. The sour and bitter tastes increased with WOF development, which was expected, since the process of lipid oxidation creates hydroperoxides, which form several compounds, including organic acids, such as acetylsalicylic acid. Furthermore, the continued interaction between lipids and proteins produces compounds with a bitter taste (Lillard, 1987). The

![Figure 1 - Mean taste (a), flavour (b), and aroma (c) attributes for warmed-over flavour in roast tenderloin.](image-url)
salty taste did not appear to influence the process, despite a significant increase, perhaps due to the dehydration that may have occurred.

Regarding the flavour attributes (Figure 1b), roast meat flavour and umami aftertaste were more important in the perceptions of the tasters and, similar to the sweet and umami taste attributes, decreased during the period of refrigerated storage before reheating. The oxidised vegetable oil flavour followed the same behaviour as the bitter and sour tastes, increasing in intensity over time. These results confirm the findings of previous studies (Byrne et al., 1999a, 1999b, and 2001), which showed a negative correlation between the attributes associated with the typical flavour of cooked meat and those related to the “off-flavour” or the strange taste of WOF.

Reinforcing the observations made, the aroma (Figure 1c) followed the same trend as flavour and taste. There was a decrease in the intensity of the roasted meat aroma, whereas the aromas of chicken, hard-boiled egg and flaxseed oil increased in intensity with increasing storage time (Table 2).

Liu et al. (1987) concluded that the concentrations of heteroatomic compounds may have been reduced during the development of WOF. Once these compounds, including many products of the Maillard reaction, appear to be the main constituents of the characteristic flavours and aromas of meat then, the reduction of roast meat flavours and aromas during WOF development is to be expected. It is also possible that the sensorial properties of these compounds can be masked by the production of other compounds that contribute to the undesirable flavour of WOF.

The same authors mention that the development of undesirable flavours may result in the production of “key components”, which may be heteroatomic compounds. This line of thought can be corroborated by the fact that furans and thiophenes containing sulphur substituted in the second position of the aromatic ring, which present characteristic “sulphur” or “burned” aromas, may exhibit the aroma of cooked meat when the replacement by sulphur occurs in the third position of the aromatic ring (Madruga, 1997).

When the data collected in the present study were subjected to PCA, the similarities and differences between the samples were revealed, clearly displaying the sensory properties of each sample. In PCA, similar samples occupy neighbouring regions in the graphic and are characterised by the vectors (specific attributes) that are closest to them. The results of the PCAs for taste, flavour, and aroma (Figure 2) confirm the results based on interpretation of the data presented in Figure 1 and of the analysis of variance and post-hoc means test (Table 2).

Table 2 - The values from the sensory team for each attribute used to evaluate roasted tenderloin

| Specific attributes | Sample | Sensory Team |
|--------------------|--------|--------------|
| Aroma              |        |              |
| Roast meat         | 1 day  | 5.47a        |
|                    | 2 days | 4.37b        |
|                    | 3 days | 3.67c        |
| Chicken meat       | 1 day  | 1.86a        |
|                    | 2 days | 2.47b        |
|                    | 3 days | 2.35b        |
| Fish               | 1 day  | 0.81a        |
|                    | 2 days | 0.73a        |
|                    | 3 days | 0.86a        |
| Flaxseed oil       | 1 day  | 1.23a        |
|                    | 2 days | 1.50b        |
|                    | 3 days | 1.48b        |
| Oxidised vegetable oil | 1 day  | 1.36a        |
|                    | 2 days | 1.45a        |
|                    | 3 days | 1.49a        |
| Nutty              | 1 day  | 0.67a        |
|                    | 2 days | 0.72a        |
|                    | 3 days | 0.69a        |
| Hard-boiled egg    | 1 day  | 1.33a        |
|                    | 2 days | 1.71b        |
|                    | 3 days | 1.70b        |
| Cardboard          | 1 day  | 1.35a        |
|                    | 2 days | 1.53a        |
|                    | 3 days | 1.48a        |
| Taste              |        |              |
| Salty              | 1 day  | 1.99a        |
|                    | 2 days | 1.94a        |
|                    | 3 days | 2.12a        |
| Sweet              | 1 day  | 1.66a        |
|                    | 2 days | 1.57a        |
|                    | 3 days | 1.19b        |
| Sour               | 1 day  | 1.49a        |
|                    | 2 days | 1.78ab       |
|                    | 3 days | 1.95b        |
| Bitter             | 1 day  | 1.04a        |
|                    | 2 days | 1.16a        |
|                    | 3 days | 1.49b        |
| Umami              | 1 day  | 3.06a        |
|                    | 2 days | 2.64b        |
|                    | 3 days | 2.19c        |
| Flavour            |        |              |
| Roast meat         | 1 day  | 5.45a        |
|                    | 2 days | 4.17b        |
|                    | 3 days | 3.41c        |
| Chicken meat       | 1 day  | 1.48a        |
|                    | 2 days | 2.00b        |
|                    | 3 days | 1.84ab       |
| Oxidised vegetable oil | 1 day  | 1.20a        |
|                    | 2 days | 1.72b        |
|                    | 3 days | 1.75b        |
| Nutty              | 1 day  | 0.80a        |
|                    | 2 days | 0.83a        |
|                    | 3 days | 0.71a        |
| Aftertaste Umami   | 1 day  | 2.67a        |
|                    | 2 days | 2.23b        |
|                    | 3 days | 1.74c        |

Values with different letters in the same column and attribute are significantly different (P<0.05).
In the PCA of taste (Figure 2), the sample from one day of refrigerated storage was characterised by the sweet and umami tastes. After three days of refrigerated storage, the samples were mainly characterised by the sour taste, as well as by the bitter taste.

In the PCA graph that characterised the flavour attributes, the same trends found for taste were observed, with the typical attributes of roast beef (roast meat flavour and umami aftertaste) characterising samples from one day of storage and the samples stored for three days characterised by oxidative attributes (nutty and chicken flavours). The samples stored for two days were, in most cases, intermediate between the samples from one and three days of storage for all of the groups of attributes and tended to be more similar to the samples stored for three days.

In the PCA graph of flavour, it can be clearly noted that the samples stored for one day were characterised by the vector representing the aroma of roast beef, whereas the sample stored for three days was mainly characterised by the fish flavour vector.

**Conclusions**

Sensory analysis showed that there was no discernible benefit in providing an alpha-tocopherol supplement to steers. The addition of alpha-tocopherol did not reduce or delay the occurrence of undesirable warmed-over flavour in cooked tenderloin. The typically desirable sensory attributes of roasted meat decreased in intensity during the three days of storage after cooking, whereas the intensity of oxidative attributes, which are distasteful to the consumer, increased. Further studies with elevated levels of alpha-tocopherol can facilitate the understanding of the effect of this supplement on warmed-over flavour in cooked meat.

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