Evaluation of the Effect of Retort Processing on the Microbiological, Sensory Evaluation and Physicochemical properties of the Ready-To-Eat Grilled Beef

Abstract

Retort processing is a method of preserving food by heating it in hermetically sealed containers like cans, glass jars and retortable pouches. **Objective:**The objective of this paper is to look at the effect of retort processing on grilled beef in retort pouches for microbiological, physicochemical, and sensory quality. **Material and method**:Ready-to-eat (RTE) grilled beef was thermally processed at different Fo values (sterilization unit) of 8, 10 and 12 at the temperature of 121 °C. Before the thermal process, beef was marinated and grilled at 200 °C, for 20 min. The filled and sealed pouches were then subjected to retort processing for optimizing the Fo value at process temperature. Grilled beef without a retort process is subjected to a control sample. The effect of different Fo values on the microbiological (total plate count, yeast& mould, *Ecoli*, coliform. *Salmonella* and *staph aureus*) sensory evaluation and physicochemical propertieswere evaluated. **Results:**Results on the microbial analysis showed that there is no growth of bacteria for all Fo values tested. The sensory evaluation scored the highest for the product processed at 121 °C, Fo 12 for overall acceptance attributes. For moisture analysis, as the Fo value increases the moisture content decreases. Retort processed grilled beef had significantly lower *L**, *a** and *b** values as the Fo value increases. **Conclusion:**It is concluded that grilled beef product retorted to Fo 12, 121 °C, had acceptable microbiological limits, highest score of organoleptic evaluation and acceptable physicochemical characteristics.

Keywords: retort processing, grilled beef, retort pouches, ready to eat (RTE), Fo values

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Introduction

"Retort processing is considered as one of the most effective methods of preserving food" (Majumdar et al. 2017). The process involves placing the food in a pouch, can, or other sealed container, and then subjecting it to high-pressure steam or water, which heats the food to a high temperature (Bindu et al. 2012). Retort processing is commonly used in the food industry for preserving a wide range of food products, including meat, seafood, vegetables, and soups. The temperature typically varies between 110 and 121 °C, influenced by the applied pressure and the nature of the product (Mieszczakowska-Frąc et al. 2021). "An effective thermal process could be designed by varying the sterility level using different times (Fo) and temperatures. Nevertheless, thermal conditions could affect the taste, appearance and nutrition as well as bioactivity of final products" (Majumdar et al. 2015). Therefore, it is necessary to determine the optimum conditions to ensure the safety, appearance and taste of each product.

The Fo value is a measure of the lethality or sterilizing effect of a retort process. It is a measurement unit used to indicate the amount of time required to achieve a specific level of microbial destruction in a particular food product at a specific temperature. The Fo value is an important parameter in the retort process, as it ensures that the product is commercially sterile and safe for consumption (Shirtz 2008). It is also used to determine the minimum processing time and temperature required to achieve a specific level of microbial destruction, which can help to optimize the retort process and minimize the impact on the sensory and nutritional quality of the food.

"In Malaysia, retort processing using pouches as a packaging material is gaining popularity over metal containers due to its unique advantages. Various researches have

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demonstrated the technical and commercial feasibility of using retortable pouches for thermal processing" (Mohan et al. 2008 and Bindu et al. 2014). "These pouches provide numerous benefits, including extended shelf stability, reduced weight and storage space, easy opening and preparation, and improved quality due to minimize heat exposure" (Majumdar et al. 2017) Additionally, retort pouches demand less heat compared to cans for achieving commercial sterility, leading to decreased cooking time and energy costs (Majumdar et al. 2017).

Ready-to-eat (RTE) food refers to any food that has been prepared, cooked, and packaged for immediate consumption without any additional cooking or preparation. These foods are usually fully cooked or processed and can be consumed straight out of the packaging or after minimal heating, such as in a microwave or oven. The demand for convenient, RTEfood products is on the rise in both developed and developing countries. Consumers are increasingly seeking high quality and convenient food options, which has led to a surge in the commercial production of RTE products (Kanatt et al. 2000; Karadag and Gunes 2008). RTE foods are becoming increasingly popular because they are convenient and save time, making them a popular choice for busy people, students, and those who do not have access to cooking facilities.

Beef is a significant component of world consumers' diets especially in European countries (Zhang et al. 2022) and it ranks as the third most favored meat variety after pork and poultry (Bassam et al. 2022). "Beef is distinguished by a high nutritional value and exceptional organoleptic properties. It is a valuable source of protein, exogenous amino acids, and micronutrients that are important for human health, such as selenium, zinc, phosphorus, bioavailable iron, and vitamin B12" (Tkacz et al. 2022). "The preparation of beef for consumption typically involves some form of thermal processing, which has evolved over the years, encompassing techniques like cook-chill, grilling, ohmic heating, laser-based packaging

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and more" (Vieges et al. 2012). "Grilling, in particular, has gained increasing interest as a thermal process that employs temperatures exceeding 150 °C through conduction and direct/radiant heat transfer" (Jezek et al. 2020). More so, the application of grilling of various types to meat products has been reported by several researchers (Farhadian et al. 2010; Kerth et al. 2003; Khan et al. 2015; Gomez et al. 2019). Frediansyah et al. (2017) and Bindu et al. (2012) reported about the thermal processing of beef and poultry products in retort pouches. In their studies, RTE meat curry products were packed in retort pouches. In their case, the product was superior in all sensory attributes and it was concluded that chettinad style goat meat product retorted to a Fo value of 12.1 min, had acceptable sensory quality characteristics. Although several researchers have studied the retort processing of beef and poultry product previously, (Lee and Shin, 2023; Vismitha Shree et al. 2022;) the information related to physicochemical, microbiological and sensory evaluation of grilled beef with different Fo value was still scarce. Hence, the present study was conducted with the objective of evaluating the physico-chemical properties and sensory acceptance of retorted grilled beef.

Material and methods

Grilled beef preparation

High quality beef was procured from the NNM Food Industries Sdn. Bhd. located in Muar, Johor. Beef was then marinated with a combination of honey,soy sauce, chili powder,black pepper, vegetable oil, salt and sesame oil. Beef was then grilled at 200 °C for 20 min before being packed. About 250 g of grilled beef was weighed and packed in retort pouches. Adequate numbers of retort pouches were fixed with glands and thermocouples and the tip of the thermocouple was inserted into grilled beef. The sealed pouches were subjected to retort

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processing with different Fo values. Grilled beef samples without a retort process were subjected as control samples.

Retort processing of grilled beef

Retort processing of grilled beef was carried out in a horizontal water immersion clutch retort (Model H60, type C50, Toyo Seikan Kaisha LTD) located at Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor. Grilled beef was packed into retail size retort pouches measuring 130 x 170 mm. The retort pouches in the present study were processed with different Fo value 8, 10 and 12 and temperature 121 °C. Three thermocouples were inserted into three of the pouches and connected to an ELLAB temperature/Fo recorder (Model CTF 84). This recorder automatically converts the heat penetration data received into Fo value directly. Beef slices of equal size were inserted to the same depth into each of the thermocouples and the pouch filled to a required solid weight of 250 g. The pouches were then placed into separate compartments in the retort trays and the product retorted at 121 °C to achieve commercial sterility, based on the lowest sterility value obtained as given by one of the thermocouples. The filled pouches were placed on the tray and loaded in the retort machine. The thermal processing was carried out to achieve different Fo values. After attaining the required Fo value, the product temperature was brought down to 50 – 55 °C by pressurized cooling (compressed air and water) in 4 – 5 min. The cooled pouches were wiped dry and examined for any visual defects. Thermocouple outputs (time – temperature data) were analyzed using a computer. The heat penetration data were plotted on a semi-log paper with temperature deficit (retort temperature – cold spot temperature) on log scale against time.

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Microbiology Analysis

In the microbiology analysis, 10 g of grilled beef samples was taken aseptically from the packaging into a sterile stomacher bag, mixed with 90 mL Peptone solution (Oxoid, UK) and homogenized for 1 min in a stomacher (Stomacher, Seward 400, UK). Next, a serial dilution of 10^1 to 10^5 was carried out using peptone solution prior to plating. For total plate count (TPC) analysis, the pour plate method was performed using the following media and culture conditions: plate count agar (PCA) (Oxoid, UK) incubated at 35 °C for 48 ± 2 h. For yeast and mould counts and *Staphylococcus aureus* analysis, the spread plate method was performed using the following media and culture conditions: potato dextrose agar (PDA) (Oxoid, UK) with the addition of 10% tartaric acid incubated at 32 °C for 48 ± 2 h and baird parker agar (BPA) (Oxoid, UK) with the addition of egg yolk tellurite emulsion incubated at 37 °C for 48 ± 2 h, respectively.

For Coliforms and *Escherichia coli*, all counts were performed using 3M Petrifilm (3M, USA) incubated at 37 °C for 48 ± 2 h. After incubation, colonies were enumerated and results reported as colony form unit (CFU)/g of sample. For *Salmonella* analysis, about 25 g of samples was placed in a sterile plastic bag containing 225 mL of sterile buffered peptone water (BPW) (Merck, Germany) as the diluent and shaken for 2 min. The diluent was then incubated at 37 °C for 24 ± 2 h for pre-enrichment. An amount of 1 mL and 0.1 mL of the pre-enriched samples were transferred into 9 mL of selenite cystine enrichment (SC) broth (Merck, Germany) and 9.9 mL of Rappaport-Vassiliadis (RV) (Merck, Germany), and were incubated at 37 °C and 42 °C,

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respectively for 24 ± 2 h. After enrichment, one loop of RV and SC broth cultures were streaked on xylose lysine deoxycholate agar (XLD) (Merck, Germany), xylose lysine tergitol-4 agar (XLT-4) (Oxoid, UK) and rambach agar (RB) (Merck, Germany) then incubated at 37 °C for 24 to 48 h ± 2 h. Isolated colonies that showed typical reactions (XLD and XLT-4; dark red colonies with black centre, RB; bright red colonies) according to manufacturer's instruction were considered as presumptive *Salmonella*.

Analysis of color

The color measurement was measured using a chromameter (CR 400 Minolta). A grilled beef piece was placed over the light source and covered by an inverted black cup supplied with the equipment and post processing L*, a*, b* values were recorded. Five readings were taken for each strip and the average values were calculated. Values are expressed using the standard Hunter L*a*b* system. In this coordinate system, L*, a*, and b* refer to the three axes of the system: a lightness axis (white – black, L*); and two axes representing both hue and chroma, one red-green (a*) and the other blue-yellow (b*). Color was expressed as L* (brightness), a* (redness) and b* (yellowness).

Moisture content

The moisture content of RTE grilled beef was analyzed by using infrared moisture analyzer (MA 35, Sartorius Lab Instruments GmbH & Co. KG). The sample was placed on an aluminum dish and tested according to the manufacturer's instructions. The sample pan or container of the

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infrared moisture analyzer was opened and the weighed sample inside was placed. The container securely closed and the drying process using the moisture analyzer started. The halogen bulb in the instrument emits infrared radiation, which heats the sample and evaporates the moisture. The instrument continuously measures the weight loss of the sample as the moisture evaporates. The instrument displayed the moisture content of the food sample after analysis completed.

Sensory evaluation

"An acceptance test was carried out on the sensory evaluation of grilled beef in the matter of color, aroma, texture, taste and overall acceptance. Thirty-five untrained panelists who declared themselves regular consumers of beef/meat, were invited to participate in this evaluation. The panelists' ages ranged from 21 to 58, possess good health and non-smokers. The evaluation was conducted at the Food Sensory Laboratory, Food Science and Technology Research Center in MARDI under ambient temperature and fluorescent light. Tissue and plain water were given to all the panelists on a tray. Then, each of the samples was served to them in plastic cups with 3-digit random numbers labeled to them. Panelists were required to rinse their mouths after each sample evaluation before the next sample. Panelists then would have to answer a sensory evaluation form which had a 7-point hedonic scale anchored by: 1 = Strongly disliked; 2 = Moderately disliked; 3 = Slightly disliked; 4 = Indifferent; 5 = Slightly liked; 6 = Moderately liked, and 7 = Strongly liked" (Granato et al., 2010). Samples with the mean scores of more than 5.00 for overall acceptability were considered acceptable.

Data analysis

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All the analysis was carried out in triplicate. The data were analyzed statistically using SAS software to find out standard deviations and significant differences between samples.

Results and Discussion

In the present study grilled beef was processed with different Fo values 8, 10 and 12 and it was as per the recommended Fo value for meat products, which was 8 – 20 (Frott and Lewis 1994). Rajkumar et al. (2010) also retorted to a Fo of 12.1 for Chettinad style goat meat curry, an Indian heritage food. Similarly, Manzoor et al. (2017) processed Rogan Josh, a traditional meat product in a retort at 121 °C using F0 values ranging from 7 to 11. Our studies are also similar with the findings of Ranganna (2000), who reported Fo values between 8 and 12 min were suited for meat products. Gopal et al. (2001) reported Fo values of 6.56 and 8.43 in Kerala style fish curry and Shankar et al. (2002) recorded Fo value of 11.5 min in heat processed seer fish curry.

Microbiological analysis

Table 1 shows the microbiological analysis of freshly grilled beef (control) and grilled beef in pouch after the retort process. The total plate count (TPC), yeast, mould, *Ecoli*, coliform. *Salmonella* and *staph aureus*) were analysed after the retort processing.In microbiology, colony-forming unit (CFU, cfu or Cfu) is a unit which estimates the number of microbial cells (bacteria, fungi, viruses etc.) in a sample that are viable, able to multiply via binary fission under the controlled conditions. Counting with colony-forming units requires culturing the microbes and counts only viable cells, in contrast with microscopic examination which counts all cells, living or dead. No microbial growth was observed in any sample with different Fo values (Table 1). This finding indicated that the recommended thermal processing parameter had achieved commercial sterilisation of the processed grilled beef. In addition, the microbial counts of grilled

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beef before the thermal process were 2.2 x 10^3, for total plate count. The absence of microbial counts observed after the retort process of grilled beef confirmed the effectiveness of the retort process in reducing the microbial load of the product. Similar to pork curry samples were retorted at 121 ^oC and Fo 11.81 did not reveal any growth of total plate counts, including *E.coli*, Salmonella *spp*, Clostridium spp and Staphylococci spp during the storage period (Girish et al. 2018). Shah et al. (2017) also reported that no microorganisms were detected after processing Rogan Josh in a retort pouch with a temperature of 121 ^oC and Fo 7 to 11. Other study by Rajkumar et al.(2010) determined total viable, anaerobic, coliform, *staphylococcal, streptococcal, clostridial* and yeast and mould count of Chettinad goat meat curry retorted to a Fo value of 12.1 min and showed that the product was commercially sterile with no bacteria exist after retort process. For products that are to be stored and distributed under tropical conditions, it has been recommended that Fo value of 12 – 15 should be given compared to a Fo value of 4 – 6 for temperate countries (Anon, 1998). The present study showed that the thermal process given was sufficient to produce commercially sterile products. Based on the microbiological examination of the samples, it was recommended that the shelf life of the product under the packaging and storage conditions described above is at least 12 months. Therefore, it can be concluded that grilled beef using different Fo values is safe for consumption and meets the standards for commercial sterilization.

Sensory analysis

Retort processing can cause changes in the attribute of sensory analysis. Among key organoleptic attributes, it is believed that color, flavor and texture show strong influence on consumers'

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overall acceptability of meat products (Hadi et al. 2017). Grilled beef in retort pouch processed to three different Fo values were analyzed on a 7 – point hedonic scale by 35 semi trained panelist. The results of the sensory are presented in Figure 2. The sensory score given by the panel for color of the product was found to be 5.27, 5.33, and 5.53 for thermally grilled beef to Fo 8, 10, and 12, respectively. In the case of flavor, panelists scored 5.37, 5.73, and 5.83 for grilled beef for Fo 8, 10, and 12, respectively. It was observed from the above result that the retort pouch-grilled beef increased significantly ($p<0.05$) in color and flavor with the increase of Fo value. This may be explained by the prolonged heating, which favors the development of color and flavor in the finished product. It is similar with the finding from Majumdar et al. (2017) where the retort processed prawn shows an increase in sensory attributes as the Fo values increase. The overall acceptability of grilled beef retorted at Fo value 12 was the most preferred by the panelist, with the score given 5.89 ± 0.21 when compared to the other samples. The high temperatures and pressure can cause proteins in the meat to denature and coagulate, resulting in a firmer texture. This is particularly true for products that have been cooked prior to retort processing, such as canned meats. However, if the meat is not cooked prior to retort processing, it can become softer due to the breakdown of collagen and connective tissue (Bak et al. 2019).

Color and moisture analysis

Retort processing also affects the color of grilled meat products. Figure 3 below shows the color profile analysis of grilled beef in different Fo values. The lower L*, a*, b* and chroma values were noticed in the product due to retort pouch processing. The result is similar with the previous

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studies by Frediansyah et al. (2017) where retort process with Fo value of 4.1 decreased the significant color value in L*, a* and b*. Shigehisa et al. (1991) reported that "the decreasing color of L* has been shown in pork muscle on different range pressures of 0.1 – 0.6 KPa". Another study by Carlez et al. (1995) was reported that the color of minced beef was decreased when using high pressure.

The decrease in L*, a*, b* and chroma values due to retort processing can be attributed to the reduction in light reflection influenced by heating. Bindu et al. (2007) suggested that "Maillard reaction between sugar and amino acid could have reduced the color scores of the retort processed product". The Maillard reaction, stemming from non-enzymatic browning reactions between amino acids and reducing sugars, is fundamental in thermally processed foods, as noted by Cho et al. (2010). This chemical process enhances the appealing sensory attributes of baked goods, cocoa and coffee roasts, and meat cooking, including color, aroma, and flavor.The rate of the browning reaction is influenced by various factors including the properties of amino acids (which are proteins that undergo the reaction), carbohydrates, temperature, pH, moisture, oxygen, metals, and sulfur oxides. When exposed to heat, the color change in food is caused by the sterilization process, where iron is oxidized to form black iron (III) compounds. Temperature changes have a significant impact on the rate of browning, with a rapid increase observed with higher temperature. In foods with a sufficient amount of sugar, the rate of browning can increase by 5 – 10 times for every 10 degree increment in temperature. Consequently, foods with higher sugar content exhibit a faster browning rate, which is further enhanced by longer heating times (Bindu et al. 2007). In the present study, marinated grilled beef was sterilized for almost 30

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minutes to achieve Fo8 with the temperature 121^{o}C. The browning rate is considered to be higher as the lightness (L) decreases.

Retort processing can cause the moisture content of meat products to decrease. The high temperatures can cause evaporation of water, resulting in a dryer product. This can be mitigated by the addition of water or other moisture-retaining ingredients. Figure 1 shows the moisture content in grilled beef with different Fo values. From the results below, it can be concluded that the moisture content significantly decreased in grilled beef as the Fo value increased. The moisture of freshly grilled beef also decreased after retort process. Sterilization process of grilled food required a high temperature (121 °C). These high-temperature processes are allowed by evaporation of moisture content in grilled beef. Frediansyah et al. (2017) also reported "a significant decrease in moisture content during retort process of dried beef rendang production. Cooking losses tended to be linear with time and temperature of cooking. The higher time and temperature of cooking, the more moisture had been lost by evaporation".

Conclusions

Grilled beef was prepared and thermally processed at three different Fo values, i.e., 8, 10, and 12. The instrumental parameters, color and moisture followed the same trend and showed decreasing trends as the Fo values increased. The organoleptic evaluation scored the highest for the product processed to Fo 12. Observations show that Fo values of 12 were found to be optimum for processing of grilled meat product in a retortable pouch. Along with the current of modernity, consumers today expect something that is quick and easy but still maintains the

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optimal taste of food products to enjoy. The retort technology will help in popularization and proper utilization of meat products and ensure a steady supply of RTE convenience products of heritage value throughout the year.

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Table 1: Microbiological analysis on grilled beef at different Fo values

Sample	TPC	Yeast and	Coliform	*E. coli*	*S. aureus*	Presumptive

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description	(CFU/g)	Mould(CFU/g)	(CFU/g)	(CFU/g)	(CFU/g)	*Salmonella* in 25g
Freshly grilled beef **(**Control)	2.2 x 10^3	<1 x 10^2	<1 x 10	<1 x 10	<1 x 10^2	Not detected
Fo 8	<1 x 10	<1 x 10^2	<25 x 10	<1 x 10	<1 x 10^2	Not detected
Fo 10	<1 x 10	<1 x 10^2	<25 x 10	<1 x 10	<1 x 10^2	Not detected
Fo 12	<1 x 10	<1 x 10^2	<25 x 10 est (1.0 x 10)	<1 x 10	<1 x 10^2	Not detected

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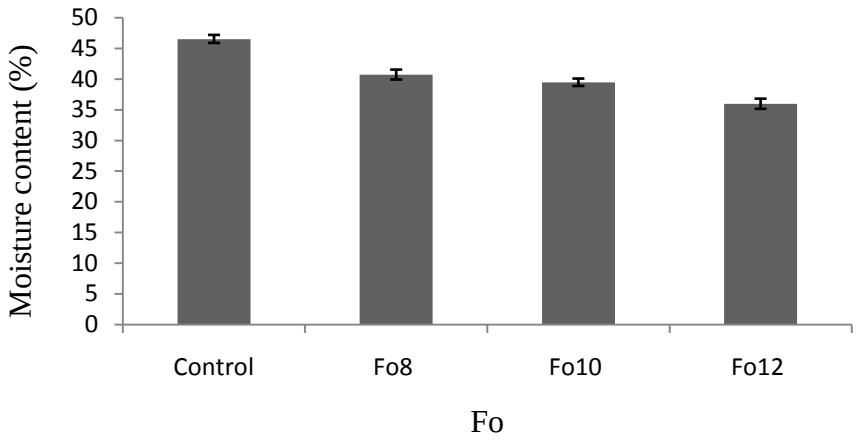

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462 Figure 1: Moisture content of RTE grilled beef in different Fo values

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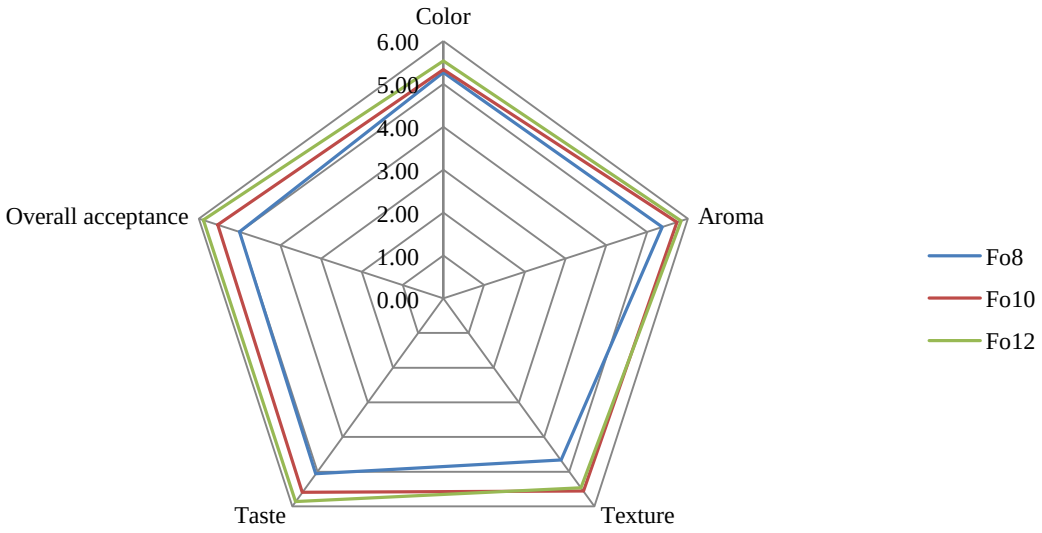

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466 Figure 2: Sensory analysis of RTE grilled beef at different Fo value
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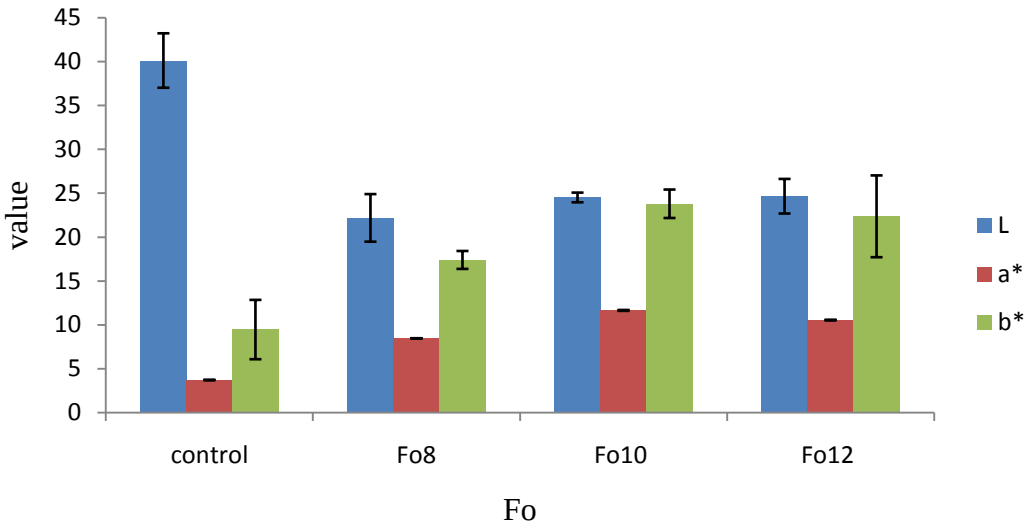

Figure 3: Color profile analysis of RTE grilled beef in different Fo values

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