THE EFFECTS OF NITRITE AND TRADITIONAL COOKING PROCESS ON THE SURVIVAL OF *CLOSTRIDIUM SPOROGENES* AND AUTOXIDATION IN KAVURMA, A TRADITIONAL TURKISH FRIED MEAT PRODUCT

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Background

Kavurma is a traditionally deep fried (coarsely ground) meat product and produced to extend the shelf life of the product for 6-9 months. In the past, this technology was used only to conserve meat, but currently it is preferred as a processed meat variety; although, some people living in many places in Turkey and the Middle-East are still processing kavurma for preservation purposes. In traditional processing, the meat from beef or mutton is ground (approximately 4x5x6 cm) and mixed with 2.0 to 5.0 % salt, and then fried or cooked in animal fat using a double sided steam cauldron. After the cooking process, it is kept in almost anaerobic condition which is provided by solidified animal fat in the container. Recently, it has also become available in vacuum-packaged forms produced in modern meat processing plants and sold in department stores and markets (Vural and Oztan, 1989; Anon., 1996; Gungor, 2000; Yetim *et al*., 2003). The popularity of ready-to-eat foods is increasing in the world because of changing life styles, and kavurma is also considered as a ready-to-eat meat product since it is generally consumed without further processing or cooking. The technological and hygienic insufficiencies during the production of kavurma may cause quality deteriorations in the product as well as a health risk associated with microorganisms.

Deteriorations occurring in kavurma are generally due to microorganisms. Although, it is heat treated, it may contain a small microbial load. Microorganisms surviving during the heat treatment of kavurma may proliferate if the product is stored under inappropriate conditions. When the microorganisms present in kavurma are pathogenic or toxic, there is a potential health risk to the consumers. For example, clostridia are considered important bacterial group in the heat treated meat products, because of their facultative anaerobic and spore forming nature; *C. botulinum* produces a deadly toxin which causes botulism.

Research on kavurma is very limited in the literature. A study on kavurma sold on local markets indicated that some thermophiles, subtilis-mesenticus bacteria and micrococci were commonly present in kavurma samples (Inal, 1992). Additionally, Sarigol (1978) determined the microbial load in kavurma stored under different conditions and reported that lipolytic and proteolytic microorganisms, yeast, mold and staphylococci were present in home type and industrial type of kavurma. Vural and Oztan (1989) investigated the effects of α - tocopherol and potassium sorbate on the enhancement of shelf life of kavurma, and determined that control and antioxidant added samples initially contained less than 10 bacteria/g while samples with antimicrobial agent had no microorganisms. They observed that total viable microorganism of the control sample continuously increased during 6-month of storage. Another study conducted for the determination of microbiological, physical and chemical qualities of kavurma sold in Bursa (Turkey) market revealed that a potential health risk might be evident resulting from the consumption of kavurma when the procuct is not hygienically produced or it is stored under improper conditions (Tiryakioglu and Yucel, 1995). On the other hand, Cetin (2000) reported some pathogenic microorganisms, such as *Salmonella*, *Morganella*, *Yersinia* or *Klebsiella* in the kavurma samples obtained from local stores in Erzurum. He pointed out that the kavurma samples containing these pathogens may pose a probable public health risk. Yetim et al. (2003) investigated the formation of nitrosamines in kavurma prepared with nitrite addition and concluded that there was no detectable amount of nitrosamine compounds in the kavurma samples.

Objectives

The objective of this study was to determine the influence of nitrite addition and the traditional cooking process of kavurma on the survival and proliferation characteristics of *C. sporogenes,* representing the *C. botulinum*, and their effects on rancidity which was evaluated by TBA, FFA values and peroxide number.

Materials and methods.

Traditional Kavurma was manufactured as described by Gokalp et al. (1994). In this study, 4 types of kavurma were prepared as illustrated in Table 1). 1) Control, 2) 100 ppm nitrite, 3) *C. sporogenes* and 4) Nitrite + *C. sporogenes* containing samples. A steam jacketed cauldron was used for kavurma processing and the internal temperature of the meat-fat mixture was approximately 98°C during cooking that took approximately 1.5 hours. Kavurma samples were filled into sterile fibrose casings and sliced and vacuum packaged under aseptic conditions before storage. All the samples were kept for 6 months under refrigerated (4±1°C) conditions.

Table 1. Kavurma samples prepared in experiment,

Sample	Kavurma Types
1	Control (contains only meat and fat)
2	Nitrite added sample (100 ppm)*
3	*C. sporogenes* inoculated sample (10^6 cfu/g)**
4	Nitrite + *C. sporogenes* (100 ppm + 10^6 cfu/g)

* : $NaNO_2$ was dissolved in distilled water and added into beef stew before cooking,
**: *C. sporogenes,* strain no: 413; the bacteria was obtained from Pendik Veterinary Control and Research Institute (Istanbul, Turkey) and proliferated in Cooked meat medium (Difco) before inoculation.

For the analysis, 25 g of Kavurma samples were homogenized using a Waring blender at 6000 rpm in 225 of ml sterile serum physiological salt solution for microbiological analysis. *C. sporogenes* count in the samples was determined by the pour plate technique using Heart infusion agar (Oxoid). Plated samples with anaerobic kit (Oxoid) in anaerobic jars (Anaerocult, Oxoid) were incubated at 37 ^oC for 48 hr. Typical *C. sporogenes* colonies were counted after the incubation period (Anon, 1994). The TBA, Peroxide and FFA values of samples were determined using the methods described by Gokalp *et al*., (1993).
The collected data was subjected to ANOVA, and means were separated using the Duncan's Multiple Range Test.

Results and discussion

C. sporogenes counts
The number of *C. sporogenes* in the control and nitrite added samples were less than <1 log CFU/g. It was 3.08 log CFU/g for *C. sporogenes* inoculated and 2.99 log CFU/g for nitrite + *C. sporogenes* containing samples. Analysis of variance results revealed that nitrite addition and *C. sporogenes* inoculation had a significant effect ($p<0.05$) on the count of *C. sporogenes*. For example, if only *C. sporogenes* inoculated samples were considered, it was observed that clostridium count was slightly lower in the samples prepared with the addition of nitrite. Duncan test indicated that there was a statistically significant difference in terms of the number of *C. sporogenes* between those two samples (Table 2). Before the processing, 6 log CFU/g microorganisms were inoculated into the kavurma sample during production, and that number was decreased about 3 log CFU/g unit with the cooking processes. The results indicated that *C. sporogenes* were not completely eliminated during the heat treatment of kavurma production and survived the storage period. It was observed that the storage time significantly affected the number of *C. sporogenes* in kavurma (Table 3). *C. sporogenes* inoculated samples initially contained 3.27 log CFU/g organism which was reduced to 2.89 log CFU/g after six months of the storage. For the nitrite + *C. sporogenes* sample; the initial count was 3.21 log CFU/g, and the count was 2.73 log CFU/g after six months.

Table 2. Duncan results (averaged over storage time) for the effect of nitrite addition on *C. sporogenes* count and oxidation parameters of the kavurma samples

Sample Type	*C. sporogenes**	TBA	Peroxide Number	FFA Value
1	$<1^c$	1.96^a	6.07^a	0.879^b
2	$<1^c$	0.43^b	4.04^c	1.036^a
3	3.08^a	1.44^a	5.38^b	0.817^b
4	2.99^b	0.25^b	3.84^d	0.981^a

1: Control, 2: Nitrite added 3: *C. sporogenes* inoculated, 4: Nitrite + *C. sporogenes* containing. *log CFU /g sample, TBA: mg malonaldehyde/kg fat, Peroxide number: meq O_2/kg fat, FFA: mg KOH/g fat, $^{a-d}$: Different letters in a column are significantly different ($p<0.05$).

Table 3. Duncan results for the effect of storage time on *C. sporogenes* count and oxidation parameters of kavurma

Storage Time (month)	*C. sporogenes**	TBA	Peroxide Number	FFA Value
0	1.97 [a]	0.80[b]	4.04[c]	0.79[b]
1.5	1.93 [a]	0.81[b]	4.69[b]	0.86[b]
3	1.82 [b]	1.03[b]	5.14[a]	0.99[a]
6	1.76 [c]	1.43[a]	5.46[a]	1.07[a]

*log CFU /g sample, TBA: mg malonaldehyde/kg fat, Peroxide number: meq O_2/kg fat, FFA: mg KOH/g fat,
[a-c] : Different letters in a column are significantly different ($p<0.05$).

It was reported in the literature (Hamilton, 1990) that the spores of *C. botulinum* did not germinate in the medium with low water activity (<0.95). Similarly, the water activity of kavurma produced in this study was less than 0.95 (the data was not reported) which may have caused no increase in the number of viable *C. sporogenes* during storage. Storage temperature of kavurma samples was 4 °C which could be another factor for the reduction or not germination of *C. sporogenes* during storage. It could also be noted that the *C. sporogenes* count was lower for the samples prepared with nitrite addition as indicated in the literature by many researchers that nitrite has an antimicrobial effect against clostridia. It was stated that the antimicrobial effect of nitrite is due to prevention of growth of bacteria rather than directly killing them (Ockerman, 1983, Cassens, 1995).

TBA value

TBA value of the samples changed in the range of 0.125 – 2.56 mg malonaldehyde/kg sample. Kaya and Gokalp (1995) stated that TBA value would be a proper indicator for determination of rancidity in kavurma. ANOVA results indicated that the addition of nitrite and nitirite + *C. sporogenes* into samples had a significant effect ($p < 0.01$) on TBA values (Table 2). It was determined by Duncan test that nitrite added kavurma (samples 2 and 4) had significantly lower TBA values compared to non-nitrite samples (1 and 3). This occurrence was probably caused by antioxidant effect of nitrite. It is known that nitrite inhibits the formation of oxidative rancidity in meat products. Oxidized Fe^{+++} present in meat catalyzes the oxidation of fat but if the meat is cured with nitrite, reaction of NO with myoglobin reduces the heme molecule to Fe^{++} state which has no effect on the oxidation of fat. It was determined that TBA value significantly increased with time during storage (Table 3). The TBA value of the samples after 6 months of storage was higher than those of the samples at any other storage time. Vural and Oztan (1989) reported that initial TBA values of the control and with 30 ppm tocopherol added kavurma samples were 1.65 and 2.10, respectively. They determined that TBA values increased during storage, and after 6 months TBA value of control group increased to 2.32 while that of antioxidant added sample reached to 1.80 mg malonaldehyde/kg fat.

Peroxide number

The peroxide number of kavurma samples ranged between 3.21and 7.10 (meq O_2/kg). The statistical analysis revealed that the addition of nitrite had a significant effect ($P<0.01$) on the peroxide number of kavurma (Table 2). It was found that sample 2 and 4 (nitrite containing samples) had significantly lower peroxide values than that of non-nitrite containing samples, 1 and 3. The change in peroxide number of sample with storage time was statistically significant (Table 3). The peroxide number of all samples increased during the storage, however the peroxide value of the samples stored 3 months was not statistically significant from that of the samples stored for 6 months. Kavurma Standard (TS 978) states that peroxide level of kavurma shall be less than 20 meq O_2/kg, and all of the current samples contanied peroxide levels under that limit. Sarigol reported that home or industrial type kavurma samples had the peroxide value of 5 ve 9 meq O_2/kg, respectively, and the number increased depending on the storage time. Tiryakioglu and Yucel (1995) investigated the peroxide value of kavurma marketed in Bursa and measured an average of 61.44 (meq O_2/kg) peroxide. Kavurma is not consumed right after the manufacture; therefore it should keep its quality during the storage. The fat used in kavurma production is an important factor for the deterioration of quality of the product since it is prone to rancidity formation due to the oxidation (Vural and Oztan, 1989). For this reason, the use of certain amounts of nitrite could be recommended in kavaurma production to retard oxidation of the fat present in kavurma.

FFA Value

It was determined that the addition of nitrite had a significant effect on the FFA (Free Fatty Acid) value of the samples. The addition of nitrite caused a significant increase in the FFA values samples (Table 2). The FFA value of the control sample was 0.879 (mg KOH/g fat) while that of sample 2 (containing 100 ppm nitrite) was 1.036. Additionally, storage time significantly affected the FFA value of samples with FFA in kavurma samples increasing during storage. As seen in Table 3, average initial FFA value of samples was 0.79, and it increased to 1.07 after 6 months of storage.

Conclusions

The results of this study revealed that *C .sporogenes* count decreased during cooking of kavurma but they were not completely eliminated and survived during storage. The number of *C. sporogenes* slightly decreased during storage which could be attributed to proper storage conditions. *C. sporogenes* was not fully inactivated during kavurma processing in the samples inoculated with the bacteria, and it was concluded that they might reproduce and might pose a potential health risk, if the product is stored under inappropriate conditions. Nitrite addition reduced the TBA value and peroxide number of kavurma. Since higher TBA value and peroxide number is related to the oxidation of lipids, nitrite addition limited the rancidity formation in kavurma by lessening the autoxidation. In conclusion, considering the facts that nitrite has a slight antimicrobial effect and inhibits the autoxidation of fat in product while enhancing the other quality characteristics of meat product, a certain amount of nitrite could be added to traditional kavurma processing.

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