

Microbiological evaluation of chicken kadinbudu meatball production stages in a poultry meat processing plant

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Summary: This study was conducted to investigate the microbiological changes occurring during the processing stages of chicken kadinbudu meatballs produced in a private poultry meat processing plant, Bursa/Turkey. One hundred and seventy samples collected from the production stages: and non-meat ingredients were examined for total aerobic mesophilic bacteria (TAMB), coliform bacteria, *Escherichia coli* (*E. coli*), *Enterobacteriaceae*, enterococci, staphylococci-micrococci, coagulase positive staphylococci, yeast and molds. In chicken ground meat, the TAMB, *Enterobacteriaceae*, enterococci, staphylococci-micrococci, yeast and mold counts were 3.75, 2.38, 2.92, 2.77, 2.59 ve 3.23 log cfu/g, respectively. There was a significant increase in TAMB counts in samples of predested, battered and breaded patties ($p<0.05$). Of the non-meat ingredients, only flour had a significant effect on the TAMB increase in predested patty samples ($R^2 = 0.55$, Beta = 0.74). There was approximately one log reduction in all sample counts after frying, with statistically significant reductions only in TAMB and *Enterobacteriaceae* counts ($p<0.05$). Samples after cooling and packaging had microbial counts under detection levels, indicating good personel hygiene, and cleaning and disinfection applications leading to zero post contamination to the product after the cooking stage. *E. coli* or coagulase positive staphylococci was not detected in any of the samples either from the production stages or the non-meat ingredients. The results indicate that neither the ground chicken meat nor the non-meat ingredients caused initial and secondary and/or cross-contaminations for the final chicken kadinbudu meatballs. Furthermore, the heat application with two stages was sufficient for the production of a non-hazardous product, and there were no post-contaminations after cooking.

Key words: Meatball, processed chicken product, microbial quality

Bir kanatlı eti işletme tesisinde tavuk kadinbudu köfte üretim aşamalarının mikrobiyolojik yönden değerlendirilmesi

Özet: Bu çalışma, Bursa'da kanatlı eti ve et ürünleri üreten özel bir işletmede tavuk kadinbudu köftelerin üretim aşamalarındaki mikrobiyolojik değişikliklerin incelenmesi amacı ile gerçekleştirildi. Bu amaçla, üretim aşamalarından ve et harici katkı maddelerinden alınan 170 örnek, toplam aerobik mezofilik bakteri (TAMB), koliform bakteri, *Escherichia coli* (*E. coli*), *Enterobacteriaceae*, enterokok, stafilokok-mikrokok, koagülaz pozitif stafilokok ile maya ve küf yönünden analiz edildi. Hammadde tavuk kıymasında TAMB, koliform bakteri, *Enterobacteriaceae*, enterokok, stafilokok-mikrokok ile maya ve küf sayıları sırası ile 3.75, 2.38, 2.92, 2.77, 2.59 ve 3.23 log kob/g düzeylerinde tespit edildi. Ön unlama, sıvı kaplama karışımı ve kuru kaplama materyali ile kaplama aşamalarından alınan örneklerin TAMB sayılarında belirlenen artışın istatistiksel olarak önemli olduğu ($p<0.05$), üretimde kullanılan et harici katkı maddelerinden sadece unun, ön unlama aşamasından alınan örneklerdeki artışta TAMB sayısı açısından önemli ($R^2=0.55$, Beta=0.74) düzeyde etkisi olduğu saptandı. Üretimde kızartma işlemi sonrası alınan örneklerde incelenen tüm mikroorganizmaların sayısında yaklaşık bir log'luk azalma şekillenmiş ancak istatistiksel olarak sadece TAMB ve *Enterobacteriaceae* sayılarındaki azalmanın önemli ($p<0.05$) olduğu belirlenmiştir. Pişirme işlemi sonrasında tespit edilebilir seviyenin altındaki düzeylerde bulunan mikroorganizmaların sayısında soğutma ve paketleme aşamalarında herhangi bir artış şekillenmemiştir. Ayrıca incelenen tüm örneklerde *E. coli* ve koagülaz pozitif stafilokok sayıları da tespit edilebilir seviyenin altındaki düzeylerde bulunmuştur. Çalışmanın sonucunda, incelenen mikroorganizmalar açısından hammadde olan kanatlı kıymasının başlangıç mikroorganizma yükünün düşük olduğu, kullanılan ingrediyenlerin sekonder ve/veya çapraz kontaminasyona neden olamayacak düzeyde mikroorganizma içerdiği belirlenmiştir. Ayrıca, üretimde kullanılan pişirme işleminde uygulanan ısı işlem derece ve süresinin yeterli olduğu pişirme sonrasında da herhangi bir kontaminasyonun şekillenmediği saptanmıştır.

Anahtar sözcükler: Köfte, işlenmiş tavuk eti ürünü, mikrobiyal kalite

Introduction

In the last decade, there has been a dramatic increase in the consumption of fresh/frozen, pre-cooked/fully cooked chicken-based meat products due to

their high nutritional value, lower cost, convenience and variety aspects for the consumer. Processing of these value-added products such as nuggets, meatballs, hamburgers, frankfurters, is also of benefit to the

processor. These types of products require strict hygiene applications throughout the processing chain, since chicken meat/chicken-based products are very susceptible to microbial spoilage and could harbor pathogens (10, 12).

Kadınbudu meatball, a traditional Turkish dish has also been produced in commercial scale as a fully cooked 'chicken-based' meat product in Turkey for over 5 years. All these types of convenience foods are industrially prepared by mixing ground raw meat with other ingredients such as spices and salt. Once the patties are formed and covered with batter and bread crumbs; they are fast deep-fried and baked in a humid steam convection oven (12). Thus, factors such as insufficient microbial quality of meat and non-meat ingredients, improper hygienic applications prior/during and post processing, inefficient heat treatments could result products potentially hazardous to consumers. Also, shorter shelf-life and recalls cause economical losses to the producer.

Currently, there are many studies investigating survival of pathogen(s) after thermal inactivation (11), including risk assessment of the product (7); survival/reduction of pathogen(s) in modified atmosphere packaging conditions (3, 13); microbial contamination sources during production stages (8); prevalence of specific pathogens (1) in further processed poultry meats. Contrarily, in Turkey, there are only a few experimental and 'processing technology development-based' studies on chicken sausage and salami (2, 6). However, up to our knowledge there are no microbiological quality reports for these types of products, particularly on kadınbudu meatballs, either on retail or during production. Therefore, this study aimed to assess the alterations in the microbiological quality of chicken kadınbudu meatballs during processing stages.

Materials and Methods

Sample collection: Samples were obtained at 10 different times over a one year period from a private poultry meat processing plant manufacturing export products in Bursa/Turkey, which obtains raw chicken meat from its own HACCP-certified slaughtering facility. Samples were collected from the production stages; ground chicken meat, ingredient added meat dough, cooled dough, formed patty, pre-dusted patty, battered patty, breaded patty, fried product, cooked product, cooled product, packaged product. In addition, samples were collected from non-meat ingredients; boiled rice, spice mix, flour, batter mix, breading material and frying oil. A minimum of 500 g from solid and 200 ml from liquid samples were aseptically taken into sterile polyethylene bags and into sterile laboratory bottles with screw caps, respectively. All samples were transferred to

the laboratory in cold chain within 1 h, and processed for microbiological analysis.

Microbiological analyses: A 10 g of subsample from each sample type was weighed into a sterile stomacher bag (Seward, London, UK), suspended in 90 ml sterile 0.1 % (w/v) peptone water (OXOID, CM0009), and was homogenized in stomacher (Laboratory Blender, Seward, London, UK) for 2 min at normal speed at room temperature. All samples were diluted up to 10^{-3} in 0.1 % (w/v) peptone water and microbiological analyses were performed by using duplicate pour plate and spread plate methods onto/into the following appropriate agar plates and incubations: Total aerobic mesophilic bacteria (TAMB) on Plate Count Agar Base (PCA, OXOID CM0325), incubated at 37°C for 24 h; coliform bacteria in Violet Red Bile (Lactose) Agar (VRBA, OXOID CM0107) and *Enterobacteriaceae* in Violet Red Bile Glucose Agar (VRBGA, OXOID CM0485) both incubated at 37°C for 24 h; enterococci on Slanetz and Bartley Medium (Merck 1.05262.0500) incubated at 37°C for 48 h; staphylococci and micrococci on Baird Parker Agar (BPA; Oxoid CM 0275) supplemented with Egg Yolk Tellurite Emulsion (Merck, 1.03785.0001) incubated at 37°C for 48 h; yeast and mold on Rose Bengal Chloramphenicol Agar Base (OXOID, CM0549) supplemented with Chloramphenicol Supplement (OXOID SR0078) incubated at 25°C for 5 d. After incubations, colony counts on plates were converted to log cfu/g. For the enumeration of *Escherichia coli* (*E. coli*), 5 typical colonies from VRBA were transferred into Lactose Broth (Oxoid CM 137) with inverted Durham tubes and incubated at 37°C for 24 h, and examined for gas formation. From gas positive tubes inoculations were carried out onto Eosine Methylene Blue (EMB; Hi-Media M317) Agar and incubated at 37°C for 24 h. Confirmation was performed by IMVIC test. Coagulase positive staphylococci were determined by testing and observing the formation of agglutination from 5 typical colonies picked from BPA with Dryspot Staphytest Plus (Oxoid DR 100M) kit.

Statistical analysis: Statistical software SPSS for Windows version 17.0 was (SPSS Inc. Chicago, IL) used. Microbial levels at production stages were analyzed by one way analysis of variance (ANOVA), and Tukey's multiple comparisons tests were applied as post-test when significant differences were determined. In addition, regression analyses were used to determine the effect of non-meat ingredients on microbial changes in production stages.

Results

The initial TAMB count, which was 3.75 log cfu/g in ground chicken meat samples, remained almost the same until pre-dusting, and increased up to 4.01 log cfu/g in pre-dusted patty samples, and then remained at similar

Table 1. Microbiological analysis results of the samples (mean count, n = 10) collected during chicken kadınbudu meatball production.

Tablo 1. Tavuk kadınbudu köfte üretimi sırasında toplanan örneklerin (aritmetik ortalama, n = 10) mikrobiyolojik analiz sonuçları.

Sample <i>Production stage x</i> <i>(Stage number)</i>	Microorganism (Mean ± SD)					
	TAMB	Coliform bacteria	<i>Enterobacteriaceae</i>	Enterococci	Staphylococci Micrococci	Yeast & mold
Ground chicken meat ^a (1)	3.75 ± 0.48	2.38 ± 0.45	2.92 ± 0.70	2.77 ± 0.54	2.59 ± 0.39	3.23 ± 0.68
Ingredient added meat dough (2)	3.94 ± 0.85*	2.68 ± 1.01	2.87 ± 0.76	3.12 ± 0.50	2.97 ± 0.74	3.28 ± 0.30
Cooled dough (3)	3.77 ± 0.80	2.74 ± 1.11	2.78 ± 0.74	3.20 ± 0.28	2.74 ± 0.50	3.08 ± 0.43
Formed patty (4)	3.70 ± 0.65	2.91 ± 0.98	2.89 ± 0.48	3.21 ± 0.35	2.99 ± 0.54	3.16 ± 0.30
Predusted patty (5)	4.01 ± 0.35*	2.87 ± 0.94	3.60 ± 0.81*	3.20 ± 0.38	3.09 ± 0.60	3.37 ± 0.38
Battered patty (6)	4.03 ± 0.64*	2.76 ± 1.00	3.47 ± 0.62	3.40 ± 0.23	3.08 ± 0.48	3.31 ± 0.33
Breaded patty (7)	4.01 ± 0.85*	2.81 ± 0.86	3.48 ± 0.51	3.24 ± 0.41	3.07 ± 0.30	3.32 ± 0.28
Fried product (8)	2.70 ± 0.82*	1.91 ± 0.57	2.33 ± 0.86*	2.80 ± 0.28	3.44 ± 0.19	2.69 ± 0.53
Cooked product (9)	<2.0 ± 0.00	<1.00 ± 0.00	<1.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00
Cooled product (10)	<2.0 ± 0.00	<1.00 ± 0.00	<1.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00
Packaged product (11)	<2.0 ± 0.00	<1.00 ± 0.00	<1.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00
F	2.98; P<0.05	0.96; P>0.05	2.93; P<0.05	2.15; P>0.05	1.30; P>0.05	1.20; P>0.05
Post Hoc ^b	8<2; 8<5; 8<6; 8<7		8<5			
<i>Non-meat ingredient</i>						
Boiled rice	2.00 ± 0.00	<1.00 ± 0.00	<1.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00
Spice mix	3.30 ± 1.08	1.70 ± 0.44	2.16 ± 0.17	2.00 ± 0.00	3.25 ± 1.77	2.73 ± 1.04
Flour	3.47 ± 0.80	<1.00 ± 0.00	2.02 ± 0.60	2.12 ± 0.24	2.57 ± 0.70	3.53 ± 1.15
Batter mix (log cfu/ml)	<2.0 ± 0.00	<1.00 ± 0.00	<1.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00
Breeding material	2.93 ± 0.74	<1.00 ± 0.00	1.45 ± 0.21	2.46 ± 0.41	<2.00 ± 0.00	2.45 ± 0.21
Frying oil (log cfu/ml)	2.61 ± 0.32	<1.00 ± 0.00	<1.00 ± 0.00	2.30 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00

^a log cfu/g unless otherwise indicated

* Significant difference found between indicated stages (p<0.05)

^b Numbers in Post Hoc test indicate production stages

levels in battered and breaded patties (p<0.05) (Table 1). In the fried product samples, there was almost one log reduction in the TAMB counts, down to 2.70 log cfu/g, which was found statistically significant (p<0.05). After this stage, counts for all cooked, cooled and packaged product samples were below detection limits.

The coliform bacteria counts, which were determined approximately 2.00 log cfu/g from the initial step until the breeding step of production, reduced one log in fried product samples to 1.91 log cfu/g, and stayed under detection limits after the cooking step. Except for spice mix, all non-meat ingredient coliform bacteria counts were below the detection limits. The *Enterobacteriaceae* count, which was 3.60 log cfu/g in the predusted patties, decreased significantly down to 2.33 log cfu/g in the fried product (p<0.05), and stayed under the detection limits in the cooked, cooled and packaged product. There was an increase in the mean enterococci counts of 2.77 log cfu/g in ground chicken meat to 3.12 log cfu/g in meat dough after the addition of ingredients. This level stayed almost constant in the further stages, reduced to 2.80 log cfu/g and dropped below the detection limits in the fried product and in the cooked product, respectively.

Mean staphylococci and micrococci count in the raw material, which was 2.59 log cfu/g, increased up to 3.09 log cfu/g in the predusted patties, and stayed at this level in the fried product samples, as well. However, numbers remained under detection limits in the cooked product samples and in the samples of the stages thereafter.

Mean yeast and mold counts were around 3.00 log cfu/g from the beginning until the breeding stage of production. This count decreased down to 2.69 log cfu/g in the fried product, and became undetectable in the cooked product. Except for boiled rice, frying oil and batter mix, all samples had mean yeast and mold levels of 2.45 to 3.53 log cfu/g. *E. coli* or coagulase positive staphylococci was not detected in any of the samples either from production stages or non-meat ingredients analyzed.

Discussion and Conclusion

Ground or mechanically deboned poultry meat (MDPM) used in poultry meat products processing is highly prone to microbial contamination due to its nutritional composition and production technology.

Therefore, hygiene status of the slaughtering facility and processing environment has direct influence on the initial microbial load of ground meat (3). In this study, the TAMB count of ground meat samples, which were used as raw material in kadınbudu meatball production was 3.75 log cfu/g (Table 1). Kozačinski et al. (9), Pipová et al. (14), and Dhananjayan et al. (3) had reported their TAMB counts from poultry ground meat, chicken ground meat, MDPM, and from turkey meat patty samples as 5.23 log₁₀ cfu/g, 2.3x10⁵ cfu/g, 10⁷ log cfu/g, and 3 log cfu/g, respectively. The TAMB count is in parallel with Dhananjayan et al. (3), and is relatively lower than the counts obtained from other indicated studies (9, 14). Here, the low initial TAMB count can primarily be linked to supplying the raw ground chicken meat in the process from the company's own, HACCP-certified slaughtering facility. Also, deboning and grinding processes, which were performed in a climate-controlled (environment temperature 12°C, meat core temperature 4°C) separate unit from the slaughter facility, and cold storage of meats immediately after handling might have helped maintaining this low count.

Presence of coliform bacteria and *Enterobacteriaceae* in meats indicate possible fecal contamination and/or exposure of meat to non-hygienic conditions. Jimenez et al. (5) indicated *E. coli* as the predominant species during poultry slaughter. Additionally, presence of enterococci on the processing equipment, originating from poultry feces or any other undesirable material in the facility, such as contaminated soil or water, can become natural flora of that environment and continuously contaminate the product. Enterococci are also known to be better indicators of hygiene and fecal contamination than coliform bacteria particularly in heat-treated products (15). In the study, coliform bacteria, *Enterobacteriaceae* and enterococci counts were found as 2.38, 2.92 and 2.77 log cfu/g, respectively (Table 1). Also the *E. coli* counts were found below the detection limits. Pipová et al. (14) reported coliform bacteria and enterococci counts in ground chicken meat and MDPM as 10⁵ and 10⁶ log cfu/g, and 10³ and 10⁴-10⁵ log cfu/g, respectively. Kozačinski et al. (9) indicated *Enterobacteriaceae* counts in their ground chicken meat samples as 1.7-3.7 log₁₀ cfu/g. The *Enterobacteriaceae* count results of our study is in accordance with the findings of Kozačinski et al. (9), whereas coliform and enterococci counts were lower than reported by Pipová et al. (14).

Ready To Eat meat and related products are of primary sources in staphylococcal food-borne intoxications. *Staphylococcus aureus* can grow and produce toxins on contaminated meat products when they reach numbers of $\geq 10^6$ cfu/g (15). In the study, staphylococci and micrococci counts were determined as 2.59 log cfu/g,

whereas level of coagulase positive staphylococci was under detectable limits. This result is considerably lower than the values reported by Pipová et al. (14), who had found staphylococci counts between 10³-10⁴ log cfu/g in MPDM. Our results show that good hygiene practices, including continuous monitoring and supervision, were being applied by the personnel working in the processing and transfer of the raw material.

Microorganisms in ambient air are mainly composed of mold spores, and depending on the physical conditions of the plant, can contaminate the product during production or cold storage (15). In our study, yeast and mold counts were found as 3.23 log cfu/g in ground meat samples, which is lower than the counts reported by Ismail et al. (4). The lower counts of yeast and mold in our study is a good indication of high quality supply both for the raw material and for non-meat ingredients. Also, the spice mix, other ingredients and the filtered processing environment air, did not pose a risk factor for yeast and mold contamination for the product.

In the beginning of the chicken kadınbudu meatball processing, boiled rice, additives and spice mix are blended with ground chicken meat, and all is mixed in cutter to conform the ingredient added meat dough. This dough, either used immediately or stored in cold room for a maximum of 24 h, is then used in the process. During these stages, there was no substantial increase in the counts of any of the microorganisms of concern (p>0.05). Statistical analyses to determine the effect of the addition of boiled rice and spice mix on the increase of the microbial load of the dough, indicated no significant effect of either of these ingredients on enterococci counts (p>0.05) (Table 1). There was no notable change in the microbial counts of the ingredient added dough, cooled dough and of the formed patty samples tested.

In chicken kadınbudu meatball processing, the formed patty is subjected to an initial pre-dusting, followed by coating with batter mix, and to breading before the frying stage. Microbiological analyses of the pre-dusted patty showed that there was increase in the TAMB, *Enterobacteriaceae*, and staphylococci-micrococci counts, where statistical analyses revealed that only TAMB and *Enterobacteriaceae* increases were significant (p<0.05) (Table 1). Also, the effect of addition of flour was determined to significantly increase only the TAMB counts (R² = 0.55, Beta = 0.74) (Data not shown in Table 1).

Our results show that there was no prominent difference in the counts of any of the microorganisms in the pre-dusted patty, battered patty, or in the breaded patty samples analysed. In addition, the batter mix and breading material used in the patty coating stages were

determined to be no sources of microbial contamination, therefore did not cause any considerable increase in the microbial load of the product.

After the breading stage, the patties are fried in 180°C frying oil for 30 sec, steam-cooked for 2.5-3 min at the same temperature, with core patty temperature of 72°C, and were finally IQF cooled at -7 to -8°C for 30 min. In our study, the fried product samples had TAMB, coliform bacteria, *Enterobacteriaceae*, enterococci, staphylococci-micrococci and yeast and mold counts as 2.70, 1.91, 2.33, 2.80, 3.44 and 2.69 log cfu/g, respectively (Table 1). When breaded patty and fried product's microbiological results were compared, there was a reduction in all of the counts except for staphylococci-micrococci, and that the reduction in TAMB and *Enterobacteriaceae* counts showed statistical significance ($p<0.05$). This finding is an indication of the possible insufficiency of heat application during the 30 sec 180°C frying, if applied solely to the product for microbial reduction. As a matter of fact, during this short heat application, the core temperature of the patty reaches only to 50-55°C. Following this stage, after the steam-cooking for 2.5-3 min at 180°C, all counts for the investigated microorganisms were found under detection limits. This result points out that this second heat application, aiming a core patty temperature of 72°C, was sufficient to kill target microorganisms in the product. Patsias et al. (13), who had reported a higher TAMB count in their cooked chicken schnitzel samples as 3.9 log cfu/g in their study, had related this finding to the high initial microbial load of the raw material or to post-cooking cross-contamination. The same researchers (13) had also implied the 2 log reduction in their TAMB counts as an expected result, and had found *Enterobacteriaceae* and yeast and mold counts under detectable levels, as similar to our findings.

Our results showed that the samples of cooling and packaging immediately after the cooking stage had microbial counts under detection levels. This is another indication of good personel hygiene, cleaning and disinfection applications to the processing environment, and therefore no post contamination to the product after the cooking stage. The low microbial counts in all non-meat ingredient samples was mainly achieved by selecting suppliers, which produce, store and transfer spices and other ingredients under required hygienic conditions to the processing plant.

Results of this study indicate that neither the ground chicken meat used as raw material nor non-meat ingredients caused initial and secondary and/or cross-contaminations for the final chicken kadinbudu meatballs. Furthermore, the heat application with two stages was

sufficient for the production of a non-hazardous product, and there were no post-contaminations after cooking.

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