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Research Article

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Investigating the Microbial Contamination of Chicken Meat Paste in Naghadeh City

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Abstract

Background and aim: The burgers, sausages, and bacon are all prepared using various techniques. MDM (Mechanically Deboned Poultry Meat) is a particular product that is frequently used to make such products. The MDM is really made up of all the chicken leftovers and wastes, including skin, bones, and useless pieces, that are transported to factories in a dirty and unhygienic state and combined together to create MDM (Mechanically Deboned Poultry Meat). The goal of the study is to ascertain the microbiological rate of a substance that will be utilized in food items that people, especially children, consume in large quantities.

Materials and Methods: 100 samples of MDM from sausage manufacturing businesses' bone removal machines were obtained. The microbiological characteristics of the MDM samples were evaluated and analyzed in accordance with Iran standard institutions as soon as they were delivered to the laboratory in cold temperature settings.

Results: The samples' contamination rates were as follows: Infected samples had the following serotypes of *Salmonella*: 25% *S. gallinarum*, 19% *S. Typhimorium*, 17% *S. enteritidis*, 15% *S. paratyphi A*, 15% *S. paratyphi C*, and 9% *S. paratyphi B*. There were also 68% *Staphylococcus aurous*, 62% *fermentative*, 59% *E. coli*, 53% various forms of *Salmonella*, and 21% *mustiness*.

Conclusion: It was evident from counting the colonies that 10% of the samples had contamination rates that were greater than allowed for and beyond the limits.

Keywords: *MDM, Buder, Salmonella, Staphaurus, E.coli*

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Introduction

The most important role of the food is to help the healthiness and stability of the body. Recent years have witnessed a growing trend in studies on the relationship between the food and the diseases, which have resulted in significant and remarkable findings on the issue (Werthmann *et al*., 2016; Gharibzahedi and Jafari, 2017). Nowadays, due to the problems caused by the industrialized world and the career issues related the fast-pacing condition of modern living, people are inclined to use ready-made foods such as sausage, baloney, and hamburger which is rapidly and remarkably increasing. Variety of ways are employed to prepare these kinds of foods, but one of the most common ways which are used in most countries especially in Iran and most developing countries is using a raw mixed materials known as MDM (Mechanically Deboned poultry Meat).

Chicken meat consumption and its related products have significantly increased in recent years. Most people prefer to use poultry instead of red meat and when formulating the meat products different amounts of meat chicken are used (Kralik *et al*., 2018; Baéza *et al*., 2022; Shan *et al*., 2017). The MDM is a raw processed material derived from the chicken, which meet an increase in its production rate recently (Bhatnagar *et al*., 2022).

The MDM has been produced since 1960 and it is used as a rudimentary and primary material in the formulation of some meat products and it is widely accepted in the formulation of different meat products due to its nutritional and technological features as well as its affordable price (Miller, 2018; Cenci *et al*., 2018). On the other hand, some shortcomings such as the color, taste, and the inappropriate tissue and its high microbe load content made it to be extremely detrimental and perishable (Iñiguez-Moreno *et al*., 2023). After killing the birds (chicken) in the slaughterhouses, the chicken is sent to the packaging units in which the bones are left and remained after the packaging, which has many chicken pieces stuck to the bones. The bones were discarded or pulverized in the past, but these days by using a machine called Budder, the meat pieces are removed from the bones under pressure and the machine will produce two kinds of MDM, one is related to that of the bones and the other contains the meat. In some other factories, there is even a simpler device, which grates the bones, and the resulted MDM is passed through a porous and perforated film or layer and the MDM is produced. Due to the fact that the chicken bones are taken from different places like restaurant, fast food restaurant and unauthorized slaughterhouses and different factories and lots of other places through unhealthy manner and are put into plastic sacks and transferred to producing units, therefore they can be a bountiful source of bacteria such as *Salmonella*, *E. coli O157: H7*, *Listeria monocyrogenas*, *Yerisinia*, *Staphylococcus aurous*, *Enterohaemorrhagic, Enterocolitica* and the putrefying agent bacteria like *Pseudomonas*. Therefore, the quality of the chicken, which is used to produce the MDM, is very important in its final quality (Akramzadeh *et al*., 2020). The *E. coli* belongs to the flora family of the intestine, but when it is exposed to the external tissues and textures such as *billiary* channels and intestinal vacuoles or genitourinary organs will cause the disease (Sarowska *et al*., 2019; Peng *et al*., 2024).

The most common type of food poisoning is caused by *enterotoxin Staphylococcus*; they immediately become resistant to various antibiotics and cause some therapeutic problems (Argaw and Addis, 2015). When *Salmonella* enters the body orally, it usually is pathogenic and will cause Enteritis, intestinal fever and systematic infestation (Bhat *et al*., 2022; Tariq *et al*., 2022; Ahmed, 2016).

Therefore, confirming and justifying the MDM is highly critical, because the population is growing so fast and they require receiving protein. As a result, authorities have always emphasized preparing the sufficient food by considering the health factors. The study aimed at finding and evaluating the MDM used for making sausages, baloney, and hamburger.

Materials and Methods

The study gathered the samples through continuous visits to factories producing sausages, baloney, and hamburger, which comprised 100 samples of MDM when preparing the MDM in the Buder in sterile condition, and where put in lidded glass containers, the sampling size was about 500 grams. The samples were transferred to the laboratory in sterile freeze condition. The sample preparation followed the Iran national standard number of 8923-2. In order to prepare the suspension, we used the blender regarding the point that the veterinary organization approved some sectors of producing and distributing the MDM, but unfortunately these sectors

did not have the laboratory and the health ministry does not confirm the consumption of this raw material. Nevertheless, the chicken packaging sectors and factories producing the sausage, baloney, and hamburger, which used this material to produce the given product, were used as the sources for the sampling. These sectors supplied the MDM samples in freeze 10 kg packages.

In the microbial analysis, the main goals are the finding the *E. coli* and *Salmonella SPP*, which must not be present in the samples under study. For this case, counting the microorganisms, which can be found in the product if they have the authorized level. To do this, the total counting of the microbes along with the *mustiness* and *fermentative* was conducted. Finding and identifying the bacteria followed the following standard numbers including 1820 for *Salmonella*, 2940 for *E. coli*, 1994 for *Staphylococcus aurous*, 997 for *mustiness* and 5272 for general microorganism counting. All culturing medium for the study prepared by Merck KGaA, Germany.

Results

After conducting the above-mentioned experiment on the samples, it became evident that 68% of the samples were infected with *S. aureus*, 62% with *fermentative*, 59% with *E. coli*, 53% with different *Salmonella* Serotypes and 21% with *mustiness.* Regarding the 53% infection of different *Salmonella* Serotypes, the *Salmonella* Serotypes test was conducted (Table 1). The study identified six different serotypes of which the most and least frequent serotypes are *S. gallinarum* and *S. paratyohi B,* respectively. Regarding the *S. aureus*, after culturing the samples on Baird Parker medium, it was revealed that 68% of the samples were infected with the bacterium. After determining the samples, the bacterium colonies counting were conducted for the *S. aureus* (Table 2). It can be said that 54% of the samples had colonies reaching to 10^3 and 2% of the samples had 4×10^3 colonies which had the highest level of infestation. In order to identify the samples with *mustiness* and *fermentative*, after culturing the samples on SDA (Sabouraud Dextrose Agar) with dilution level of 10^{-2}, it was clear that 21% of the samples were infected with *mustiness* and 62% with *fermentative*. Counting the colonies number and diversity was conducted after sample infection rate was measured (Table 2). The infected sample had the highest and lowest level of *mustiness* from 400 to 100, respectively. This is important because the lowest and highest infection rate for the *fermentative* were 1000 and 4000 colonies, respectively. The microorganism overall counting with different dilution rate on plate count agar medium is shown in table 3. It is witnessed that 21% of the samples were infected with all kinds of microorganism and 14% were infected free. Regarding the existing colonies of these 14 samples, it was clear that the number of existing microorganisms was lower than that of the healthy meat.

Serotypes	Group	Somatique	H Phase I	H Phase Ц	Serotypes frequency	The serotypes sample infected with *Salmonella*
S.gallinarum	D	12, 9.1	-	-	13%	25%
S.typhimorium	B	12.5, 4.1	I	1.2	10%	19%
S.enteritidis	D	12, 9.1	G, m	1.7	9%	17%
S.parathphi A	A	1.2, 12	A	-	8%	15%
S.parathphi C	C	6.7 Vi	C	1.5	8%	15%
S.parathphi B	B	12.5, 4.1	B	1.2	5%	9%
Total					53%	100%

Table 1. The antigenic structure and number of different serotypes of *Salmonella* in the MDM samples.

Number of colonies	*Mustiness*		*Fermentative*			*S. aureus*		
	Infected positive sample to *mustiness* (F)	Positive sample infection (%)	Number of colonies	Infected positive Sample of *femenative* (F)	Positive sample infection (%)	Number of colonies	Infected positive sample to *S. aureus* (F)	Positive sample infection (%)
-1×10^2	1 (10%)	47%	$1\text{-}10\times10^2$	23%	37%	-1×10^3	54%	79%
$10^2\text{-}2\times10^2$	6 (6%)	29%	$10\text{-}20\times10^2$	17%	28%	$10^3\text{-}2\times10^3$	9%	14%
-3×10^2	3 (3%)	14%	-30×10^2	12%	19%	-3×10^3	3%	4%
2×10^2			20			2×10^3		
-4×10^2	2 (2%)	10%	-40×10^2	10%	16%	-4×10^3	2%	3%
3×10^2			30			3×10^3		
Total	21 (21%)	100%	Total	62%	100%	Total	68%	100%

Table 2. The number of colonies of *mustiness*, *fermentative*, and *S. aureus* in MDM (%).

Colonies number	Frequency (%)
10^3	17 %
10^3–10^4	23 %
10^4–10^5	39 %
10^5–10^6	11 %
10^6–10^7	6 %
10^7–10^8	3 %
10^8–10^9	1 %
Total	100

Table 3. Microorganism overall counting in MDM (%).

Discussion

Regarding the 50 years MDM production in the world, there have been many studies on the MDM characteristics. Greenwood and Swami introduced a study and reported that 22% of the MDM infection was due to *Salmonella*. Conley conducted a study on the proliferation of *Salmonella* regarding the Frankfort chicken sausage producing line (Conley, 2014). 3% Of MDM infection, containing baloney produced in this factory was reported to have *Salmonella* infection (Fimbres-García *et al*., 2022; Baker *et al*., 2012). There are legal and systematic rules and instruction for the MDM characteristics, production, storage, and formulation. For example, the 2073/2005, NoEC 583/2004 of European union instruction proposes a set of guidelines on the microbial features, sampling, storing, producing and formulating and also the rules regarding the labeling of the product containing the MDM with FSIS USDA'S of America (Commission Regulation (EC) No 2073/2005 , 2005; Commission Regulation (EC) No 835/2004, 2004). Controlling the food health and safety to provide users' health is highly important. In order for providing healthy food and product, the ingredients should be monitored, controlled, and standardized. The results of the study showed a higher degree of microbial contamination in MDM, which is congruent with the study conducted by China, which evaluated 100 freeze samples of MDM and reported to contain bacterium infection of *Salmonella*, *E.coli*, *Staph.aureus* and *mustiness*. The most common part in MDM infection was related to the killed chicken especially in the killing process in the slaughterhouses (China, 2020). Many studies were conducted in Iran, which confirmed the chicken carcass infection including the following. In a study conducted, 100 slaughtered chickens were studied in the Ahvaz slaughterhouses and it was reported that the samples were infected with *Salmonella* at 12% level (Miahi *et al*., 2005). Niazi Shahraki et al. also studied the slaughtered chicken in different slaughterhouses in Tehran and reported that 69% of the samples were infected with *Salmonella* (Niazi Shahraki *et al*., 2007). Another research was also conducted by Wijesinghe et al. on the infection load changes of *Staphylococcus aurous* at different stages of bird killing in the slaughterhouses and the results indicated that different stages including plucking, , emptying the viscera, cold water immersing in chiller tank are considered to be the most critical stages in slaughterhouses and the average *Staphylococcus aurous* counting at emptying viscera stage was 4.3×10^4 and 90% of the chicken carcasses showed *Staphylococcus aurous* contamination (Wijesinghe *et al*., 2023). The contamination of chicken at deboning stage can be transferred the MDM. Therefore, maintaining the health related factors and conditions during the MDM preparation stage could serve as a barrier in the infection increase. The most important point in preparing the MDM is the thorough cleaning and securing the chicken carcass before it is put in the deboning machine. The rectum, viscera, gizzard, heart, liver and different poultry parts should be completely removed from the chicken. In addition, sustaining the cold temperatures during the production stage to cool the chicken immediately after slaughtering at 4˚C is important in reducing infection load. Iran developed the standard procedures for maintenance, preservation, and mixing in 2005 and the microbial and Physiochemical properties in 2007. Therefore, there are not many researches on the production status of the MDM. We can only refer to a research which confirmed the microbial contamination of the MDM (Vollmers *et al*., 2022). The MDM is produced by sausage, baloney, and MDM producing factories. In this study, samples of MDM were selected during the deboning stage in Sausage and baloney factories in Tehran

province and were tested using the standard methods and the results were compared with Iran National standard. The measured factors included the total counting of the microorganisms per gram, with the authorized limit of 5×104, *E. coli* counting per gram with the authorized limit of 5×10, positive *Staphylococcus coagula* per gram with the authorized limit of 1×10 and *Salmonella* in 25 g with the negative authorized limit (D'amico, 2014). After counting the number of *S. aurous* bacterium colonies, it was determined that the rate of contamination in 14% of the samples was far beyond the standard authorized limit while, based on the standard, the number of *Staphylococcus aurous* colonies in the samples should be 10^3. Because there was not any comprehensive study on this bacterium in the country, so an accurate comparison cannot be done in this case. However, regarding the *mustiness* and *fermentative* colonies counting, it can be said that contamination rate of the samples were 11 and 39% above the standard level (Yilma, 2012). Based on the standard level, the *mustiness* and *fermentative* colonies number in the sample must be eventually 10^2 and 10^3, respectively. After the total counting of microorganisms, the results showed that 10% of samples were infected. Regarding the standard authorized limit, the total counting of microorganisms should be 10^5 for the Sausages and 10^6 for the hamburger (Silva *et al*., 2023). In this study, 53% of samples were infected with different types of *Salmonella* indicating the high contamination of these samples and if they are not completely cooked, the produced food can be very problematic. These bacteria can cause infection due to their adhesion capability to the surfaces of equipment, tools, and people, which also raise the possibility of transmission (Querido *et al*., 2019). Also due to *Staphylococcus aurous* bacterium having the ability to produce heat resistant toxins, it is one important cause of food-borne illnesses. *E. coli* and *Salmonella* belong to the normal flora in the human digestive system and other animals, which are excreted through the feces and can cause environment pollution (Abulreesh, 2012; Zhang *et al*., 2020). The contamination present in these samples with *E. coli* and *Salmonella* showed a high contamination through fecal specimens. The MDM due to specific production processes, transportation, and processing develop contamination at different stages. Therefore, besides the typical microbes in the meat, we should expect the secondary infection type to the other factors, which are also pathogenic. Consequently, in some samples, the microbial infection load was extremely high. Unfortunately, the type of bacteria can cause serious risks for human. For example, considering the *Salmonella* contamination which is very high, we should not imagine that this is the general infection statistics, because 25 gram of the sample is selected for the study, and should know that a few ten gram of MDM is obtained from the bones, so a few infected ones would infect all the MDM. Cynically speaking, it should be stated that the non-contaminated samples must be prepared in an exceptional process or about 59% of samples were contaminated with *E. coli*. Based on the Laboratory experiments, samples of chickens in factory would rarely be discarded due to *E. coli* contamination and if the total counting of microorganisms is high, the infection in poultry is reported. The 14% of the tested samples had extremely low infection rate; The microbial load of these samples taken from the thigh and chest were also lower which made clear that the samples were put into the *Cholera* before packaging which is also not permissible since the serious dangers will follow.

Conclusion

In conclusion, the results show that the raw material (MDM) is not recommended and also acceptable to be used from both the microbial contamination and the chemical quality; But because this research has been done on a limited number of samples, it cannot accurately determine the contamination level discovered in the product, so it is essential that comprehensive investigations across the country associated with this product be conducted. It is hoped that comprehensive programs for producing healthy production or preventing the MDM production would be resulted using these information.

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مقاله پژوهشی

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بررسی آلودگی میکروبی خمیر گوشت مرغ در شهرستان نقده

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چکیده

زمینه و هدف: همبرگرها، سوسیس‌ها و بیکن‌ها با تکنیک‌های مختلفی تهیه می‌شوند. خمیر گوشت مرغ محصول خاصی است که اغلب برای تولید چنین محصولاتی استفاده می شود. این خمیر در واقع از باقیمانده‌ها و ضایعات مرغ از جمله پوست و اسکلت آن تهیه می‌شود. هدف از این مطالعه تعیین میزان بار میکروبی در این نوع از محصولات غذایی بود که قرار بود توسط مردم به ویژه کودکان استفاده شود.

مواد و روش‌ها: ۱۰۰ نمونه خمیر گوشت مرغ از شرکت‌های تولید کننده سوسیس و کالباس جمع‌آوری شدند. این نمونه بلافاصله در شرایط دمای سرد به آزمایشگاه منتقل شدند و ویژگی‌های میکروبی آن‌ها بر اساس موسسه استاندارد ایران ارزیابی و آزمایش شد.

یافته‌ها: میزان آلودگی در نمونه‌ها عبارت بود از ۶۸ درصد استافیلوکوکوس اوروس، ۶۲ درصد از نوع تخمیری، ۵۹ درصد اشریشیا کولی، ۵۳ درصد انواع سالمونلا، ۲۱ درصد کپک‌زدگی و نمونه‌های آلوده به سروتیپ‌های مختلف *سالمونلا* شامل ۲۵ درصد *سالمونلا گالیناروم*، ۱۹ درصد *سالمونلا تایفی‌موریوم*، ۱۷ درصد *سالمونلا انتریتیدیس*، ۱۵ درصد *سالمونلا پاراتایفی A*، ۱۵ درصد *سالمونلا پاراتایفی C* و ۹ درصد *سالمونلا پاراتایفی B* بود.

نتیجه‌گیری: بر اساس شمارش تعداد کلنی‌ها مشخص شد که میزان آلودگی در ۱۰ درصد نمونه‌ها فراتر از حد مجاز بود.

واژه‌های کلیدی: *خمیر گوشت مرغ، برگر، سالمونلا، استافیلوکک، اشرشیاکلی*

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