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ASSESSMENT OF SOME PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY PARAMETERS OF RAW MEATBALLS PREPARED WITH HIGH PRESSURIZED MEAT

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ABSTRACT
Raw Meatball (Çiğ Köfte) is a traditional Turkish food prepared from raw beef or lamb meat, bulgur, tomato and/or pepper paste, spices, and condiments that is treated with any process and consumed raw. In this study, high hydrostatic pressure (HHP) was applied to post-rigor minced beef meat at 50–300 MPa for 20 mins at room temperature to improve safety of Çiğ Köfte. Physicochemical (a_w, moisture, pH, and colour) and total aerobic mesophilic bacteria analyses were performed on both minced beef and raw meatball samples. Significant (P <0.05) differences caused by the HHP treatment were observed in the physicochemical and microbial analyses: Total aerobic mesophilic bacteria counts decreased as HHP levels increased. Therefore, it is concluded that HHP treatment is a promising process for preparing Çiğ Köfte while maintaining its traditional recipe. The application of the current study is expected therefore, would be a new insight for ready-to-eat food sector.
Keywords: Food safety, high hydrostatic pressure, raw meatball

YÜKSEK BASINÇ UYGULANMIŞ ETLE HAZIRLANAN ÇİĞ KÖFTELERİN BAZI FİZİKOKİMYASAL VE MİKROBİYOLOJİK KALİTE PARAMETRELERİNİN DEĞERLENDİRİLMESİ

ÖZ
Geleneksel bir Türk gıdası olan Çiğ Köfte dana veya koyun eti, bulgur, domates ve/veya biber salçası, baharat ve çeşniler ile hazırlanmakta ve hiçbir prosese tabi tutulmadan çiğ olarak tüketilmektedir. Bu çalışmada, Çiğ Köftenin güvenliğinin arttırılması için post-rigor dana kıyma etine 50-300 MPa'da 20 dakika oda sıcaklığında yüksek hidrostatik basınç uygulanmıştır. Dana kıyma eti ve Çiğ Köfte örneklerinde fizikokimyasal (a_w, nem, pH ve renk) analizler ile toplam aerobik mezofilik bakteri analizleri yapılmıştır. Yüksek hidrostatik basınç uygulanması fizikokimyasal ve mikrobiyolojik analizlerde önemli (P <0.05) değişikliklere neden olmuştur. Toplam aerobik mezofilik bakteri sayıları yüksek hidrostatik basınç değerleri arttıkça azalma göstermiştir. Bu nedenle, Çiğ Köftenin geleneksel formülasyonunu koruyarak hazırlanmasında yüksek hidrostatik basınç uygulamasının gelecek vaat eden bir proses olduğu sonucuna varılmıştır. Bu çalışmada yer verilen uygulamanın tüketime hazır gıda sektörüne yeni bir bakış açısı getireceği beklenmektedir.
Anahtar kelimeler: Gıda güvenliği, yüksek hidrostatik basınç, Çiğ Köfte

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INTRODUCTION

High hydrostatic pressure, irradiation, pulsed electric fields, high-intensity light pulses are among the non-thermal technologies. Some foods are requiring to be processed with these technologies because of avoiding negative effect of thermal treatment, such as non-enzymatic browning, accumulation of undesirable compounds (furfural, acrylamide, etc.) and nutritional and sensorial losses (San Martín et al., 2002). Çiğ Köfte is one of these foods that requires a non-thermal technology to conserve its originality. Because it is prepared from raw meat and consumed as is, in several hours. This traditional raw meatball appetizer called Çiğ Köfte is commonly consumed in Turkey. It is prepared with raw minced beef or lamb meat, bulgur, tomato and/or pepper paste, spices, and condiments. Consumers are strictly advised to cook meat due to its high perishability and food poisoning risk, however Çiğ Köfte is prepared from raw meat, which raises major food safety concerns (Var and Kabak, 2005).

Due to the time-consuming nature of its preparation, consumers prefer to buy Çiğ Köfte from markets. Moreover, in the last decade Turkish regulations prohibited the sale of Çiğ Köfte that contains raw meat. However, consumer interest in Çiğ Köfte endures and the popularity of a meatless form of the dish has recently increased to follow the sensorial perception of the product. Çiğ Köfte is traditionally prepared and consumed during a folk ceremony that includes music in a process that lasts no more than two hours. A new processing method for raw meat is therefore needed to increase the level of food safety for Çiğ Köfte preparation.

High hydrostatic pressure (HHP) is the most popular cold pasteurisation process used in non-thermal technologies (Mert et al., 2013). Pressure is usually applied in a range of 100–1000 MPa (Bárcenas et al., 2010). The quality of pressurised foods has been documented to be better than thermally pasteurised products (San Martín et al., 2002), and regulatory agencies in several countries (including the United States) have approved its commercial use (Bermúdez-Aguirre and Barbosa-Cánovas, 2011).

The aim of the current study was to assess the quality of Çiğ Köfte that prepared with HHP-treated with meat. Because, there was a need to implement a non-thermal technology to prepare a safe Çiğ Köfte. The HHP treatment was applied to post-rigor minced beef at 50–300MPa for 20 mins at room temperature, and the physicochemical (aw, moisture, pH, colour) and microbiological parameters of the fresh meat were determined.

MATERIALS AND METHODS

Peptone water 0.1%, tryptic soy agar (TSA), and 1,2-Propanediol were used (Sigma-Aldrich, United Kingdom). Minced beef, onion, spring onion, tomato paste, black pepper, and salt were purchased from local markets in Reading (United Kingdom). Meat samples were purchased from two different retailers. Bulgur and gamma-irradiated red pepper were purchased from Antalya (Turkey).

High hydrostatic pressure treatments

Fifty grams of minced beef samples were sealed in polyethylene (PE) bags using a Multivac A300 instrument. Samples were placed in a Stansted high-pressure rig (Stansted Fluid Power Ltd, Stansted, United Kingdom). 1,2-Propanediol was used as a pressurizing medium. Minced beef samples were then separately pressure treated at 50 MPa, 100 MPa, 200 MPa, and 300 MPa for 20 mins at room temperature at the University of Reading Food and Nutritional Sciences Department's food processing centre. High pressurized meat samples were then divided into two groups. (I) Raw meat ball (Çiğ Köfte), and (II) Minced beef.

Preparation of the Çiğ Köfte samples

To determine the contribution of ingredients (other than minced beef) to the microbiological load of Çiğ Köfte, group (I) was used in Çiğ Köfte preparation, while the remained group (II) was evaluated as its control and kept at 4°C until further analyses. The following materials were used to prepare the Çiğ Köfte samples: bulgur

(36.43%), minced beef (29.13%), onion (11.65%), irradiated red pepper (8.74%), tomato paste (7.28%), spring onion (5.83%), salt (0.73%), and black pepper (0.21%). The onion and spring onion were chopped and dipped in a citric acid–water solution (2.5% w/v) for 1 hr to decrease the amount of initial microbial flora. All materials were hand kneaded for about half an hour on a metallic bench. Samples were then aseptically placed in PE bags and sealed using a Multivac A300 packaging unit at the food processing centre. Duplicate bags were sampled in analyses.

Physicochemical analyses

A Rotronic HygroLab C1 (Bassersdorf, CH) instrument was used to conduct a_w (water activity) measurements at a temperature of 20°C. A Mettler Toledo HE53 (Port Melbourne, Australia) Moisture Analyzer (set at 105°C) was used to determine the samples' moisture content, and ColorQuest (Hunter Lab, United States) was used to measure the samples' colour (the reflectance mode was set, the observation angle was 10°, and the illuminant was IIIA). Reflectance specula included, the measuring spot size was 9 mm. Triplicate readings were evaluated for CIE *L** (lightness), *a** (redness), and *b** (yellowness) parameters. Ten grams of each sample were homogenised in 90 mL of distilled water to determine their pH values, which were recorded using a pH meter (SevenEasy, Mettler Toledo) averaging three measurements (AOAC, 2000).

Microbiological analysis

A total of 10-g samples were weighed in sterile stomacher bags containing 90 mL of peptone water (0.1%) and homogenised in a stomacher (Seward 400, UK) at 230 rpm for 2 min. Serial decimal dilutions were prepared and spread onto the TSA. Plates were incubated at 37°C for 24–48 hr prior to counting colonies. TSA was used according to the reference; (Clavero and Beuchat, 1995).

Statistical analysis

Each trial was repeated twice, and duplicate samples were tested at each sampling time. In order to determine the effect of HHP treatment, an analysis of variance (ANOVA) was conducted to evaluate the data using SPSS, version 21(IBM, United States). Duncan's post hoc test was applied at a significance level of P <0.05 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

In this study, it is found that preparation of Çiğ Köfte from HHP-treated meat is a new and contributively finding. In the colour analyses, CIE *L** values were lowered by decreased levels of HHP treatment, indicating that the samples became darker when less pressure was applied. The lower pressure values for both sample sets (raw meatball and minced beef) were found to be similar to the control group's (untreated samples) values, meaning that lightness was lower than in the samples that were exposed to increased levels of pressurisation. Differences were found significant (P <0.05) among each sample set of *L** values (Table 1). *L** values of the minced beef samples were found to be higher than those of the raw meatball samples.

It has already been stated that HHP treatment results in changes to the lightness of meat at pressures of 200–400 MPa (Jung et al., 2003). The findings—that *L** values increased from 200 to 300 MPa—agreed with this. Moreover, the addition of ingredients (e.g., tomato paste and red pepper) eliminated colour losses. This is consistent with a similar study of Uzunlu and Niranjan (2016)'s findings, which were observed visually. Where, they treated minced beef samples for use in a raw meatball preparation with 400 MPa of pressure for 15 min at 22°C. In earlier studies of raw meatballs, both Dogan et al. (2014) and Kozan and Sariçoban (2016) found higher *L** values in their control group samples (prepared by tap water) compared to ours. These differences are likely the result of the different formulations (including materials and proportions) used in each study.

The redness (CIE *a**) of the samples was found to be similar for the raw meatball samples. However, different pressures of HHP treatment resulted in significant (P <0.05) differences between minced beef samples. The *a** values decreased as levels of HHP treatment increased, indicating that the redness of the meat samples was lost when exposed to greater pressure (Table1). The redness of the minced beef samples decreased after 100–300 MPa of HHP treatment, it was higher at 50 MPa of treatment compared to the control samples. For the preparation of Çiğ Köfte, the consumers who buy meat pay attention to their original reddish colour. For this reason, high-pressure processors must consider the pressure level. For example, moderate pressures (e.g., 130 MPa) improve meat colour by increasing redness during the first few days of chilled storage (Jung et al., 2003), which is why the current research investigated low-pressure (50-and 100-MPa) application for retail purpose. However, higher pressures (>130 MPa) are required to sanitise meat in industrial usage; therefore, pressures of 200 and 300 MPa were used in the current study.

Table 1. Physicochemical and microbial changes of RMB and MB as affected by HHP [a]

	Control	50 MPa	100 MPa	200 MPa	300 MPa
a_w					
RMB	0.93±0.00	0.93±0.00	0.94±0.01	0.95±0.02	0.93±0.00
MB	0.96±0.01	0.97±0.00	0.97±0.00	0.97±0.00	0.98±0.00
Moisture (%)					
RMB	27.14±1.35	24.92±1.63	28.74±1.3	26.73±1.22	26.78±0.31
MB	33.22±0.07 [a]	33.66±0.37 [a]	36.41±1.03 [b]	34.78±0.73 [ab]	36.97±0.49 [b]
pH					
RMB	5.17±0.01 [b]	5.1±0.00 [a]	5.1±0.00 [a]	5.15±0.02 [ab]	5.16±0.01 [b]
MB	5.58±0.02 [b]	5.49±0.00 [a]	5.51±0.02 [a]	5.51±0.02 [a]	5.54±0.01 [ab]
*L**					
RMB	33.12±1.53 [b]	33.15±0.12 [ab]	35.10±0.83 [ab]	35.52±0.03 [ab]	36.16±0.05 [a]
MB	37.96±0.03 [bc]	37.29±1.00 [c]	38.28±0.83 [ac]	40.66±0.11 [b]	44.92±0.09 [a]
*a**					
RMB	8.85±1.24	10.24±0.76	9.45±0.08	9.52±0.39	9.42±0.63
MB	12.17±0.26 [ab]	13.14±0.69 [b]	11.30±0.40 [ab]	11.65±0.32 [ab]	10.14±1.51 [a]
*b**					
RMB	6.14±0.16 [b]	7.22±0.09 [a]	7.39±0.23 [a]	7.81±0.24 [a]	7.47±0.10 [a]
MB	4.54±0.26 [b]	5.30±0.15 [a]	5.50±0.24 [a]	5.27±0.30 [a]	5.65±0.17 [a]
TAMB					
RMB	6.05±0.15 [a]	5.31±0.25 [b]	4.50±0.255 [c]	3.60±0.055 [d]	0.15±0.05 [e]
MB	6.00±0.095 [a]	5.23±0.035 [b]	4.14±0.09 [c]	3.23±0.015 [d]	0.1±0.00 [e]

[a] Values are means ± SD.
Values in rows with different letters indicate significant differences ($P < 0.05$). No letters indicate no significant differences ($P > 0.05$) in rows.
RMB: Raw meatball, MB: Minced beef, TAMB: Total aerobic mesophilic bacteria (expressed as log CFU/g)

The yellowness (CIE *b**) of both sample sets decreased when lower levels of pressure were applied. The yellowness of the raw meatball samples was higher than that of the minced beef samples, and the HHP treated samples were significantly (P <0.05) different than the control samples indicating that yellowness were higher than control samples (Table 1). Although, the *a** values of meat form a more significant parameter than *b** values according to consumer choice, the addition of ingredients such as bulgur (boiled and pounded wheat product) resulted in higher *b** levels in the raw meatball samples. Both Dogan et al. (2014) and Kozan and Sariçoban (2016) found the *b** values of the raw meatball samples in their control groups to be higher than ours, which is related to the different formulations used.

The a_w values of the minced beef samples were found to be higher than those of the raw meatballs (Table 1). It is likely that the highly crude materials (e.g., bulgur, paste, and spices) added to the minced beef became bound to free water, leading to decreased values (0.4±0.1 unit lower). Doğan et al. (2014) and Kozan and Sarıçoban (2016) reported a_w values of 0.94 and 0.94–0.95, respectively, for their raw meatball control samples, both of which are very close to the current reported values. While there were no significant differences to the moisture content of the raw meatball samples, significant differences (P <0.05) were observed in the minced beef samples according to the level of pressure used. The HHP treatment resulted in an increase to the moisture content of the minced beef samples. For example, a 4% increase was observed in the sample that was exposed to the highest level of pressure (300 MPa) compared to the control sample. As reported already, the HHP treatment resulted in increases to water content caused by drip losses (Jung et al., 2003). Both Doğan et al. (2014) and Kozan and Sarıçoban (2016) reported moisture content levels about twice as high as those found in the current study; this was caused by the amount of water used in their formulations. Different levels of HHP treatment resulted in significantly different (P <0.05) pH values for each sample set (raw meatball and minced beef). While these differences were significant, the pH values for each sample set remained very close. In keeping with the previously documented pH of post-rigor meat (5.4–5.8), it is found that a pH level of 5.5 in the minced beef samples. The addition of ingredients to minced beef during the preparation of raw meatballs resulted in a pH decrease of about 0.4 units. However, Erol et al. (1993), Uzunlu and Yıldırım (2003), Doğan et al. (2014), Kozan and Sarıçoban (2016) have reported the pH of raw meatball samples to be between 5.5–5.8. This difference could be attributed to the water used in their formulations, which was omitted from the recipe.

Meat and fish species are susceptible to oxidation caused by HHP treatment, which could be perceived by a sensory panel. Both the metal (primarily iron) ions and free radicals released by the pressure treatment are responsible for this increased rate of oxidation (Ma and Ledward, 2013). In a similar study, Uzunlu and Niranjan (2016) treated minced beef samples for use in a raw meatball preparation with 400 MPa of pressure for 15 min at 22°C. However, this level (400 MPa) stands for a critical pressure by making the polyunsaturated fatty acids in the fresh meat more susceptible to oxidation. If 400 MPa upward is required, metal chelating agents, such as ethylene diamine tetra acetate (EDTA), might be used to effectively inhibit increases to lipid oxidation (Ma and Ledward, 2013). Gecgel (2013) identified C18:1 and C18:2 as the main unsaturated fatty acids in raw meatballs and found that storage at 4°C for three weeks resulted in a very negligible (0.05) decrease to the percentage of total polyunsaturated fatty acids present (Gecgel, 2013).

One important parameter for the sensorial judgment of meat is its tenderness. Ma and Ledward (2013) reported that the use of moderate pressures (100–200 MPa) and high temperatures (60–70°C) tenderised post-rigor meat better than treatments at room temperature. This practice could be adopted by raw meatball vendors. Although the meat might appear cooked after such a treatment, the addition of ingredients (tomato paste, red pepper, bulgur) could mask that appearance if consumers like the Çiğ Köfte at sensory evaluation of further research.

Total aerobic mesophilic bacteria counts were substantially affected by the HHP treatment. The growth of microorganisms decreased as the level of HHP treatment increased. For both sample sets, different levels of HHP treatment resulted in significant (P <0.05) differences (Table 1). Earlier studies have reported that Çiğ Köfte has a high microbial load, which is consistent with the current findings. For instance, total aerobic mesophilic bacteria were reported in the range of 4–7 logs, and coliform group bacteria and *Staphylococcus aureus* were reported in the range of 2–5 logs (Pekel et al., 2003; Uzunlu et al., 2004; Ardic and Durmaz, 2008; Cetin et al., 2008; Cetinkaya et al., 2012; Dogan et al., 2014). However, the threat to public health comes not only from the indigenous microbial load found in Çiğ Köfte but also from food handlers' improper hygienic practices. For example, in a study of the recently introduced meatless form of Çiğ Köfte, Taban (2012) found that 12 of the 70 samples purchased in Ankara, Turkey were contaminated with *Listeria monocytogenes*.

Proper hygienic practices are required to increase the safety of Çiğ Köfte consumption. However, the source of risk from raw meat majorly could be removed by using HHP or partly be removed by using natural antimicrobials. One such study reported that plum sauce and pomegranate sauce resulted to one log cycle decrease in Çiğ Köfte samples when compared to control samples (Var and Kabak, 2005). Again, using yoghurt or yoghurt serum inhibited growth of Coliform group bacteria and *S. aureus* in Çiğ Köfte samples, while showed slight antimicrobial effect on total aerobic mesophilic bacteria (Dogan et al., 2014).

The reason that the current study made use of elevated pressure levels (200–300 MPa) at room temperature was to provide microbiological safety to the meat used to prepare Çiğ Köfte so that it could be safely consumed raw, as is tradition. The use of an HHP treatment at 300 MPa resulted in a 6 decimal microbial reduction, which achieved the targeted levels (5-log) of safety in the Çiğ Köfte samples. This level of pressure meets the 5-log microbial reduction specified by the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF), which was released for high-pressure processors (Daryaei and Balasubramaniam, 2012). In addition, the 300-MPa treatment falls in the range of 300–600 MPa, which has been found to cause the inactivation levels of vegetative cells to increase (Rastogi et al., 2007). Conversely, the 200-MPa treatment provided only a 3-log decrease of cells. However, a 200-MPa treatment would be enough to sanitise a meat product with an indigenous microbial load of 3 logs at the time of sale. Uzunlu and Niranjan (2016) found 3 logs of indigenous load in minced beef that was to be used to prepare Çiğ Köfte. Additionally, they reported that a 400-MPa treatment inactivated this initial microbial load (Uzunlu and Niranjan, 2016). The current study found that the 50- and 100-MPa treatments resulted in decreases of 0.8 and 1.8 logs, respectively.

Spore-forming bacteria are another threat to food safety posed by raw meat consumption because bacterial spores are highly resistant to high pressures (above 1200 MPa). Therefore, thermally assisted HHP (using a thermal treatment during the HHP treatment) and non-thermal methods, such as a pre-treatment ultrasound, should be evaluated for microbial safety (Rastogi et al., 2007). As is stressed by Rastogi et al. (2007), HHP treatment should not be considered a replacement for traditional processing methods.

CONCLUSIONS

The current study used low levels of pressure (50 and 100 MPa) to prevent the loss of redness in raw meat for retail purposes; however, these levels of HHP treatment were insufficient to inactivate 6 logs of the minced meat samples' initial microbial load. Elevated levels of pressure (200 and 300 MPa) were therefore used to substantially decrease the microbial load in order to create a treatment suitable for industrial (i.e., catering) purposes. While a loss of redness occurs in meat processed at these elevated pressures, the Çiğ Köfte prepared is safer for consumption than the untreated (control) samples and its original appearance remains protected owing to the ingredients added (i.e. tomato paste, red pepper). Therefore, it is found that HHP to be an effective process for the preparation of Çiğ Köfte.

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