Influence of meat batter addition in ground beef on structural properties and quality parameters

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Abstract
The determination of the amount of non-intact cells (ANIC) in ground beef products is usually performed using a time-consuming and subjective histometric approach neglecting structural properties, which is why more objective and faster methods including evaluation of quality parameters are needed. To determine, whether the addition of meat batter increases the histologically determined ANIC ground beef samples containing increasing shares of meat batter (non-intact cells) were investigated histologically and results were compared to other methodological approaches, namely lactate dehydrogenase activity (LDH), soluble protein content, metmyoglobin content, drip loss, firmness, and cooking loss. Histological measurements showed that ANIC increased linearly with the addition of meat batter to ground beef. The quality parameters drip loss ($r = -0.834, p < 0.01$) and firmness ($r = -0.499, p < 0.01$), and the structural parameter metmyoglobin content ($r = 0.924, p < 0.01$) revealed significant correlations with the amount of added meat batter, and detected differences between ground beef samples when the difference in the amount of added batter-like substance was $\geq 25\%$. Therefore, those methods might be useful to estimate and extrapolate ANIC, and assess product quality of ground beef samples in a faster and simpler way. The cooking loss was not affected by meat batter addition, whereas LDH activity revealed non-repeatable results. Taken together, histometric methods are useful to measure ANIC, nevertheless, it is limited in terms of characterization of morphological and structural changes in the meat. However, other parameters were correlated and could, in addition, be used for assessing the quality of ground meat.

Keywords Hamburger · Ground beef · Characterization · Quality parameters · Histology · Chemical properties

Introduction
Despite the growing trend of meat alternatives [1, 2], the consumers’ interest in meat products especially hamburgers are still high [3, 4]. However, quality changes in ground meat products are reported [5]. To find the initial cause of the quality alteration, basic information on morphological changes is needed but had not yet been investigated.

Industrial production of hamburgers mechanically stresses meat due to compression, wall friction, shear forces, and applied pressures [6] thereby significantly changing the meat structure and thus being a key parameter in cell structure disintegration [7–9]. A combination of mechanical methods cumulatively increases the amount of non-intact cells (ANIC) [7, 10]. The ANIC in meat products is legally regulated in Germany by the German Foodstuff code on meat and meat products [11], and influences product quality properties, functionality, and sensorial perception [7, 10]. As an example, the consistency and granularity changes upon increased ANIC leading to a more pasty and soft mouthfeel [7, 12]. According to the German “Leitsätze für Fleisch & Fleischerzeugnisse” (number 2.507) [11], the ANIC is evaluated by histometric approaches and a maximum of 20 Vol% of non-intact cells are allowed in ground meat products [7]. This technique is officially used to classify ground meat products quality in the German regulations but is a time-consuming and subjective method. A fast, simple, more accurate, and objective alternative method is required but currently lacking [10].
It is known that a mechanical rupture of the meat cells opens its internal structure thus making the proteins available for extraction [13] and increasing the amount of soluble proteins in meat extract. There are chemical and physical analyses well established to assess meat quality such as the determination of LDH activity or the drip loss. LDH is a sarcoplasmic protein that is released upon a structural breakdown of the cells [8, 14, 15]. As an example, LDH activity increases upon protein hydrolysis in aged meat [14] or by freeze-thawing [8]. Thus, it is assumed that LDH activity might increase throughout grinding [8] and therefore be used to estimate the ANIC in ground meat products. Myoglobin is a sarcoplasmic heme protein [16] that is oxidized to metmyoglobin with extended exposure to oxygen thereby changing its color from red to brown [17]. It is assumed that more intense processing increases the oxygen exposure of the meat mass, which increases the concentration of metmyoglobin.

It is reported that disintegration of muscle structure upon grinding alters quality parameters such as drip loss, cooking loss, or firmness of the samples and increases the leakage of intracellular compounds [8, 13]. Based on that, those parameters might be useful as a new approach to estimate the ANIC of ground meat samples. The water holding capacity, the reciprocal of the drip loss, describes the ability of meat to retain part or all of its own and added water [18, 19]. The amount of released water increases with greater ANIC [6, 13]. Both meat quality and sensorial perceptions of the consumer are closely linked to the samples water holding capacity and are strongly dependent on the changes of cellular structures during processing [18].

Upon cooking, meat proteins denature [20], whereby the connective tissue protein fraction shrinks at temperatures of 55−60 °C, causing increased loss of water, fat, or jelly [19]. In cooked, ground meat products, sarcoplasmic proteins form a strong and ordered protein gel network embedding fat and water [20–24] via intermolecular interactions upon heating [25]. Depending on the gel network’s strength, the capacity of retaining water differs [25]. Meat processing adds energy to the system, thereby solubilizing proteins [23, 24]. Thus, it is hypothesized that more intense processing leads to an increased cooking loss when the amount of solubilized proteins are incapable of holding the liberated water, whereas the cooking loss is reduced when the amount of solubilized protein is sufficient to entrap the released water.

It is the aim of this study to characterize morphological changes in ground meat resulting from meat batter addition, the impact on the material properties and quality parameters, and to also assess the methods’ usability as an alternative evaluation criterion. In addition, to the histological reference method, ground beef samples were analyzed for their drip loss (DL), the firmness and the cooking loss (CL), the lactate dehydrogenase (LDH) activity, metmyoglobin content (MetMb) and the soluble protein content (SPC). When combinations of those parameters are considered instead of evaluating ANIC alone, a more objective, comprehensive, and rapid quantification of ground beef quality should be obtained.

**Materials and methods**

**Materials and sample preparation**

Cuts from flank of heifers (*M. transversus abdominis, M. obliquus externus abdominis, M. obliquus internus abdominis*) [26] were visually standardized to fat content of 20%, cut into beef cubes of 5 × 5 × 5 cm, and mixed in a paddle mixer (RC-40, Equipamientos Cárnicos, S.L., Mainca, Barcelona, Spain) for 1 min at 32 rpm. The meat was stored overnight at 1 °C, then first ground to 13 mm particle size (Forschungsautomatenwolf Typ AE 130, Maschinenfabrik Seydelmann KG, Aalen, Germany) with a speed of 20 rpm at the feeding screw and 187 rpm at the grinder screw equipped with a three-fold grinding system (Precutting device (2031809T, Maschinenfabrik Seydelmann KG, Aalen, Germany), with 4-wing knife (TC93094, turbocut Joop GmbH, Bad Neustadt an der Sale, Germany), 13 mm end-hole plate (TC3090278.1, turbocut Joop GmbH, Bad Neustadt an der Sale, Germany)), mixed with the paddle mixer for 30 s at 32 rpm and ground to 2.4 mm particle size using a three-fold grinding system (Cup spring spacer (Maschinenfabrik Seydelmann KG, Aalen, Germany), with 5-wing pendulum knife (TC90820, turbocut Joop GmbH, Bad Neustadt an der Sale, Germany), 2.4 mm end-hole plate (TC3093457.1, turbocut Joop GmbH, Bad Neustadt an der Sale, Germany) with the same grinder settings.

4.5 kg part of the ground beef was chopped for 2 min at 3000 rpm using a chopper (K20, Maschinenfabrik Seydelmann KG, Aalen, Germany) to form meat batter. Batches containing 0%, 5%, 10%, 25%, 40%, and 100% meat batter were produced by carefully mixing ground meat of 2.4 mm particle size with the respective amount of meat batter by hand until homogeneously distributed. Samples were stored airtight and cooled at 1 °C until further analysis. The exact sample preparation is summarized in Fig. 1.

For this study, the term “base material” is defined as ground beef with a particle size of 2.4 mm and the term “meat batter” as finely, batter-like chopped ground beef.

**Determination of the proximate composition**

To determine the chemical composition of the raw material the meat batter was analyzed. The water content was determined according to the procedure described in §64 LFGB method L 06.00-3 [27] using the sea-sand method. Following of the water determination, the samples were utilized for the fat determination according to the procedure described
in §64 LFGB method L 06.00-6 using Soxhlet-extraction (Büchi 810, Büchi Laboratoriums-Technik AG, Flawil, Switzerland). The protein content was determined according to the procedure described in §64 LFGB method L 06.00-20 using rapid nitrogen analysis according to Dumas combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) [27]. A nitrogen to protein conversion factor of 6.25 according to Mariotti and Tomé et al. [28] was applied.

Histochemical analyses of ANIC

Histochemical analysis of the meat samples was performed to assess the amount of non-intact cells (ANIC) according to the procedure described in §64 LFGB method L 06.00-13 [27]. Cryo-cuts of 5 µm thickness were dyed using picri-indigo carmine (CALLEJA) coloring agent and transferred into high-resolution images (Labor Kneissler, Burglen- genfeld, Germany). Histometric analyses were done for 6 images per sample by point-counting non-intact cells of the cross-section scans with software (NDP.view 2.7.52, Hamamatsu Photonics K.K., Shizuoka, Japan).

The upper and lower limit of ANIC is calculated for two images using the following Eq. 1 [27]:

$$
\hat{p}_u \cong \hat{p} - \frac{1}{2n} - 2.3263 \cdot \sqrt{\frac{\hat{p} \cdot \hat{q}}{n}}
$$

$$
\hat{p}_o \cong \hat{p} + \frac{1}{2n} + 2.3263 \cdot \sqrt{\frac{\hat{p} \cdot \hat{q}}{n}}
$$

With \( \hat{p}_u \) = lower limit, \( \hat{p}_o \) = upper limit, \( \hat{p} = \frac{\hat{z}}{n} \), \( \hat{q} = \frac{n-\hat{z}}{n} \), \( n \) = number of non-intact cells counted, \( x \) = number of total cells counted. The mean and the standard deviation of the 3 determinations are calculated.

The linear correlation of the ANIC and the amount of added meat batter can be described with Eq. 2 as

$$
f(x_{\text{meat batter}}) = ANIC_0 + k \cdot x_{\text{meat batter}}
$$

With \( ANIC_0 \) being the y-axes intercept, \( k \) being a slope factor and \( x_{\text{meat batter}} \) being the amount of added meat batter.

Meat extract preparation

Extracts of the beef samples were prepared by modifying existing procedures of Farouk and Wieliczko et al. [22], Wang and Abouzie et al. [29] and Trout [30] to be used for further analyses. Samples were diluted in 10 mM potassium phosphate buffer pH 7 at a ratio of 1:10 in brown glass,
incubated for 20 min at 7 °C and 85 rpm (innova® 42R, New Brunswick Scientific/Eppendorf AG, Hamburg, Germany), and stored for 1 h at 7 °C for further extraction. Meat was separated from the extract by folded filters (Rotilabo®-folded filters, type 113P, Carl Roth GmbH & Co. KG, Karlsruhe, Germany). Extracts were stored at 7 °C in brown glass bottles until further analyses.

**Determination of lactate dehydrogenase activity (LDH)**

The LDH activity of the meat extracts was photometrically determined at 450 nm using an enzyme detection kit (Lactate dehydrogenase activity assay kit MAK066, Sigma-Aldrich Chemie GmbH, Munich, Germany). It is based on an indicator reaction where LDH reduces NAD+ to NADH+H+ [31] which interacts with the probe resulting in the formation of a colored compound photometrically quantifiable at 450 nm [32]. For this purpose, the meat extract was diluted in a ratio of 1:400 using 10 mM potassium phosphate buffer pH7 and then further diluted to a final dilution ratio of 1:40,000 using the LDH sample buffer (part of the enzyme kit). The test was carried out in triplicate of each sample according to the manufacturer’s instructions. The enzyme activity was calculated as stated in the instructions.

**Determination of soluble protein content (SPC)**

The amount of soluble protein in the meat extract was quantified in triplicate through rapid nitrogen analysis according to Dumas combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) [27]. A nitrogen to protein conversion factor of 6.25 according to Mariotti and Tomé et al. [28] was applied.

**Determination of metmyoglobin content (MetMb)**

The metmyoglobin content of the meat extracts was photometrically detected in triplicate using a modified method of Trout [30]. Therefore, 100 µL of each meat extract was transferred to a 96-well transparent plate (NuncTM Delta Surface, Thermo Fisher Scientific, Roskilde, Denmark). The absorption spectra of the extracts were recorded at 25 °C (Biotek Synergy HT, Biotek Instruments, Inc., Winooski, USA). The metmyoglobin content (MetMb) was calculated with Eq. 3 according to Trout [30].

\[
\text{MetMb} \left( \frac{\text{mg}}{\text{mL}} \right) = \left( 1.395 - \frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \cdot 100 
\]

with \(A_\lambda\) = absorbance at \(\lambda\) nm.

A blank of 10 mM potassium phosphate buffer pH 7 at the specific wavelength was subtracted from each measurement.

**Determination of drip loss (DL)**

The drip loss of the meat samples was analyzed in triplicate using the centrifugation method previously described by Honikel and Hamm [18]. 10 g meat sample were weighed into tubes (Nalgene 50 mL PP tubes, Nalgene Nunc International Corporation, New York, USA), and centrifuged for 20 min at 5 °C and 16,000 rpm (Z32HK, Hermle Labortechnik GmbH, Wehingen, Germany). The excess meat juice was removed by placing the meat pellet on a tissue for 1 min. The difference in weight before and after centrifugation was used to determine the percentage weight loss of the meat sample, as shown in Eq. 4:

\[
\text{DL(\%)} = \frac{m_{\text{before centrifugation}} - m_{\text{after centrifugation}}}{m_{\text{before centrifugation}}} \cdot 100 
\]

With \(m_{\text{before centrifugation}}\) = weight of meat sample before centrifugation [g] and \(m_{\text{after centrifugation}}\) = weight of meat sample after centrifugation [g].

**Determination of firmness**

The firmness was analyzed in quintuplicate by forward extrusion method. For this, a cylinder of 50 mm diameter with a bottom hole opening of 7.5 mm diameter was carefully filled with the meat mass at 1 °C, thereby trying to avoid entrapped air. A texture measurement device (Instron, Model 3365, Instron Engineering Corporation Ltd, Massachusetts, USA) equipped with a plunger of 49 mm diameter and a crosshead speed of 20 mm/min was used to press the meat mass through the hole opening thereby recording the required force.

**Determination of cooking loss**

To analyze the cooking loss, approx. 40 g meat sample was placed into a Nalgene can (60 mL PP screw cap container, Nalgene Nunc International Corporation, New York, USA), compressed with 20 bar for 5 s (Ham Press Typ Mini, Waser Johann GmbH formerly Barth und Seibold, Aalen) and the exact sample weight was noted. The cans were closed, heated in a water bath for 60 min at 90 °C, and then cooled in ice water for 10 min. Meat and meat juice was separated using a sieve before the weight of the cooked meat was determined. The difference in weight before and after cooking was used to determine the percentage cooking loss of the meat sample, as shown in Eq. 5:
CL(%) = \frac{m_{\text{before cooking}} - m_{\text{after cooking}}}{m_{\text{before cooking}}} \cdot 100 \quad (5)

With \( m_{\text{before cooking}} = \) weight of meat sample before cooking [g] and \( m_{\text{after cooking}} = \) weight of meat sample after cooking [g].

**Statistical analyses**

The experiment was performed in duplicate. The mean and standard deviation was calculated using MS Excel (Microsoft, Redmond, WA, USA) and plotted using OriginPro 2020 (OriginLab Corporation, North Hampton, MA, USA). Statistical analyses were performed with SPSS (IBM SPSS Statistics 25, IBM Deutschland GmbH, Ehningen, Germany). Normal distribution of data and variance homogeneity were tested using Shapiro–Wilk, Levene test, and QQ-plots, respectively. All data showing significance values were normally distributed. For data showing variance homogeneity, a significance analysis using the univariant ANOVA (analysis of variance) was conducted. The Tukey post hoc test with a confidence interval of 95% \((\alpha = 0.05)\) was applied. For data showing no variance homogeneity, a significance analysis using the Welch-ANOVA (analysis of variance) was conducted. The Games-Howell post hoc test with a confidence interval of 95% \((\alpha = 0.05)\) was applied.

Statistical linear correlation analysis between two variables was conducted using Pearson’s correlation (PC) test, usually applied to normally distributed data. The correlation coefficient \(r\) was used to assess the power of the correlation whereas the \(p\)-value was used to assess the significance of the correlation.

**Results and discussion**

**Basic composition**

The shares of meat batter addition were chosen to simulate increased amounts of non-intact cells due to relevance in application. Shares between 40 and 100% meat were used to validate the analytical methods. The proximate composition of the base material was investigated to ensure product quality as well as constant and comparable sample composition throughout different experiments. On average, the base material was composed of 60.40 ± 0.85 g/100 g moisture, 20.40 ± 0.57 g/100 g fat, and 19.50 ± 1.61 g/100 g protein. Fat content of approximately 20 g/100 g is commonly used for hamburger manufacturing, as it is advantageous for palatability [33].

**Determination of amount of non-intact cells (ANIC)**

The histological analysis was carried out to detect differences in ANIC among the differently treated samples. Furthermore, it served as a reference method to compare the suitability of the alternative physical and chemical methods to detect cell disruption. It was assumed that an increasing amount of added meat batter increases the ANIC of the sample.

As shown in Fig. 2 the ANIC increased significantly with increasing amount of added meat batter as indicated by a highly significant, strong, positive linear Pearson correlation \((r = 0.947, p < 0.01)\) (Table 1). Thereby the ANIC increases from 20.61 ± 1.75 Vol% in the base material \((x_{\text{meat batter}} = 0\%)\) to 97.38 ± 1.25 Vol% in the meat batter \((x_{\text{meat batter}} = 100\%)\). Figure 2 also depicts histological images of the samples with 0, 5, 40, and 100% meat batter addition. As indicated by the arrows, the amount of destructed, irregular shaped muscle cells, the ANIC, increases with increasing share of added meat batter. The number of cell fragments increased, whereas their size decreased, leading to a less ordered system. The results are in accordance with literature expectations reporting cell disintegration in meat upon mechanical treatment [34]. Beneke [7] stated that there is a histologically detectable increase in ANIC due to the increased share of batter-like substances caused by the mechanical load on ground meat. However, there are limitations with respect to the informative value of the histological approach that should be pointed out. These limitations include, inter alia, strong dependence on base material properties, dependence on the sampling process and sampling size, and the subjectivity of the method. For this study, the correlation between the ANIC and the amount of added meat batter is linear in the range up to 25% added meat batter which is described as \(f(x_{\text{meat batter}}) = 1.542 + 18.648 \cdot x_{\text{meat batter}} (R^2=0.9922)\). At higher meat batter additions, polynomic correlations were observed. Because meat batter additions of 0–25% represent the likely occurring range of ANIC, the correlation with the alternative methods is still given.

It is known that ANIC0 is defined by the base material and thus prone to raw material fluctuations resulting in an individual graph intercept. It is further known that meat batter has a high ANIC of 99–100% due to strong mechanical treatment thus forming a fixed endpoint of the graph. As the slope factor \(k\) is defined by the graphs’ intercept and endpoint the correlation between the ANIC and the amount of added meat batter varies among the used base material. There is no general correlation between the parameters, thus a universal conclusion from the histologically determined ANIC to the relative amount of added meat batter is not possible without taking the base material characteristics into account. The dependency of the correlation on the base material properties was proven in preliminary
studies, in which coarse particle size of the base material (13 mm) resulted in ANIC values lower than 15 Vol% (data not shown).

Because an increased ANIC leads to multidimensional morphological changes creating a heterogeneous system, it is suggested that further material and quality parameters should be considered when evaluating the material properties. The influence of those morphological changes might cause changes in the material and quality parameters of the samples.

### Influence of meat batter addition on structural properties of ground meat samples

Because higher amounts of meat batter increase ANIC (Fig. 2), an increased release of intracellular compounds like proteins, pigments, and enzymes is hypothesized, assuming

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**Table 1** Pearson correlation coefficients $r$ and significance levels $p$ of amount of added meat batter $x_{\text{meat batter}}$ amount of non-intact cells (ANIC), soluble protein content (SPC), metmyoglobin content (MetMb), drip loss (DL), Firmness, and cooking loss (CL)

| $x_{\text{meat batter}}$ (%) | ANIC (Vol%) | SPC (%) | MetMb (mg/mL) | DL (%) | Firmness (N) | CL (%) |
|-----------------------------|------------|---------|---------------|--------|--------------|--------|
| $x_{\text{meat batter}}$ (%) | $r$        | $p$     | $r$           | $p$    | $r$          | $p$    |
| ANIC (Vol%)                 | $0.947^{**}$ | $0$     | $-0.573^{**}$ | $0.924^{**}$ | $-0.834^{**}$ | $-0.499^{**}$ | $-0.275$ |
| SPC (%)                     | $-0.573^{**}$ | $0.670^{**}$ | $1$           | $-0.526^{**}$ | $0.472^{**}$ | $0.049$ | $0.500^{**}$ |
| MetMb (mg/mL)               | $0.924^{**}$ | $0.870^{**}$ | $-0.526^{**}$ | $0.001$ | $0.004$ | $0.779$ | $0.002$ |
| DL (%)                      | $-0.834^{**}$ | $-0.749^{**}$ | $0.472^{**}$ | $-0.787^{**}$ | $1$ | $0.533^{**}$ | $0.268$ |
| Firmness (N)                | $-0.499^{**}$ | $-0.389^{*}$ | $0.049$ | $-0.441^{**}$ | $0.533^{**}$ | $1$ | $0.247$ |
| CL (%)                      | $-0.275$ | $0.132$ | $0.002$ | $0.098$ | $0.120$ | $0.153$ |

*The correlation is significant on a level of 0.05

**The correlation is significant on a level of 0.01

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LDH, SPC, and MetMb to increase as well. Based on this principle, LDH is already used as a biochemical marker for muscle damage in humans [35] and the identification of frozen meat [8].

Lactate dehydrogenase (LDH)

The LDH of the extracts ranged from $884.07 \pm 514.50$ units/mL ($x_{\text{meat batter}} = 0\%$) to $427.75 \pm 342.78$ units/mL ($x_{\text{meat batter}} = 100\%$) (Fig. 3A). Although homogeneous sample material was ensured, measurement data strongly fluctuated. Due to high standard deviations and non-normal distributed data (Fig. 4), no statistical significance and no correlation could be calculated. Keller [36] reported LDH changes in bovine serum upon injuries and Kumar and Nagarajan et al. [31] found increased LDH with increasing damage of cell plasma membrane. In contrast to that, significant correlation between the ANIC and LDH could not be identified in this study. It is reported that the activity of LDH in meat is not only affected by the morphological changes upon processing but also by age, sex, breed, and origin of the cattle, storage time after slaughtering, and temperature during production or storage [8, 37, 38]. It is therefore assumed, that the LDH changes caused by processing are overlayed by other influencing parameters, making this method unsuitable for an ANIC estimation.

Soluble protein content (SPC)

Increasing the amount of meat batter slightly reduces the SPC from $5.96 \pm 1.31\%$ ($x_{\text{meat batter}} = 0\%$) to $3.83 \pm 0.32\%$ ($x_{\text{meat batter}} = 100\%$) (Fig. 3B). The correlation analysis revealed a highly significant, negative linear correlation between the amount of added meat batter and the SPC ($r = -0.573, p < 0.01$) and a highly significant, negative linear correlation between the ANIC and the SPC ($r = -0.670, p < 0.01$), (Table 1), being contrary to the initial hypothesis. This might be traced back to the fact, that not only particle sizes are reduced but also the morphology and texture changes alter the molecular interactions in the sample. The properties of meat samples are also altered by salt concentration [21]. As the meat batter was prepared without salt addition, the natural ionic strength of meat is not altered. Therefore, mainly water-soluble, sarcoplasmic proteins are present in solution. Reduced SPC might be explained by a stronger involvement of the proteins in network formation by interacting with fat, protein, and water components [39]. Due to molecular interactions upon network formation, extractability might be reduced. The higher the amount of added meat batter the more solubilized proteins might interact in the network, thus reducing SPC.

Fig. 3 Characterization of the material properties A Lactate dehydrogenase activity (LDH), B Soluble protein content (SPC) and C Met-myoglobin content (MetMb) of the ground beef samples extracts as a function of the amount of added meat batter $x_{\text{meat batter}}$. Data points with different letters are significantly different ($p < 0.05$)
Metmyoglobin content (MetMb)

MetMb, as a function of added meat batter, increased from 98.37 ± 27.82 mg/mL (x_{meat batter} = 0%) to 267.77 ± 4.67 mg/mL (x_{meat batter} = 100%) (Fig. 3C). MetMb shows not only a highly significant, positive linear correlation with the amount of added meat batter (r = 0.924, p < 0.01) but also with the ANIC (r = 0.870, p < 0.01) (Table 1). Significant differences between extreme samples (x_{meat batter} = 0% vs. x_{meat batter} = 100) were found, indicating that MetMb might be suitable to roughly estimate the ANIC in this study. The MetMb increase might be caused by additive, linear mixing effects of the base material and meat batter as it is an oxidative product of the intracellular compound myoglobin [40]. Intense meat processing also enhances the oxygen incorporation [34], accelerating the oxidation rate of myoglobin to MetMb. Besides myoglobin, fats and other components are also oxidized by higher oxygen exposure. Literature reports similar oxidation mechanisms for lipids and myoglobin based on lipid and oxy-free radical generation [40, 41], wherefore MetMb might be used as a quality degradation indicator. Unlike the SPC, MetMb increases with meat batter addition, as the pigment is not involved in network formation and is freely available for extraction.

Among the structural properties in this study, only MetMb revealed a good correlation to the amount of added meat batter and the ANIC.

Influence of meat batter addition on quality parameters of ground meat samples

Because meat batter addition increases ANIC leading to more opened, disrupted cells and more released intracellular compounds, the samples drip loss (DL), firmness, and cooking loss (CL) were assumed to increase as well.

Drip loss (DL)

The DL of the samples was linearly reduced from 5.09 ± 0.71% (x_{meat batter} = 5%) to 1.50 ± 0.31% (x_{meat batter} = 100%) with significant differences between the samples (Fig. 5A). Correlation analyses between the amount of added meat batter and the DL revealed a highly significant, strong negative correlation (r = −0.834, p < 0.01) and a highly significant, strong negative correlation (r = −0.749, p < 0.01) (Table 1) between the DL and the ANIC, indicating an increased water holding capacity of the meat samples with increased ANIC. Honikel and Hamm [18] reported correlations between changes in cell structure and the drip loss of meat samples. The drip loss is influenced by both the amount of released intracellular proteins and morphological changes based on the mixing ratio of base material and meat batter [39, 42]. ANIC is higher in the meat batter than in the base material (Fig. 2). Upon chopping, intracellular components like proteins are solubilized, being available for network formation and water binding thus causing lower drip loss with increasing meat batter addition [24, 43, 44]. As DL strongly negatively correlates with the amount of added meat batter and ANIC, it is a suitable parameter for quality estimation in the study.

Firmness

The firmness of the samples increased from 258.95 ± 20.94 N (x_{meat batter} = 0%) to 319.28 ± 1.21 N (x_{meat batter} = 25%) and then decreased to 210.41 ± 27.66 N (x_{meat batter} = 100%) (Fig. 5B). The turning point at 25% meat batter addition indicates a change of the predominant morphological structures. Below 25% meat batter addition, the solubilized proteins might form three-dimensional networks embedding fat particles, cells, or connective tissue fragments. These molecular and interparticle interactions probably increase the samples' firmness. At shares of > 25% meat batter, the number of particles, the length of muscle cells, and the degree of entanglement decreased and, the three-dimensional network became weaker, thus the firmness of the sample would be reduced. At the same time, the amount of entrapped water (Fig. 5A) in the protein network increased which additionally softens the texture [45].
Cooking loss (CL)

The CL of all samples ranged between 32.29 and 35.55% without any statistically significant differences (Fig. 5C). The results indicated that the amount of added meat batter and the ANIC did not influence the samples CL. This is supported by the non-significant correlation of the CL and the amount of added meat batter ($r = -0.275, p = 0.110$) as well as the CL and the ANIC ($r = -0.260, p = 0.132$) (Table 1). The findings are contrary to the initially expected increase of the cooking loss with increasing amount of meat batter as Berry [46] reported a slightly higher cooking loss in chopped (cooking loss 39%) than in ground (cooking loss 36%) hamburgers. As the amounts of cooking losses detected by Offer and Knight et al. [47] (up to 40%) and Berry [46] (36–39%) are comparable to the results of this study and the differences were also quite small, the results are in accordance. Therefore, it is concluded, that the denaturation behavior of the protein structures are unaffected by the degree of comminution and the particle size. Although the ANIC differs by more than 80 Vol%, the important quality parameter CL was not affected. This underlines, that besides the ANIC, several other parameters should also be evaluated to fully categorize the ground meat quality.

DL and Firmness revealed a significant correlation between the amount of added meat batter and the ANIC (Table 1), thus being able to conclude about the quality parameters, whereas no correlations were found for CL and amount of added meat batter within this study.

Underlying mechanism

Based on the previous findings, the following mechanism for the changes in the ground meat samples upon meat batter addition is proposed (Fig. 6).

Base material ($x_{\text{meat batter}} = 0\%$)

The base material properties ($x_{\text{meat batter}} = 0\%$, ANIC$_0 = 20.61 \pm 1.75$ Vol%) shows dispersed system characteristics predominantly defined by particle interactions between mainly big, intact muscle cells and particular components surrounded by some cell fragments and a small amount of solubilized proteins. The typical ground meat is characterized by a rather loose structure, weaker interactions, and thus exhibits less cohesion. Therefore, water retention (e.g., DL) and firmness of the meat sample are lower when more intact cells are present.

Meat batter ($x_{\text{meat batter}} = 100\%$)

In contrast, the meat batter ($x_{\text{meat batter}} = 100\%$, ANIC$_{100} = 97.38 \pm 1.25$ Vol%), is a finely chopped meat
mass in which particle sizes are hence reduced [21]. Thus, meat batter is mainly defined by the characteristics of an emulsified system with predominantly small cell fragments and a high concentration of solubilized proteins [25]. In meat batter, the stronger molecular interactions of the dissolved proteins allow for better water retention (e.g., lower DL). Nevertheless, structuring components, such as larger, intact cells or parts of connective tissue are missing. The cell fragments are too small to form a strong and three-dimensional network, which is why the meat batter is less firm than the base material.

**Mixed samples (ANICΔ)**

In mixed samples (ANIC\(_{Δ}\)), consisting of the base material and meat batter, mixing effects occur caused by two main factors. (i) First, the mechanically induced morphological changes of the cell structure: those are more important in systems with higher amounts of base material forming the dispersed particle phase. (ii) Second, the amount of solubilized sarcoplasmic proteins: those are more pronounced at higher shares of meat batter and are available for molecular interactions and functionality [25]. Mixed samples contain a mixture of intact and non-interact cells of different sizes, particular components, cell fragments, and solubilized proteins. Their molecular and particle interactions enable three-dimensional network formation. The embedding of different amounts of particulate components in the network alters quality and structural properties of the samples. Therefore, the bulk properties of mixed systems are mainly defined by the predominant component. It is assumed, that exceeding 25% meat batter addition marks a critical amount at which the predominant system properties change from dispersed to emulsified characteristics, as the quality parameters DL & firmness significantly change at higher meat batter amounts.

**Conclusions**

The addition of meat batter to the base material led to linear mixing effects, thus increasing ANIC as expected and causing changes in structural and quality parameters. Those changes are assumed to be mainly based on morphological changes due to the mechanical disintegration of meat structures. Correlations of MetMb, DL, and firmness with the share of meat batter addition qualify them to estimate ground beef quality quickly and easily in this model system. It was demonstrated that the ANIC highly depends on the base material characteristics and should therefore not be exclusively used to rate the ground beef quality and material properties, wherefore those parameters could serve as additional methods. The suitability of the alternative methods to characterize cell disintegration in ground meat products generally is limited, as significant differences were mainly found between extreme samples (\(x_{\text{meat batter}} = 0\%\) vs. \(x_{\text{meat batter}} = 100\%\)). A more precise differentiation in the practically occurring range (up to ca. 35 Vol% ANIC) was not possible.
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Declarations

Conflict of interest  The authors declare no conflict of interest.

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