Process, Structure and Function Relationship in Ground Meat

Dissertation to obtain the doctoral degree of Natural Sciences (Dr. rer. nat.)

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Co-Authors

The scientific work presented was partially conducted in cooperation with other scientists. Prof. Dr. Jochen Weiss supervised the complete doctoral thesis as project leader and contributed substantially to the conception and interpretation of this work. Apl. Prof. Dr. rer. nat. Monika Gibis was involved as project coordinator in the conception, funding acquisition, experimental planning, writing, and proof-reading of the manuscripts. Dr. Nino Terjung supported the conception and was involved in project administration and funding acquisition.

- **Chapter I:** All authors contributed to conceptualization of the review as well as the planning and carrying out parts of the writing. Lisa Berger, Franziska Witte contributed to writing, editing, and visualization equally (shared first authorship). All authors performed proof reading.
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List of Publications

All manuscripts marked with a superscript asterisk (*) are included in this dissertation.

Peer-reviewed publications with first authorship

Berger, L.M.; Gibis, M.; Witte, F., Terjung, N.; Weiss, J. (2022)*: Influence of Meat Batter Addition in Ground Beef on Structural Properties and Quality Parameters. *European Food Research and Technology*, 248, 2509–2520. doi: 10.1007/s00217-022-04065-0

Berger, L.M.; Witte, F.; Terjung, N.; Weiss, J.; Gibis, M. (2022)*: Influence of Processing Steps on Structural, Functional, and Quality Properties of Beef Hamburgers. *Applied Sciences*, 12, 7377. doi: 10.3390/app12157377

Berger, L.M.; Böckle, C.; Gibis, M.; Herrmann, K.; Terjung, N.; Weiss, J. (2023)*: Effect of Manufacturing and Frozen Meat Temperatures on Structural and Functional Properties of Hamburgers. *Journal of Food Engineering*, 353, 111526. doi: 10.1016/j.jfoodeng.2023.111526

Berger, L.M.; Böckle, C.; Gibis, M.; Terjung, N.; Weiss, J. (2023)*: Effect of Re-fed Meat in Beef Hamburger Production. *JSFA Reports*, 3(12): 646-654. doi: 10.1002/jsf2.174

Berger, L.M.; Adam, F.; Gibis, M.; Witte, F.; Terjung, N.; Weiss, J. (2023)*: Effect of Cutting Set Variations on Structural and Functional Properties of Hamburgers. *European Food Research and Technology*. doi: 10.1007/s00217-023-04414-7

Berger, L.M.; Witte, F.; Tomasevic, I.; Heinz, V.; Weiss, J.; Gibis, M.; Terjung, N. (2023)*: A Review on the Relation between Grinding Process and Quality of Ground Meat. *Meat Science*, 205:109320. doi: 10.1016/j.meatsci.2023.109320

Other peer-reviewed publications

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Witte, F.; Sawas, E.; **Berger, L.M.**; Gibis, M.; Weiss, J.; Röser, A.; Upmann, M.; Joeres, E.; Juadjur, A.; Bindrich, U.; Heinz, V.; Terjung, N. (2022): Influence of Finely Chopped Meat Addition on Quality Parameters of Minced Meat. *Applied Sciences*, 12, 10590. doi: 10.3390/app122010590

Tomasevic, I.; Witte, F.; Kühling, R.E.; **Berger, L.M.**; Gibis, M.; Weiss, J.; Röser, A.; Upmann, M.; Joeres, E.; Juadjur, A.; Bindrich, U.; Heinz, V.; Terjung, N. (2023): Effect of Frozen to Fresh Meat Ratio in Minced Pork on Its Quality. *Applied Sciences*, 13, 2323. doi: 10.3390/app13042323

Ebert, S., **Berger, L.M.**, Moll, P. (2022). Potential of upcycled Side-Streams. *Cereal Technology*

Forthcoming publications

Berger, L.M.; Esch, M.; Eckelt, A.; Herrmann, K.; Gibis, M.; Weiss, J. (2023): Einfluss unterschiedlicher Mischzeiten auf die Struktur und Funktionalität von Hamburgern. To be published in *Fleischwirtschaft*

Oral presentations

Berger, L.M. (2019): Sitzung des Projektbegleitenden Ausschusses AiF 20384N „Hackfleisch und Hackfleischerzeugnisse“, Biberach, Germany

Berger, L.M. (2019): Process, Structure and Function Relationship in Ground Meat, *Seminar of Food Material Science for Graduate Students and Doctoral Candidates*, Hohenheim, Germany

Berger, L.M. (2019): Meat in General, *M.Sc. Course: Advanced Meat Science and Technology*, Hohenheim, Germany

Berger, L.M. (2020): Expert Panel Discussion and Workshop „Workshop des ILT und der ALTS-UAG Lebensmittel-Histologie zur Vereinheitlichung von Bewertungen bei Strukturveränderungen in Hackfleisch, Lemgo, Germany

Berger, L.M. (2020): Sitzung des projektbegleitenden Ausschusses AiF 20384N „Hackfleisch und Hackfleischerzeugnisse“, Online, Germany

Berger, L.M. (2020): Meat in General, *M.Sc. Course: Advanced Meat Science and Technology*, Hohenheim, Germany

Berger, L.M. (2020): Dry fermented Sausages & dry-cured ham, *M.Sc. Course: Advanced Meat Science and Technology*, Hohenheim, Germany

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Berger, L.M. (2021): Fertig mit Studium/ Promotion. Und nun?, *Studi-Kongress der Gesellschaft Deutscher Lebensmitteltechnologen e.V.*, Online, Germany

Berger, L.M. (2021): Sitzung des projektbegleitenden Ausschusses AiF 20384N „Hackfleisch und Hackfleischerzeugnisse“, Online, Germany

Berger, L.M. (2022): Introduction into Food Product Development, *M.Sc. Course: Food Product Development – From Concept Ideation to Product Launch*, Hohenheim, Germany

Berger, L.M. (2022): Process, Structure and Function Relationship in Ground Meat, *Seminar of Food Material Science for Graduate Students and Doctoral Candidates*, Hohenheim, Germany

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Berger, L.M. (2022): Digitalisierung in der sensorischen Qualitätskontrolle und Produktenwicklung, *B.Sc. Course: Sensorische Methoden in Produktentwicklung und Qualitätssicherung*, Hohenheim, Germany

Berger, L.M. (2022): Meat in General, *M.Sc. Course: Advanced Meat Science and Technology*, Hohenheim, Germany

Berger, L.M. (2022): Dry fermented Sausages & Dry-Cured Ham, *M.Sc. Course: Advanced Meat Science and Technology*, Hohenheim, Germany

Berger, L.M. (2022): Introduction in Food Systems, *M.Sc. Course: AgFoodTech*, Hohenheim, Germany

Berger, L.M. (2022): Insights into a sustainable Food Start Up, *Start-Up Garage*, Hohenheim, Germany

Berger, L.M. (2022): Insights into a sustainable Food Start Up, *ELLS Spring Camp*, Hohenheim, Germany

Berger, L.M. (2022): Storytelling and Pitching, *Social Impact Hub Stuttgart*, Stuttgart, Germany

Berger, L.M. (2022): Expert Discussion Panel: “Tackling Food Waste: Defining the Problem, Innovating Solutions, *IFT 2022*, Chicago, USA

Berger, L.M. (2023): Introduction into Food Product Development, *M.Sc. Course: Food Product Development – From Concept Ideation to Product Launch,* Hohenheim, Germany

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Berger, L.M.; Gibis, M.; Witte, F.; Terjung, N.; Weiss, J. (2023): Mixing time influencing morphological structure, physico-chemical and functional properties of ground beef. Oral presentation at Research Showcase Stage at *IFT.First23 Annual Event and Expo*, Chicago, USA

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Berger, L.M.; Herrmann, K.; Gibis, M.; Weiss, J. (2023): Mechanical and structural characterization of industrial ground beef and hamburger processing line. Poster presentation at the *ICoMST2023*, Padua, Italy

Herrmann, K.; **Berger, L.M.**; Gibis, M.; Weiss, J. (2023): Comparative studies of pilot plant and industrial trials on the addition of re-fed meat on quality in hamburgers. Poster presentation at the *ICoMST2023*, Padua, Italy

Table of Content

List of Figures

List of Tables

Symbols and Abbreviations

Symbol/ Abbreviation	Definition	Unit
%	Percent	
°C	Degree celsius	
AiF	Arbeitsgemeinschaft industrieller Forschungsvereinigungen	
ANIC	Amount of non-intact cells	Vol.%
ANOVA	Analysis of variance	
CL	Cooking loss	%
CLSM	Confocal laser scanning microscopy	
cm	Centimeter	
d	Diameter	
DL	Drip loss	%
e.g.	For example (*Latin*: exempli gratia)	
FEI	Forschungskreis der Ernährungsindustrie e.V.	
h	Hour	
i.e.	That is to say (*Latin*: id est)	
J	Joule	
K	Kelvin	
kg	Kilogramm	
L	Litre	
LDH	Lactate dehydrogenase	units/mL
m	Meter	
Mb	Myoglobin content	mg/mL
MetMb	Metmyoglobin content	mg/MmL
min	Minute	
mL	Milliliter	
mm	Millimeter	
µm	Micrometer	
N	Newton	
n.d.	Not determined	
p	Probability	

Pa	Pascal	
pH	Potential of hydrogen	
rad	Radian	
rpm	Rounds per minute	
s	Second	
SME	Specific mechanic energy	J/kg or kJ/kg
SPC	Sobuble protein content	%
t	Time	
T	Temperature	°C
V	Volt	
Vol.%		
Vol%	Volume percent	
Vol.-%		

Summary

Ground beef has enjoyed high popularity with consumers because it is convenient to use and facilitates a rapid preparation of a large variety of different meals. Meat processing was initially carried out by butchers in a craft-style setting, but high demand has given rise to manufacturing that takes place in larger food enterprises. Large-scale manufacturing of ground beef and its products involves a combination of several unit operations such as separation, size reduction, forming, freezing, and packaging leading to the formation of a ground meat mass that is composed of small, distinct meat and fat particles. Ideally, the original underlying cellular meat or fat structure in such masses is preserved as much as possible so that important quality attributes such as water holding, texture, appearance, and color are optimized. However, the effect of varying conditions and parameters in modern processes on the quality of ground meat has not yet been investigated in detail. According to the current German “Leitsätze für Fleisch und Fleischerzeugnisse”, hamburgers must not contain more than 20 Vol.% of non-intact cell structures to be sold without further declaration. Therefore, this work aimed to identify process, structure, and function relationships in ground meat production to facilitate a gentler processing of in particular hamburgers. To investigate these effects systematically, a standardized production method for hamburgers was developed and a pilot plant scale meat grinder was set up with the possibility to record process-relevant data.

In the **first part of this thesis,** studies were performed to gain a better understanding of the basic system properties of ground meat. Specifically, the relationship between the structure and functionality of ground meat was investigated in **Chapter II**. A model system with increasing amounts of added meat batter was used to simulate changes in meat structure due to cell disintegration. A new term, i.e., the amount of non-intact cells (ANIC), was introduced to quantify the amount of disintegrated meat cells during processing. It was shown that changes in the structure due to a higher or lower ANIC resulted in altered physicochemical and functional properties of the ground meat system. This was explained by morphological changes in the system, altered mixing effects, and interactions of the system components.

A systematic screening of the influence of raw material properties on the structure and function of ground meat was performed in the **second part of the thesis**. In **Chapter III** the effect of frozen meat content and temperature on the structure and function of hamburgers was investigated to verify the above-obtained correlation to an application-relevant setting. As the specific cutting resistance is significantly higher in frozen than in chilled meat, it was assumed, that the impact on the ground meat’s structure and function differed accordingly. Indeed, this could be verified and resulted in more pronounced structural changes (higher ANIC) and altered product parameters.

In hamburger manufacturing, it is common practice to re-fed imperfectly molded patties, e.g., in a frozen, coarsely crushed state. In contrast to the findings of **Chapter III**, the use of up to 20 % re-fed material in hamburger manufacturing did not result in any noticeable differences (**Chapter IV**). The results showed that neither the specific mechanical energy input (SME) nor the ANIC was significantly influenced by the addition of up to 20 % re-fed material. This can be attributed to three main reasons, which are (1) the formation of smaller fragments, (2) an already small pre-grinding particle size of re-fed material, and (3) a temperature equalization between the frozen and cooled material causing a softening of the frozen particles.

Summarizing the **second part of the thesis**, it was demonstrated, that some raw material variations can have an impact on both structure and function of hamburgers. Especially, temperature effects and associated changes in the cutting resistance of the raw material had the strongest influence on structure and function of ground meat. However, if structural differences were found, they were not sufficient to manifest in differences in sensory evaluation. This means that the consumer perception and thus the quality of the hamburger was not influenced.

The **third part of the thesis** focuses on the process parameters and their impact on the structure and function of hamburgers. **Chapter V** investigates the impact of the four main processing steps pre-grinding, mixing, grinding, and forming. An increased ANIC was determined with progressive processing, whereby the first and second grinding steps accounted for the strongest increase. Mixing and forming were of minor importance for structural and functional changes. This was also underlined by most of the analytical parameters. It could be shown that there is a close relationship between applied mechanical forces, structure, and functionality.

By varying the angle of the drill holes of the hole plates, the size of the drill holes, and the number of cutting levels, the influence of the cutting set compositions on the structure and function of hamburgers was assessed in **Chapter VI**. The SME and the ANIC increased if more cutting levels were used. This can be attributed to higher shear stress applied to the meat, which has to pass through more levels and has thus more contact with drill holes and knives. However, the angle of the drill holes and the hole size of the middle hole plate did cause no or only negligible changes in the ANIC and SME. Although an impact of the cutting set composition on the structure could be found, no or only marginal effects on the function and the sensory and optical quality of the hamburgers were found. This indicates that less cutting levels result in gentler processing.

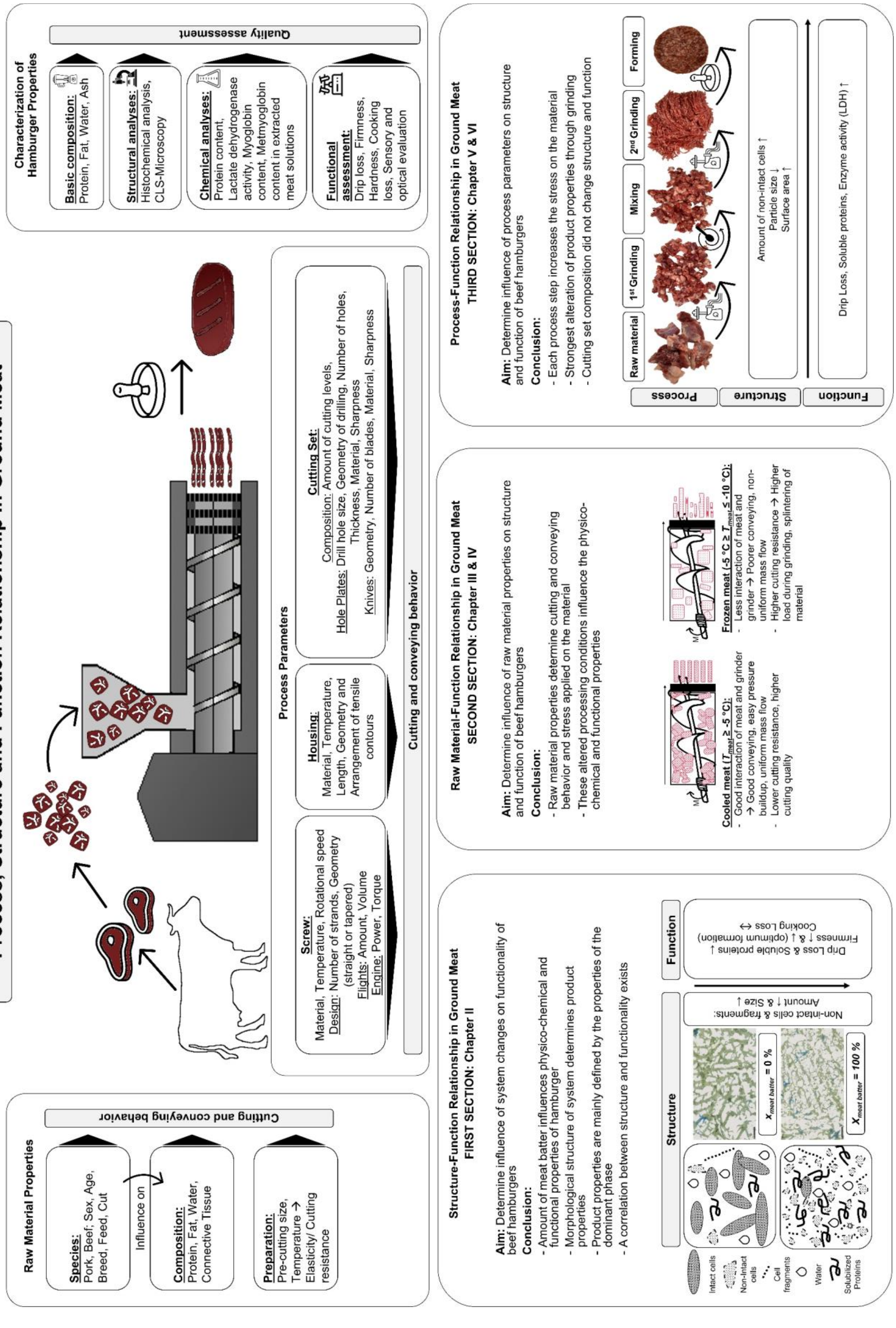
It can therefore be concluded from the **third part of the thesis** that the shear forces acting on the meat during grinding have the strongest influence on the structure and function of beef. By reducing the acting shear forces, the grinding can be designed to be gentler resulting in lower ANIC. Despite the influence on the process-control (SME, pressure, torque) and the structural parameters (ANIC), it needs to be emphasized that the influence on the function and quality of the hamburgers is small in application-relevant ranges (**Chapter II**). In application-relevant ranges this relationship is only slightly pronounced (**Chapters V & VI**). Comparable results were found in **Chapters III & IV**, as raw material variations only partially caused structural, functional, and quality effects in the hamburgers. This in turn means that changes in structure cannot always be linked to a shift in perceived quality. In order to carry out an integrated evaluation of the product, structural parameters and quality parameters must be defined, assessed separately, and merged into a combined overall sample assessment.

Hohenheim, 11.07.2023

Place and Date

Signature

Graphical Abstract



Zusammenfassung

Aufgrund des niedrigen Preises und der Vielseitigkeit ist Hackfleisch seit jeher eines der beliebtesten Rindfleischprodukte. Das Wolfen von Fleisch kann als Zerkleinerungstechnik beschrieben werden und ist eine komplexe Kombination verschiedener Verfahren wie, z.B., Fördern, Pressen, Schneiden und Scheren. Ziel ist es, die Partikelgröße des Fleisches zu verringern und die Zellstrukturen teilweise aufzubrechen. In den deutschen Leitsätzen für Fleisch und Fleischerzeugnisse ist spezifiziert, dass Hamburger nicht mehr als 20 Vol.% an nicht intakten Zellstrukturen enthalten dürfen, um ohne weitere Änderung der Deklaration verkauft werden zu können. Ziel dieser Arbeit war es, einen Zusammenhang zwischen Prozess, Struktur und/oder Funktion der Hackfleischprodukte mit Fokus auf die Hamburgerherstellung zu identifizieren. Um die Einflüsse systematisch zu untersuchen, wurde ein standardisiertes Herstellungsverfahren für Hamburger definiert und ein Fleischwolf mit der Möglichkeit zur Aufzeichnung prozessrelevanter Daten entwickelt.

Der **erste Teil der Dissertation** wurde durchgeführt, um grundlegende Kenntnisse über die Systemeigenschaften von Hackfleisch zu gewinnen. So wurde in **Kapitel II** der Zusammenhang zwischen Struktur und Funktionalität von Hackfleisch untersucht. Ein Modellsystem mit steigendem Anteil an brätartiger Fleischmasse simulierte eine Veränderung der Fleischstruktur durch Zelldesintegration. Der Begriff des Anteils nicht intakter Zellen (*engl.* Amount of non-intact cells, ANIC) wurde als Maß für die Menge der während der Verarbeitung desintegrierten Fleischzellen definiert. Es wurde gezeigt, dass Veränderungen in der Struktur zu veränderten physikochemischen und funktionellen Eigenschaften des Hackfleischsystems führten. Dies wurde durch morphologische Veränderungen im System, veränderte Mischeffekte und Wechselwirkungen der Systemkomponenten erklärt.

Im **zweiten Teil der Dissertation** wurde ein systematisches Screening des Einflusses von Rohmaterialeigenschaften auf die Struktur und Funktionalität von Hackfleisch und Hamburgern durchgeführt. In **Kapitel III** wurde der Einfluss von Gefrierfleischanteil und -temperatur auf die Struktur und Funktionalität von Hamburgern untersucht. Da der spezifische Schneidewiderstand bei gefrorenem Fleisch höher ist als bei gekühltem Fleisch, wurde postuliert, dass sich die Auswirkungen auf die Struktur und Funktionalität des Hackfleisches entsprechend unterscheiden. Dies konnte bestätigt werden und führte zu ausgeprägteren strukturellen Veränderungen und veränderten Produktparameter.

Bei der Herstellung von Hamburgern werden Produkte, die äußerliche Mängel aufweisen, in gefrorenem, grob zerkleinertem Zustand dem Prozess wieder zugeführt. Im Gegensatz zu den Erkenntnissen aus **Kapitel III** führte die Verwendung von bis zu 20 % rückgeführtem Material bei der Herstellung von Hamburgern in **Kapitel IV** zu keinen nennenswerten Unterschieden. Die Ergebnisse zeigten, dass weder der SME noch der ANIC durch die Zugabe von bis zu 20 % wiederverwendetem Material beeinflusst wurden. Diese Beobachtungen lassen sich auf drei Gründe zurückführen: (1) die Bildung kleinerer Fragmente, (2) die bereits geringe Partikelgröße des wieder zugegebenen Materials, und (3) einem Temperaturausgleich zwischen gefrorenem und gekühltem Material, der eine Erweichung der gefrorenen Partikel bewirkt.

Somit wurde im **zweiten Teil der Dissertation** gezeigt, dass einige Rohstoffvariationen einen Einfluss auf die Struktur und die Funktionalität von Hamburgern haben können. Vor allem Temperatureffekte und damit verbundene Änderungen des Schneidewiderstands hatten den stärksten Einfluss. Allerdings reichten die gefundenen strukturellen Unterschiede nicht aus, um Unterschiede in der sensorischen Bewertung zu bewirken. Dies bedeutet, dass die Verbraucherwahrnehmung und damit die Qualität des Hamburgers innerhalb der untersuchten Parameter nicht beeinflusst wurde.

Im **dritten Teil der Dissertation** wurden die Prozessparameter und deren Einfluss auf die Struktur und Funktionalität der Hamburger untersucht. In **Kapitel V** wurden die Auswirkungen der vier Hauptverarbeitungsschritte Vorwolfen, Mischen, Wolfen und Formen untersucht. Dabei nahm der ANIC mit fortschreitender Verarbeitung zu, wobei das erste und zweite Wolfen den stärksten Anstieg bewirkten. Mischen und Formen waren für die strukturellen und funktionellen Veränderungen von geringer Bedeutung. Dies galt auch für die meisten analytischen Parameter. Es konnte somit gezeigt werden, dass ein Zusammenhang zwischen den wirkenden mechanischen Kräften, der Struktur und Funktionalität besteht.

In **Kapitel VI** wurde durch die Variation des Bohrwinkels der Lochplatten, der Größe der Bohrungen und der Anzahl der Schneidebenen der Einfluss des Schneidsatzes auf die Struktur und Funktionalität der Hamburger untersucht. Es konnte gezeigt werden, dass SME und ANIC zunahmen, wenn mehr Schneideebenen verwendet wurden. Dies führte zu einer höheren Scherbeanspruchung des Fleisches, da mehrere Stufen durchlaufen werden müssen und somit mehr Kontakt zu Bohrungen und Messern auftritt. Der Winkel der Bohrlöcher sowie die Lochgröße der mittleren Lochplatte verursachten jedoch keine oder nur geringe Veränderungen bei ANIC und SME. Obwohl ein Einfluss der Schneidsatzzusammensetzung auf die Struktur festgestellt werden konnte, wurden nur geringfügige Auswirkungen auf die Funktionalität und die sensorische und optische Qualität der Hamburger ermittelt. Dies deutet darauf hin, dass weniger Schneideebenen zu einer schonenderen Verarbeitung führen.

Aus dem **dritten Teil** kann daher geschlossen werden, dass die Scherkraft, die während des Wolfens auf das Fleisch einwirkt, den stärksten Einfluss auf die Struktur und Funktionalität von Rinderhackfleisch hat. Wird die Scherkraft reduziert, führt das schonendere Wolfen zu geringeren Mengen an nicht intakten Zellen (ANIC). Trotz des Einflusses auf die Prozesssteuerung und die Strukturparameter ist hervorzuheben, dass der Einfluss auf die Funktionalität und sensorischen Qualität der Hamburger in anwendungsrelevanten Bereichen gering ist (**Kapitel II**). In den für die Anwendung relevanten Grenzen ist dieser Zusammenhang nur gering ausgeprägt (**Kapitel V & VI**). Vergleichbare Ergebnisse wurden in **Kapitel III & IV** ermittelt, da Rohstoffvariationen nur teilweise strukturelle, funktionelle und qualitative Auswirkungen in den Hamburgern verursachten. Dies wiederum bedeutet, dass Veränderungen in der Struktur nicht immer mit einer Veränderung der Qualität und der Verbraucherwahrnehmung in Verbindung gebracht werden können. Um eine ganzheitliche Bewertung des Produkts vorzunehmen, sollten Strukturparameter und Qualitätsparameter getrennt betrachtet und zu einer Gesamtbewertung der Probe zusammengeführt werden.

Hohenheim, 11.07.2023

Place and Date

Signature

Introduction

Fundamentals of Ground Meat Manufacturing

Meat products such as sausages, ham, and ground meat products represent a very important product class in the German meat industry. In Germany, approximately 29 kg of meat products is consumed per capita (Bundesanstalt für Landwirtschaft und Ernährung, 2022; Deutscher Fleischerverband, 2020). Nowadays, the meat industry is changing from small-scale manufacturing to high-capacity industrial processing. Consequently, manufacturing practices are shifting from craft-style to industrial scale and from small butcher shops to larger supermarkets and globally operating retailers. With available expertise in mechanical engineering and continuing advances in tool design, new machines are emerging that can fulfill new requirements in terms of scale, efficiency, and hygiene. For this reason, there is a need for a better understanding of the different operations involved in the production of meat preparations and products, in particular the grinding operation.

The following introduction provides a brief overview of the basics of ground meat and ground meat products. Detailed information can be found in the subsequent review article (**Chapter I).**

Raw Materials for Ground Meat and Ground Meat Products

The German guidelines for meat and meat products define meat as "mammalian and avian skeletal muscles with adherent or embedded fat and connective tissue, lymph nodes, nerves, vessels, and porcine salivary glands" (Bundesministerium für Ernährung und Landwirtschaft, 2019). According to various sources, the term beef is understood as meat from cattle of different breeds, ages, or sexes. It ranges from 6-month-old calves, heifers, young bulls, bulls, oxen to cows. As a natural product, the quality of the meat can vary greatly depending on the type of meat used. In turn, processing characteristics and quality of the product may vary (Branscheid et al., 2007). While the meat of older animals and bulls has a rather strong muscle fiber structure and a higher content of connective tissue, the meat of young animals, e.g., heifers, young bulls, or calves, contains finer muscle fibers and is more tender (Kögel et al., 2003).

Following the German guidelines for meat and meat products, hamburgers are usually produced from ground beef, mainly composed of lean meat from beef carcasses, that have been roughly freed from the tendons without the addition of spices, salt, or other additives (Bundesministerium für Ernährung und Landwirtschaft, 2019). As such and in this thesis, the term "hamburger" refers to "beef hamburgers". Ground beef without additives contains approximately 65 % moisture, 20 % protein and 14 % fat, and 1 - 2 % minerals (Claus and Sørheim, 2006; Meza-Márquez et al., 2010; Souci et al., 2022). The restriction to certain beef cuts is not legally prescribed, but often the meat from flanks of heifers is used (*M. transversus abdominis, M. obliquus externus abdominis, M. obliquus internus abdominis;* Prändl et al. (1988)).

The selection of the cuts of meat does not only determine the chemical composition of the hamburger but also its sensory characteristics (Blackmon et al., 2015; Kerth et al., 2015). The sensory properties are also depended on the feed, the breed, and the age of the animal, e.g., young cattle or cow beef. Hamburgers made from the meat of young cattle (age < 24 months) were rated higher in palatability and tenderness than hamburgers made from the meat of older cattle (age > 24 months) (Berry and Abraham, 1996; Cross et al., 1976). In contrast to the German guidelines, the recipes and production of hamburgers can vary in different countries. For example, an Egyptian traditional burger recipe contains onions, garlic, and starch as additives, and the ingredients are then homogenized with beef (Abd-Elhak et al., 2014). In Great Britain, the addition of cereals or other protein additives is common. This can affect the mechanical and textural properties of produced burger patties (Jones et al., 1985).

Although the composition may vary due to different recipes, Miller et al. (1993) compared low-fat beef hamburgers with 10 % and regular hamburgers with 22 % fat, whereby the protein content increases with decreasing fat content (Miller et al., 1993). Many studies involved a typical fat content of 20 % in beef patties (hamburgers), as the beef flavor, juiciness, and tenderness increased with higher fat content (Berry, 1993, 1994; Egbert et al., 1991). Overall, higher fat content correlated with better sensory evaluation of hamburgers (Berry and Leddy, 1984; Cross et al., 1980).

Besides fat, other components such as lean meat, connective tissue, or tendons determine the consumer perception of the product but also its processability. Each component has specific chemical structures and physical properties, e.g., cutting resistance influencing grinding characteristics. E. Haack et al. (2003b) reported the highest penetration depth into the drill holes for lean pork meat whereas connective tissue, tendons, cartilages, and parts of the skin possess had lower penetration properties (E. Haack et al., 2003b). This affected the cutting efficiency and quality of the cutting set. As an element of transferring the energy between the conveying screw and the housing of the grinder, the meat is responsible for most of the energy consumption and force generation during the grinding (E. Haack and Sielaff, 2005; O. Haack, 2007). Thus, the properties of the raw material are of the highest importance for grinding efficiency and quality.

Temperature is known to change the physico-chemical properties of materials. In meat, it determines rheological properties such as viscoelasticity thereby affecting cutting resistance (King, 1996). At lower temperatures of -15 to -30 °C the fracture behavior is mainly attributed to the brittle character of ice (Munro, 1983). Brown et al. (2005) found a fundamental relationship between meat temperature and cutting forces. A decrease in temperature increased the cutting and friction forces of the meat, and meat at -5 °C had significantly higher values of shear forces, than meat at +5 and +15 °C. It was stated, that the cutting force of beef might deviate, due to the variable amounts of fat or connective tissue (Brown et al., 2005). The freezing procedure and temperature during freezing influence the structure of beef muscles due to ice crystal formation. The lower the freezing temperature, the fewer cells are damaged due to the formation of predominantly small and intracellular ice crystals (Rahelić et al., 1985). Typically, beef is pre-ground at about -20 °C and finally ground at temperatures slightly below the freezing point (-5 to -2 °C), as this temperature range was detected to be optimal for a size reduction of lean beef (Farag et al., 2009). The temperature of the raw material before the grinding impacts the grinding pressure and product flow rate. Using beef at -2 °C instead of +4 °C significantly increased the pressure by 25 % resulting in a higher extrusion rate and enhancing the visual appearance (Wild et al., 1991). A series of studies by Sheard et al. (1989), Sheard et al. (1990) and Sheard et al. (1991) found that lower product temperature results in smaller particles. The influence of the temperature on the particle size properties of ground meat can be explained by complex interactions between the ice content, the viscosity of the unfrozen fluid parts, and the difference in mechanical characteristics of the meat (Sheard et al., 1991).

Legal Framework in Germany

In Germany, meat products must fulfill specific quality standards such as texture, taste, and appearance. Regulatory requirements are specified in the German "Leitsätze". According to the definition of the “Leitsätze für Fleisch und Fleischerzeugnisse” hamburgers are defined as “shaped and portioned products made from ground or similarly comminuted, roughly stretched meat of bovine animals” (Bundesministerium für Ernährung und Landwirtschaft, 2019). Usually, no other ingredients or additives, e.g., salt and spices, may be added. Moreover, the guidelines also define physio-chemical requirements for the hamburger, e.g., the content of meat protein free from connective tissue protein (german: Bindegewebsfreies Fleischeiweis, BEFFE), fat, and abrade muscle substance (= amount of non-intact cells, ANIC). For hamburgers, the guidelines specify at least 13.5 % (w/w) BEFFE and an ANIC of a maximum of 20 Vol.% (Bundesministerium für Ernährung und Landwirtschaft, 2019). If this value is exceeded, labeling is mandatory. For this purpose, the food's sales description must be complemented by the addition of "meat partially comminuted “ (*german*: “Fleisch z.T. zerkleinert”) or similar wording in order to prevent consumers from being misled (Arbeitskreis der auf dem Gebiet der Lebensmittelhygiene und der Lebensmittel tierischer Herkunft tätigen Sachverständigen, 2015; European Union, 2015). For this purpose, the food's sales description must be complemented by the addition of "meat partially minced" or similar wording in order to prevent consumers from being misled.

The abrade muscle substance ANIC is defined as a finely comminuted mass of meat that contains mainly disintegrated cell structure (Beneke, 2018). It is formed when meat cells are disintegrated and lose their structure due to high mechanical stress being superimposed and intracellular muscle protein being released (Ballin and Lametsch, 2008; Tyszkiewicz et al., 1997). The quality of the ground meat is directly related to the mechanical energy input during the production process.

Histological Analysis of Meat Structure

The ANIC is an important quality parameter of ground meat and can be determined via histological analysis (Hildebrandt and Jöckel, 1980; Schering, 2015a). Histological analyses comprise the microscopic examination of biological tissue samples (Ross and Pawlina, 2016; Schering, 2015a) and are often applied in medicine, e.g., to diagnose and classify tumors. In food analysis, histology is used, among others, to verify whether samples match the declaration and list of ingredients or to detect food adulteration (Schering, 2015a). Histological analyses enable the visual identification and evaluation of different tissue and cell structures, thus allowing the determination of the tissue composition of a food.

Usually frozen or paraffin sections are prepared (Schering, 2015b). For frozen sections, the samples are frozen at -20 °C in a cryostat, cut into 2 x 2 cm cubes, and subsequently sectioned into thin slices of 12 µm, collected on glass microscope slides, and stained (Raudsepp et al., 2017; Schering, 2015b). For paraffin sections, the samples are first fixed with formalin to stop structure changes and then embedded in paraffin. Thereby the water present in the tissues is replaced by the embedding medium paraffin via several intermediate steps. Cubic paraffin blocks are obtained after cooling down and cross sections of 10 µm are cut, placed on microscope slides, deparaffinized, and stained (Schering, 2015b). According to the official German methods (§64 LFGB method L 06.00-13, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (2006)), two sections each from at least three fixed blocks must be prepared. Thus, in total six cross-sections of 2 x 2 cm² size and a total cross-section area of 24 cm² are assessed for each sample (Schering, 2015b).

Calleja staining is the most frequently used staining technique in the compositional analysis of processed food. It stains skeletal muscles green, connective tissue blue, and cell nuclei purple, enabling good differentiation between muscle structures (Sifre et al., 2009). Histological cross-sections might be evaluated qualitatively or quantitatively. Qualitative evaluation estimates the quantity of a tissue type and assigns it to one of the frequency classes (predominant (> 50 Vol.%), abundant (35 - 50 Vol.%), medium (20 - 35 Vol.%), moderate (5 - 20 Vol.%), low (≤ 5 Vol.%), or sporadic (not regularly occurring in every section)) (Schering, 2015b). For a quantitative, histometric evaluation the point-count method or planimetry is used. In the point-count method, a grid of regularly arranged points covers the section plane. At each of these points, the underlying tissue structure is evaluated and counted. The examiner must decide whether the cell is structured and intact or unstructured and not intact. For the assessment of the structures, a guideline can be used (Schering, 2015b). However, it is evident, that the classification relies on subjective assessment of the examiner and is time-consuming. In planimetry, areas of disintegrated muscle structures are manually marked in digital cross-sections and the covered area is calculated (Beneke, 2018). In contrast to the point-count method, planimetry provides additional information on particle number, mean particle size, and vacuole diameter. However, the point-count method is often preferred over planimetry, as it requires less work and covers a wider measuring range (Hildebrandt and Jöckel, 1980). A new immunohistochemical approach, which uses antibodies for staining, offers the possibility of optimization as it provides better image contrast and quality, has a higher specificity of staining, and achieves a more objective and transparent measurement compared to conventional methods (Raudsepp et al., 2017). For ground meat products such as hamburgers, histological analysis is, at the moment, the only official method used to evaluate product structure. However, since the method is very time-consuming and subjective, it is of substantial interest to find a more objective and time-saving analytical method.

Ground Meat Processing and Basics Principle of Meat Grinders

The unit operation grinding is widely used in the meat industry and describes the size reduction of meat and fat. The importance of grinding has increased since the middle of the last century a fact that can be attributed to the increased demand for ground meat products for home use and the use of ground meat in sausage production. However, it is at present difficult for meat manufacturers to meet the legal requirements of max. 20 Vol.% ANIC, as fundamental knowledge about the process, structure, and function relationship in ground meat and ground meat products is lacking. It is not known, to which extent the process steps contribute to the cell disintegration and formation of ANIC. Further, no critical process steps have been defined to adjust the extent of ANIC and the resulting changes in product characteristics.

As production lines for hamburgers and ground meat combine the main process steps of grinding, mixing, and shaping, which apply different mechanical forces to the meat, a systematic screening of the influencing parameters is necessary to understand the process. Besides the process-related parameters, also raw material characteristics need to be considered since meat is a natural product and prone to fluctuations in quality depending on the age, breed, or maturing time of the beef meat (Branscheid et al., 2007).

Free conveying meat grinder

The working principle of a meat grinder can be described as a system in which the raw material is conveyed through a cutting system by a rotating screw and is thereby cut (Rust and Knipe, 2014). In its simplest configuration, the cutting system consists of a rotating knife and a fixed hole plate, but also multi-stage arrangement, e.g., composed of two or more knives and a variety of hole plates, is possible (Mialki, 1951). As the knives are attached at the end of the screw, they have the same rotational speed whereas the hole plates are fixed in the screw casing and remain static (E. Haack et al., 2003a).

During grinding the diameters of the drill holes in the hole plates decrease progressively to achieve a gentle gradual reduction of the raw material particle size (Eberhard Haack et al., 2003; Krickmeier et al., 2012; Rust and Knipe, 2014). A variety of hole plate geometries, differing in the arrangement of the holes, e.g., straight or inclined, number of holes per plate, plate thickness, and angle of the drill holes, is available (turbocut Jopp GmbH, 2013). The size reduction of meat in the meat grinder is realized by a rotary shear cut (Mialki, 1951; Schnäckel et al., 2011; Tscheuschner, 2017), which describes the separation of raw material by two cutting elements moving past each other. The rotary shear cut happens between the cutting edge of the knife and the edge of the drill holes (Bersźan, 1989). As a result, the raw material is cut off by shear forces as soon as the specific shear strength of the raw material is exceeded (Weck and Brecher, 2005). Cutting in the grinder includes compression, friction, and disintegration of the meat (Schnäckel et al., 2011). First, the compression forces deform the meat elastically and plastically. When this compression force exceeds the resistance of the meat, the knife penetrates the meat. Strong stresses, such as pressure, pull, and thrust, cause slow crack growth and ultimately a separation in the meat. Due to the contact between the cutting elements and the material friction occurs which causes an increase in temperatures due to heat dissipation (Krickmeier, 2015; Tscheuschner, 2017).

Vacuum filler grinder systems

The vacuum filler grinder is a special, modern design of a meat grinder and combines the processing steps of grinding and filling, and/or portioning in one device. Using different outlet designs a variety of products can be produced, e.g., ground meat, sausages, or formed meat products (Irmscher et al., 2013; Irmscher et al., 2016; Irmscher et al., 2015). The vacuum filler grinder system can be described as a combination of a vacuum filling machine and a meat grinder. It consists of a raw material hopper which is usually combined with a vane cell feed system applying a vacuum to the product. The product is pulled into the vane cell, compressed, and conveyed into the cutting set of the attached grinder, where the particles are cut to the final size. It simultaneously evacuates the product thus reducing the oxygen content, increasing color stability and shelf life of the product (Irmscher et al., 2015; Weiss et al., 2010).

Compared to the free conveying grinder system, the vacuum filler grinder has numerous advantages. First, it combines several production steps in one system, wherefore less equipment is necessary (Irmscher et al., 2013; Irmscher et al., 2015). Second, due to the short distances and residence times of the meat in the system and a cutting set without a screw, less frictional and shear forces act on the meat leading to gentler processing (Büchele, 2009). It is also reported, that less frictional loads lead to a clearer cut of meat and fat, reduce smearing, and thermal energy dissipation (Büchele, 2009; Irmscher et al., 2013). Third, by connecting a wide variety of attachments, accessories, outlet geometries, or other modules, the vacuum filler grinder becomes a very versatile food processing apparatus.

Besides ground meat production, vacuum filler grinders are also used for the production of coarse raw fermented sausage (e.g., salami), meatballs, sausages with finer structure, or plant-based alternatives (Irmscher et al., 2016; Irmscher et al., 2015).

However, in industrial hamburger production, the traditional meat grinder is equipped with a forming machine facilitating a higher throughput and the ability to process partly frozen meat masses down to -25 °C frozen meat temperature (Seydelmann and Geisen, 2019).

Analogy of Meat Grinders and Extruders

Extrusion involves compression and describes the process of forcing a material mechanically through an opening hole (Choton et al., 2020; Dobraszczyk et al., 2005; Godavarti and Karwe, 1997; Kazemzadeh, 2012; Morales Alvarez, 2020). Depending on the thermal conditions, one can distinguish between cold (mechanical process) and hot (thermo-mechanical process) extrusion (Choton et al., 2020; Dobraszczyk et al., 2005; Lazou, 2022). A widely used example of hot extrusion is extrusion cooking, where high temperature and pressure are applied to the material during extrusion. Thermal-extrusion processes are, i.e., used to texturize plant proteins for meat alternatives or produce extruded snacks, breakfast cereals, or pasta (Choton et al., 2020; Dobraszczyk et al., 2005; Fang et al., 2013; Lazou, 2022). Due to their composition and way of working, meat grinders are also considered to be extruders and are a typical example of cold extrusion (Heinz and Hautzinger, 2007; Heiss, 2004). Grinding is defined as a mechanical way of comminution (Eberhard Haack et al., 2003) and is widely used for size reduction (Rust and Knipe, 2014).

In extrusion, material properties such as structure and functionality are altered, either thermo-mechanically in hot extrusion or solely mechanically in cold extrusion (Choton et al., 2020; Lazou, 2022). This is why extrusion processes are considered multiple input and multiple output processes, in which several input and process parameters define the output parameters (Lazou, 2022). Depending on their combination, the properties of the product are defined. In meat grinders, input parameters such as raw material composition and meat grinder setup and process parameters such as pressure and specific mechanical energy (SME) determine output parameters such as macroscopic, physio-chemical, sensorial, and functional product parameters (Lazou, 2022). It was already shown, that by changing the raw material and process parameters during meat grinding, the properties of the product can be modulated (Bakieva et al., 2019; Berger, Gibis, et al., 2022; Berger, Witte, et al., 2022; Dobraszczyk et al., 2005; Lazou, 2022; Oppen et al., 2022; Suchenko et al., 2017; Tomasevic et al., 2023; Witte et al., 2022; Xiong and Kenney, 1999).

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Aim of the Study

This dissertation focuses on the characterization of the relationship between process, structure, and function in ground meat and ground meat products to gain an overall insight into the influence of mechanical stresses on the physicochemical and functional properties. The aim was to generate a mechanistic overview of the processing of beef into ground meat products, in particular hamburgers, and to draw conclusions for process optimization and gentler processing of ground meat. In addition, the generated knowledge should be used to adjust raw materials and process parameters in a targeted way so as to induce certain product properties. The underlying idea was that consumers place different demands on ground meat, depending on its intended use. If the ground meat is to be used for a Bolognese sauce later, it should have a granular and loose structure, whereas if it is to be used for hamburgers, a stickiness of the ground meat mass and thus a certain cohesion in the product is desired. At present, it is not possible to specifically prepare ground meat for a particular use, as there is a lack of knowledge about the effects of processing and raw material properties on product properties. In order to cover these requirements, the present dissertation investigated influencing factors that are postulated to contribute to the physicochemical, functional, and qualitative properties of ground meat. In order to investigate the relationships systematically, the work was divided into 3 parts: i) Structure-Function Relationship in Ground Meat, ii) Raw Material-Function Relationship in Ground Meat, iii) Process-Function Relationship in Ground Meat.

It was suggested initially that there is a correlation between the amount of non-intact cells (ANIC) and quality attributes such as, e.g., serum loss in ground meat. To examine this, the basic effects of the ANIC on the physicochemical and functional attributes of ground meat were investigated in **Chapter I** using a model system. This study aimed at gaining a basic understanding of the ground meat system and the interactions of its constituents. Thus, the fundamental relationship between structure and functionality was to be investigated there.

Based on this, the **second and third parts** explored whether there is an additional relationship between process, structure, and functionality. First, raw material characteristics and second, process parameters were systematically varied to assess whether they can impact structure and thus functionality of products.

For this, the influence of the raw material temperature was discussed in **Chapter II**. Due to altered specific cutting resistances in the frozen meat, increased mechanical loads during processing were suspected, which, based on the findings from **Chapter I**, were likely to cause changes in the product properties. In **Chapter III**, the effects of adding re-fed material on product characteristics were investigated. This is a common practice in ground meat processing and should therefore provide the most practical reference.

Whether variations in the process can influence the characteristics of the final product should be investigated in the **third part**.

In order to gain a basic understanding of the hamburger production process, **Chapter IV** first examined the individual process steps and characterized their respective effects. Based on the knowledge gained in **Chapter IV** that particle size reduction during grinding has the greatest influence on the change in the structure of the meat, the influence of different cutting sets was to be investigated in **Chapter V**.

Each chapter of this study was thus designed based on specific research questions that were developed and intended to be investigated.

i) How does the amount of added meat batter correlate with the histologically analyzed amount of non-intact cells (ANIC)? Does the structure (in terms of ANIC) of the meat influence its physicochemical and functional properties (e.g., drip loss, amount of soluble protein, firmness)? If yes, which model applies to the correlation (**Chapter I**)?

ii) How does the raw material temperature influence the processing of the meat? Is the applied mechanical load higher when using higher amounts and colder meat? Does a higher processing load alter product properties (**Chapter II**)?

iii) Does the use of re-fed material change the processing load and influence product attributes? Is there a limit beyond which the product properties change fundamentally and can a guideline for gentler industrial processing be derived from this (**Chapter III**)?

iv) How does the processing steps in hamburger manufacturing contribute to structural changes in the beef meat? To which extend are they influencing physicochemical and functional properties of the product? Is there a processing step which accounts for the strongest changes, and if yes, which one (**Chapter IV**)?

v) Which effect does the cutting set composition have on structure and functionality of products? Is it possible to modulate product characteristics by the cutting set composition? Is there a possibility to design a gentler grinding process by the choice of the cutting set (**Chapter V)**?

Each of these chapters represents a study that was published (**Chapters II, III, V**) in a peer-reviewed journal or has been submitted for publication (**Chapters IV & VI**).

I. Chapter:

A Review on the Relation between Grinding Process and Quality of Ground Meat

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Highlights

- The forces and movement of the meat in a meat grinder is well understood.
- The quality of ground meat relates on the raw material, ingredients, and preparation technique.
- There is a research gap regarding novel processing of ground meat.

Abstract

This review is providing an overview of the actual and past research in the field of ground meat. The forces that are acting in the meat grinder are well understood. The higher the forces that are acting on the meat while grinding, the stronger the disintegration of the meat cells after the process. These forces can be calculated as energy transfer in meat grinders using specific mechanical energy (SME). The amount of non-intact cells (ANIC) can be used to describe the extent of disintegrated cells. Different methods are available to rate the quality of ground meat, which is mainly influenced by the raw material and processing. Over the past decades of industrialization, the landscape of ground meat production has changed. However, the effects of the process adjustments on the quality of ground meat are not yet sufficiently described in the literature.

Keywords: ground meat technology, ground meat quality

1. Introduction

Ground beef is typically composed of 65 % moisture, 20 % protein and 14 % fat, and 1 – 2 % minerals (Meza-Márquez et al., 2010; Souci et al., 2022). As meat is a natural product, the quality varies depending on the cut, the breed, or the age of the animal (Belitz et al., 2007; Branscheid et al., 2007). Such raw material fluctuation, as well as processing conditions (Haack et al., 2003c), influence the quality characteristics of the product, i.e., water-holding capacity, tenderness, sensory attributes, or the amount of non-intact cells (Berry and Abraham, 1996; Cross et al., 1976; Huff-Lonergan and Lonergan, 2005; Schering, 2015a; Tornberg, 2005). The latter is an important quality parameter of ground meat and is histologically determined (Berger, Gibis, et al., 2022; Schering, 2015a).

We understand ground meat quality as multifactorial influences of the composition of the macro elements protein, fat, and moisture, the microbiological activity in combination with the acidity (pH-value), color, structure, and sensorial evaluation. This is supported by Huff-Lonergan and Lonergan (2005) statement that the eating quality includes both flavor and aroma, as well as texture/tenderness, and juiciness. Damez and Clerjon (2008) stated that several fast, non-invasive sensors will support the assessment of meats' structure and thus the eating quality indirectly or directly using biophysical methods.

Still, the quality of ground meat is defined by consumer acceptability including flavor, juiciness, and tenderness (Brewer, 2012). According to Robbins et al. (2003), tenderness and flavor consistency are the most important traits which define consumer acceptance of beef. In addition, according to Brewer and Novakofski (2008) the appearance, which is defined by the amount of fat and visible moisture, significantly impacts consumer acceptance. Moreover, when ground meat is purchased, it remains unclear whether the consumer will prepare a lose product such as Bolognese or a firm product such as meatballs. Independent of further ingredients, the cooking device and procedure as well as the origin of the ground meat, it should work optimally depending on the desired application. Therefore, ground meat needs to satisfy consumer on several occasions. Thus, the functionality of ground meat varies broad and can be influenced by varying several processing parameters. However, it was shown, that sometimes even drastic production variations did not necessarily change the perception of the ground meats quality (Berger et al., 2023; Berger, Gibis, et al., 2022; Berger, Witte, et al., 2022; Tomasevic et al., 2023; Witte et al., 2022). During grinding meat is conveyed through a cylindrical housing towards a cutting system by a rotating screw. Following, the particle size is reduced (Rust and

Knipe, 2004) due to a rotary shear cut (Mialki, 1951; Schnäckel, Krickmeier, Schnäkel, et al., 2011; Tscheuschner, 2017). Production lines for ground meat and ground meat products not only involve grinding, but also mixing, and shaping. Thus, different mechanical forces are acting on the meat. Due to industrialization, meat manufacturing shifted from small-scale to industrial processing within the last decades. However, only a few studies focused on the changed requirements including the influence of processing conditions on quality parameters, packaging, and hygiene. Thus, this review aimed to collect all data dealing with ground meat and hamburger production, the process engineering basics, the changed requirements due to production automatization as well as the characterization of the product quality parameters and their classification regarding their meaningfulness to the quality of ground meat and the derived products.

2. Realization of the literature work

The literature search was carried out from 2018 until May 2023 with intensified research in 2023. The source search was done using Google Scholar, Scopus, and SciFinder with the keywords “ground meat”, “minced meat”, “ground meat processing”, “extrusion”, “food extrusion”, “meat extrusion”, “cutting”, “grinder”, “filler grinder”, “Packaging AND minced meat”, “Cleaning AND minced meat”, “inline sensors AND meat”, “TTI”, “meat quality”, “ground meat quality”, “minced meat quality”. No temporal limitation of the literature was made. Results of intensively processed ground meat were excluded. However, studies including grinding for sausage production were evaluated only for ground meat. The focus was set to ground pork and ground beef, whereby other meat origins were not explicitly excluded, except for vegan or vegetarian ground “meat”.

3. One general parameter for the analysis of ground meat quality?

When grinding meat, the analysis of the amount of non-intact cells (ANIC) is used to assess the quality of the product and the process. The authors are aware that the analysis is not used in all countries, still the ANIC, is worth to discuss since this analysis is able to provide a direct indication about the integrity of the muscle cells. A reason that this analysis is not widely applied could be that the assessment of ANIC depends on the evaluator (Schering, 2015a) and is poorly reproducible (Sifre et al., 2009), further properties should be analyzed (Schering, 2015b). Still a laboratory comparative study of histometry with trained evaluators on cooked cured meat products, e. g. cooked ham, showed that differences in results due to evaluators are

15 Vol.-% when the product is fresh. However, when the meat used was frozen and thawed several times, the accuracy of the evaluation is reduced (Grünewald et.al., 2013), In particular when trying to assess the ground meat quality based on the ANIC, it is just not feasible (Witte et al., 2022). However, an increasing grinding degree contributes to the less abundant transverse mesh of fibres, as the cell structure passes into an amorphous state because the longitudinal and transverse boundaries of the fibres disappear (Sukhenko et al., 2017). The grinding should not result in a too strong disintegration of the cells (as it should be to produce a Bologna-style sausage in the bowl shopper). To determine this, light microscopy and a point-count-method are used.

Witte et al. (2022) study aimed to characterize if the ANIC contributes to any inference on ground meat quality. Moreover, to prove if the processing intensity alters the ground meat quality negatively, finely chopped lean pork loin was used as intensively processed meat batter and mixed with ground pork shoulder. It was found that the ANIC increased with an increased amount of meat batter, whereas the ground meat quality did not alter with the same magnitude. Authors concluded that the addition of up to 30 % meat batter to ground meat does not result in significant differences to solely ground meat. This conclusion bases on the results of drip loss, cooking loss, firmness, and sensory firmness, sensory juiciness as well as the sensory rating of inner cohesion and inner structure. Due to these findings, it was stated that the ANIC shall not be used exclusively for the evaluation of ground meats quality, especially as multiple factors, e. g. muscles' properties and the production process, affect the ANIC but not the ground meat quality.

In their study and Berger, Gibis, et al. (2022) found similar effects and proved that with increasing content of meat batter addition significantly increased the ANIC in ground beef samples. The morphological changes (separate finely chopped meat which was purposefully added) consisting of only pork or beef, respectively, the ANIC increases resulted in altered physico-chemical (lactate dehydrogenase activity, soluble protein content, metmyoglobin content) and functional properties (drip loss, firmness, cooking loss) of the beef samples. These results were traced back to linear mixing effects based on mechanical disintegration of meat structure. It was further reported that the ANIC is also increasing with increasing processing steps (Berger, Witte, et al., 2022). For this, the authors compared physico-chemical and functional properties of beef samples after each main processing step during ground beef manufacturing, namely raw material, first grinding, mixing, second grinding and forming. The results indicate that the grinding steps have the greatest effect on the structure and function of the ground beef.

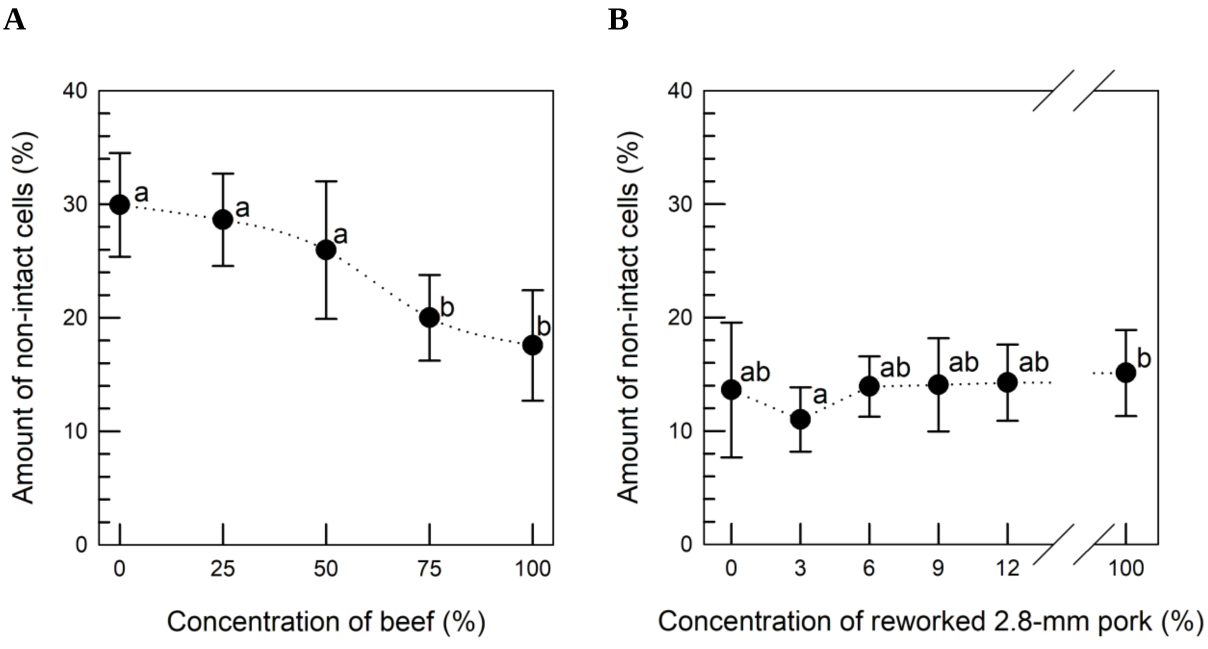

Figure I-1: Amount of non-intact cells (A) with increasing ratio of beef to pork and (B) with increasing reworked 2.8-mm pork concentration. Different letters indicate significant differences ($p < 0.05$) determined according to Witte et al. (2022) and Tomasevic et al. (2023). The material and methods are in line with these studies.

Other studies indicate that an increasing amount of frozen pork (Tomasevic et al., 2023) or beef (Berger et al., 2023) and a higher ratio of pork to beef (**Figure I-1A**) also increases the ANIC and effects physico-chemical and functional properties. Beef was added to pork prior grinding to investigate the impact of beefs muscle fibres on the ground meat quality while maintaining the same production process. The ANIC was significantly higher, when the ratio of pork and beef consisted of 50 % or more pork. These significant differences were not shown for the drip loss, firmness, and sensory evaluation of the firmness, juiciness, inner cohesion, and inner structure. The effect of frozen beef was studied by adding increasing ratios of frozen meat at two different temperatures. The authors found increasing batch processing temperatures at higher contents of frozen beef, which results in higher specific mechanical energy input during grinding, causing higher mechanical stress acting on the meat. This ends in increased ANIC, drip loss and myoglobin content and decreased firmness and hardness. It was shown that the influence of the frozen meat content was more pronounced than the frozen meat temperature (Berger et al., 2023). The sensory assessment of the hamburgers was not and optical evaluation only slight affected by the frozen meat temperatures and content.

However, neither the ANIC does not increase with increasing amount of reworked ground meat – even up to 100 % [data not published] (**Figure I-1B**), nor other physico-chemical or functional properties [data under publication]. This is somehow surprising, and it seems, that the already ground meat particles are moving through the hole plates without any further destruction. Hildebrandt and Jöckel (1980) studied methods to prove the addition of meat batter in ground meat products such as hamburgers using the combination of different production devices (grinder, bowl chopper), processes (variation of hole plate, time, mixing), additives, and raw materials (fresh meat, frozen meat, meat batter). Based on these investigations, they concluded that it is technologically unavoidable to obtain an ANIC with less than 10 Vol. %. Moreover, if frozen meat instead of fresh meat is used, an ANIC of less than 20 Vol.-% is technologically unavoidable (Hildebrandt and Jöckel, 1980). Caused by the changing industrial process of ground meat production (Irmscher et al., 2013) due to a higher production volume and therewith the need to produce faster, ANIC increases (Beneke, 2018; Tichaczek-Dischinger and Otto-Kuhn (2015)). Tichaczek-Dischinger and Otto-Kuhn, 2015 analyzed ground meat produced either industrially or artisanal among others by use of histometric. They found that 5.9 % (2 out of 34) of artisanal and 44.4 % (8 out of 18) of industrially produced ground meat obtain an ANIC above 20 Vol.-%. Thus, they stated that ground meat with an ANIC above 20 Vol.-% cannot be considered as ground meat. Beneke (2018) summarized how the industrial production of meat and meat products impacts muscles' structure and stated, based on histometric results, that especially the meat grinder and the bowl chopper contribute to an altered muscle structure. The altered meat structure due to industrial processing is especially obvious in ground meat or ground meat products but also in e. g. raw fermented sausages or cooked ham.

Comparing the control samples of each study among the studies, published and unpublished, it is interesting that the ANIC varies depending highly on the raw material batch. The raw material for all studies was purchased in the same way and as similar as possible: fresh pork shoulder, tendons removed, diced, delivered one day prior production (Tomasevic et al., 2023; Witte et al., 2022), still the ANIC varies between 8 and 34 Vol.-% [data not published]. A varying ANIC, while obtaining specified raw material quality, has not been described in the literature yet – probably because changes in ground meat quality parameters in dependence of the batch are of higher importance than changes in the ANIC while remaining the ground meat quality.

Based on the relation between ANIC and sensory evaluation (Berger et al., 2023; Tomasevic et al., 2023; Witte et al., 2022), contrary to Beneke (2018), an increased ANIC might not be sensorial perceptible to a reasonable extent of less than 50 Vol.-% and therefore, in contrast to

Tichaczek-Dischinger and Otto-Kuhn (2015), those products could still be considered as ground meat. However, an ANIC above 50 Vol.-% was only found in Witte et al. (2022) and Berger, Gibis, et al. (2022), but not in the unpublished data about the addition of beef to pork and the reworking of ground meat (**Figure I-1B**). An approach to optimize the histological examination of mechanically separated meat is the objective and more rapid computerized image analysis of histological sections (Sifre et al., 2009).

Aiming to classify the quality of machine separated meat (poultry) in-line and to assess the muscle fiber degradation automatically, an advanced automated image analysis system based on the histochemical method was investigated (M. Christensen et al., 2015). The image analysis is done by the immunological detection of the muscle protein meromyosin and the basement membrane protein laminin, which is superior for quantifying the degree of muscle degradation to monochromatic methods for mechanically separated poultry objectively and accurately. Due to the muscle degradation, it was possible to differentiate between two groups. This was caused by the specificity of the staining, good image contrast, and objectivity and transparency of the measurement (Raudsepp et al., 2017). The approach to differentiate degradation classes could also be a useful tool to analyze the quality of industrial ground meat products objectively and accurately - especially when considering the results described and the accuracy and subjectivity of the histochemical determination of the ANIC.

To summarize, the histology is feasible to visualize the muscle cells and to identified and calculate the ANIC. The histology can be used to indicate the intensity of meat processing. As shown in the literature and our own data, this analysis does not correlate with the ground meat quality, such as water loss. The ANIC differs when industrially ground meat is compared to ground meat retrieved from a grinder with just one pre-cutter knife and one hole plate. The questions remain what forces act on the meat during grinding causing the destruction of the cells.

4. What is actually happening during ground meat processing?

4.1. Forces and pressure during ground meat processing

Meat can be considered a natural, anisotropic biopolymer with an ordered, fibrous structure (Haack et al., 2003b, 2003d; Lepetit and Culioli, 1994; Sukenko et al., 2017). It is characterized as a plastic-elastic material that can store force acting on it to a certain degree (Haack and Sielaff, 2005). The anisotropic nature of meat determines the thermal and mechanical properties (Lepetit and Culioli, 1994), which vary depending on the direction of the muscle fibers.

Basic meat grinders are typically single-screw extruders powered by a motor and consisting of a raw material hopper, a housing with a rotating grinding screw, and a cutting set (Barbut, 2015; Brennan, 2005; Choton et al., 2020; Lazou, 2022; Maskan and Altan, 2012; Rust and Knipe, 2004). A permanent, free-feeding movement of the material towards the cutting set is generated in the meat grinder as the material transfers the energy between the rotating screw and the housing (Dobraszczyk et al., 2005; Haack et al., 2003b, 2003d; Krickmeier, 2015) once the flights of the rotating grinding screw are filled with the raw material. The way of working is thus comparable to the flighted Archimedes screw principle (Harper, 2019). Choton et al. (2020); Dobraszczyk et al. (2005); Haack et al. (2003a); Haack et al. (2007); Krickmeier (2015); Morales Alvarez (2020) described that the net movement of the material through the housing relies on a combination of the frictional force between material and housing, the cross-channel flow, and the pressure flow. This pressure increases along the transport of the material from the hopper to the cutting set, due to the reduced volume in the flights of the screw (Choton et al., 2020; Harper, 2019; Morales Alvarez, 2020). Later in the process, pressure differences between the hole plate and the knife cause a further drag of the material flow, thus the meat is transported into the cutting area and subsequently cut by the rotating knife causing a particle size reduction (Dobraszczyk et al., 2005; Haack et al., 2003a, 2003d; Rust and Knipe, 2004). The rotatory shear cut takes place between the outer edge of the drill holes of the hole plates and the cutting blade edge of the knife (Krickmeier, 2015; Schnäckel, Krickmeier, Oktaviani, et al., 2011). In the cutting set, maximum pressures of 6 – 8 bar acts on the lean meat (Haack et al., 2003b) which drop after the material passes the cutting set (Berger, Witte, et al., 2022; Choton et al., 2020; Haack et al., 2003a, 2003b; Harper, 2019) through the drill holes of the hole plates.

By investigating the effect of processing steps on ground beef properties, it was found that the pressure during grinding is lower in the first grinding step to medium coarse particle sizes of 13 mm (0.5 – 1.5 bar) and is higher when grinding the meat to the final particle size of 2.4 mm

(4 – 6 bar) (Berger, Witte, et al., 2022). This can be explained by a shift from elastoplastic to more viscoelastic properties of the meat upon size reduction. A higher pressure at the cutting set is not only disintegrating the muscle cells but is crucial for the working principle in a meat grinder (Choton et al., 2020; Haack et al., 2003b, 2003d; Morales Alvarez, 2020). In a free-feeding grinder, a blockage of the cutting set can occur when heavily comminuted material parts return from the cutting set into the screw against the direction of the material flow and interferes with the pressure buildup (Barbut, 2015). Since the operating principle is based on the pressure buildup, the cutting quality of the material can be reduced and even an interruption of the material flow can be caused. The pressure in the meat grinder depends on the material properties (e.g., elasticity), the screw speed, and the constructive setup of the housing and screw. Therefore, the optimal adjustment of process parameters to material properties is crucial for a high-quality of final product (Haack et al., 2003d). At the end of the process the pressure is released when the material leaves the grinder through the cutting set. The cutting set consists of several hole plates and rotating knives. When forcing the meat through the cutting set, it is mechanically sheared and cut into smaller pieces, thus reducing the particle size (Haack et al., 2003a; Rust and Knipe, 2004).

Table I-1 summarizes the influence of different process parameters on the structure and functionality of ground meat.

Table I-1: Influence of process parameters on the structure and functionality of the ground meat.

Parameter	Influences…	Explanation	Source
Cutting set composition	Pressure build-up Conveying quality & volume flow Cutting quality & product functionality Product particle size	VARIABLE PARAMETERS: Amount of cutting parts (knives and hole plates) Shape and thickness of hole plates Size of drill holes of the hole plates Geometry of knives Geometry of drill holes Sharpness of knives and plates Cutting set counteracts the product flow and builds up pressure. Cutting set composition influences extent of pressure build-up. Cutting set restricts the outlet area, thus it determines the conveying quality & volume flow Quality of cut determines properties of the end product and is influenced by: • Size of drill holes (meat penetrates further into bigger holes) • Contact area of knives and cutting edge • Sharpness of cutting set (dull knives cause fat smearing etc.) • More gentle grinding with inclined drilled hole plates Size of hole plate determines the particle size of the product Continuous cutting in cutting set creates drag flow of the material Without continuous cutting material blocks in the screw and higher mechanical forces are applied which increases material disintegration	Barbut (2015); Büchs (1994); Haack et al. (2003a, 2003d); Roth et al. (1999); turbocut Jopp GmbH (2013)

Grinding screw geometry	Conveying quality & volume flow Pressure build-up	VARIABLE PARAMETERS: Geometry of screw (Single-stranded, double-stranded) Amount, size, and geometry of flights Material of screw Screw enables energy transfer from the engine to product and facilitates conveying Screw conveys towards the cutting set. If conveying is altered, pressure build-up is changed. Changes in the screw layout change interaction of product and screw and thus conveying quality, volume flow, pressure build-up, and the efficiency of grinder	Haack et al. (2003d); Morales Alvarez (2020)
Rotational speed of grinding screw/ volume flow	Volume flow & residence time Mechanical load acting on the meat	Higher rotational speed cause higher volume flow and lower residence time Higher rotational speed causes higher shear forces → more cell disintegration (screw) Rotational speed determines the speed of material transported. As the grinder volume stays constant, an increase in rotational speed is proportional to the increase of the volume flow and reciprocally proportional to the residence time Rotating screw → compression is built → energy transferred into material → dissipation into heat energy Best conveying when material has medium particle size (reduced volume flow in 2nd grinding of pre-ground meat) → too fast rotational speed causes increased cell disintegration and counteract the conveying (screw) → medium screw speed better	Dobraszczyk et al. (2005); Godavarti and Karwe (1997); Haack et al. (2003d); Harper (2019); Lazou (2022)

The resulting pressure gradient develops a drag flow, which is the basic mechanism of conveying in the meat grinder (Dobraszczyk et al., 2005; Morales Alvarez, 2020). One important parameter in the process of grinding and transportation is the flow properties of the ground meat. Rheological measurements can be used to obtain insights into the alterations in the microstructure of the ground muscle. Generally, different processing factors affect myofibrillar proteins. During grinding, the muscle fibrils are cut destroying the sarcolemma and releasing the myofibrils and myofilaments. The extent of this release is depending on the degree of grinding. Ground meat consists of fragments of still intact fibers, membranes, myofilaments, various subcellular particles, particles of connective tissue and fat, and of sarcoplasmic fluid – determining the rheological behavior of the complex system (Hamm, 1975).

During grinding meat is exposed to different (mainly mechanical) forces. Each mechanical action (force) or unit operation (e.g., mixing, shearing, conveying, cutting) on the product results in the alteration of technological parameters and physical properties. **Table I-2** summarizes the main processing steps in meat grinding, their function and the forces acting on the meat. Since a certain amount of energy needs to be applied to the meat during grinding to obtain the desired particle size, the ground meat quality changes (Kabulov et al., 2019; Kamdem and Hardy, 1995).

Kabulov et al. (2019) detected changes in the yield stress of the ground meat (mixture of beef and pork) by variation of processing conditions (e.g., rotating seed of knife, duration of processing). According to the authors, this rheological parameter can be correlated to quality attributes for ground beef as changed rheological properties indicate altered processing conditions. However, during grinding some of the energy is dissipated into thermal energy which increases the material temperature (Dobraszczyk et al., 2005; Haack et al., 2003b). The highest dissipation occurs in the cutting set due to the shear forces between rotating knives, hole plates, and meat (Haack et al., 2003b). In their article series, the authors aimed to detailly describe the basic processes during ground meat production. The article describes that the meat is the basic component of energy transmission and thus its properties therefore its properties have a decisive influence on the transport behavior and the process characteristics. Further, they describe the principle of transportation is mainly depending on frictional forces and pressure differences and that too high pressures during processing result in higher ANIC and reduced product properties. But also when conveying the material from the hopper to the cutting set, shear force occurs between the material and the screw, within the material particles, and between the material and the housing (Haack et al., 2003d; Harper, 2019; Morales Alvarez, 2020). The latter might also be called wall friction. Shear force is the basis for conveying in a free-feeding meat grinder. The energy is transferred from the rotating screw to the material, which is then pressed against the housing wall due to the rotation. The combination of the geometry of the screw, the shear force and wall friction, and the properties of the raw material (solid, anisotropic) enable a forward movement thrust of the material (Haack et al., 2003d; Morales Alvarez, 2020). When the rotating knife cuts the meat strands at the end of the hole plate the meat is exposed to shear force leading to several small meat parts (Haack et al., 2003b). As reported by Bekeshova et al. (2022), the quality of such ground meat parts is influenced by the grinding process and the machines used. The authors associate meat quality mainly with its physical and structural–mechanical properties, i.e., yield stress and water-holding capacity. To evaluate that, the authors varied the rotational speed and the gap between the rotary knives of the grinder and found that the yield stress and the water-binding capacity of ground chicken meat increased with increasing rotational speed, as well as the power consumption of the machinery. Even if grinding machines used to grind meat using the same set up, the duration depends on the geometrical and kinematic machine characteristics (Dorokhov et al., 2011). Generally, grinding results in different shapes and proportions of muscle fiber disintegration, caused by the raw material composition as well as the dwell time and grinding steps. Berry et al. (1981) also reported that the processing method (grinding vs. flaking vs. several combinations) impacts the cooking loss of hamburgers, whereas chopping causes a greater reduction in the hamburger height than grinding (Berry, 1980). A combination of initial flaking followed by grinding is recommended (Berry et al., 1981). The final drill hole diameter is known to influence the ANIC and the physico-chemical and functional properties, as shown in the study of Berger et al. (2022), where the ANIC in ground meat with 2,4 mm particle size was more than twice as high as in 13 mm ground meat. However, the usage of inclined drill holes did not result in significantly lower ANIC and better functionality [data under publication].

To summarize the forces, acting on the meat, are well described: The pressure in the system affects the meat quality. The mechanical forces influencing the ground meat are multiple, as shown by the insignificant effects on the ground meat quality. Only fewer processing, as described above, results in fewer cell disintegration.

Table I-2: Processing stages in meat grinding and their function.

	Stage	Processing	Purpose	Forces acting on the material	Effects (macroscopic)	Source
External	Meat standardization	Mechanical	Removing tendons and fat Separating different cuts Mixing to standardize composition	Shear forces (cutting material – knife & mixing: material – mixer, material-material)	Reduction of particle size Increasing surface area → increased risk of spoilage): *E. coli 0157:H7*, *Salmonella spp.*, Pseudomonades, lactic acid bacteria are present, might be distributed and grow	Castelo et al. (2001); Hui (2012); Koutsoumanis et al. (2006); Roberts and Weese (1998)
	Meat preparation	Thermal	Pre-cooling or freezing to modulate cutting behavior and product properties Maintain microbiological quality and stability		Disintegration of cells due to ice crystals No clear cut of meat and fat Increasing temperature up to 10°C (without addition of frozen meat) Controlling temperature during grinding (microbiological stability) Increasing cutting resistance of meat	Krickmeier (2015); Ranken (2000); Wild et al. (1991)
		Mechanical	Pre-cutting to reduce particle size and enable uniform feeding from hopper to screw (particle size depends on raw material and meat grinder size)	Shear forces (cutting)	Reduction of particle size Increasing surface area (increased risk of spoilage)	

Within meat grinder	Feeding and conveying	Mechanical	Conveying material with rotating screw from hopper to cutting set Mixing & homogenization of pre-cut raw material Pressure build-up, formation of drag flow	Shear forces (material – housing, material – screw, material-material) Friction Pressure (compression of material)	Temperature increase Disintegration of cells and formation of “non-intact cells” Pressure in feeding screw Shear forces/tearing screw – housing	Brennan (2005); Dobraszczyk et al. (2005); Ranken (2000)
	Passing cutting set	Mechanical	Size reduction Altering product properties	Pressure Shear forces	Size reduction Disintegration of meat cells formation of “non-intact cells” Altering meat properties	Hui (2012)

4.2. A standard parameter for the assessment of ground meat production

Calculating energy transfer in meat grinders using the specific mechanical energy (SME) input can serve as a standard parameter to assess ground meat production. Grinding meat in a meat grinder means mechanically comminuting and breaking the fibrous structure, whereby the meat is transferred from a viscoelastic into a more rubbery texture (Dobraszczyk et al., 2005; Ranken, 2000). The energy input during grinding can be recorded with the SME, as widely applied in hot extrusion (Fang et al., 2013; Lazou, 2022; Morales Alvarez, 2020) and extrusion of polymers (Villmow et al., 2010).

According to Fang et al. (2013); Godavarti and Karwe (1997); Morales Alvarez (2020); Villmow et al. (2010) the SME (J/kg) during meat grinding can be calculated with the following Eq. I-1 and I-2.

$$SME = \frac{P}{\dot{m}} \qquad \text{I-1}$$

$$P = (M - M_{empty}) \cdot n_{rad} \qquad \text{I-2}$$

With P = average power during grinding process (W), M = average grinding screw torque during grinding (Nm), M_{empty} = idling torque of grinding screw (Nm), $\dot{m} = \frac{m}{t}$ being the mass flow (kg/s), $n_{rad} = \frac{2\pi \cdot n_{grinding\,screw}}{60}$ being the radial rotational speed of the grinding screw (s^{-1}) and $n_{grinding\,screw}$ = rotational speed of the grinding screw (s^{-1}). The average torque during grinding is determined as the torque during the plateau phase of grinding.

The SME serves as a measure of the energy transferred from the motor of the grinder to the material (Berger et al., 2023; Morales Alvarez, 2020). As the SME is highly dependent on the process setup, it is a direct connector between the mechanical grinder and product quality parameters (Godavarti and Karwe, 1997; Morales Alvarez, 2020; Villmow et al., 2010). It characterizes the grinding process and can be related to the quality attributes of the product (Godavarti and Karwe, 1997; Morales Alvarez, 2020; Villmow et al., 2010) such as the degree of cell disintegration during grinding (Godavarti and Karwe, 1997).

In their study, Berger et al. (2023) investigated different SME values for grinding meat in a pilot plant scale grinder. For grinding pre-cooled (T = 1 °C) meat from 13 mm to 2.4 mm particle size, SME values of 1.36 ± 0.06 kJ/kg were reported. With increasing frozen meat content, the SME for grinding meat to 2.4 mm increased from 1.60 ± 0.33 kJ/kg in samples with 15 % frozen meat addition at -6 °C to 2.49 ± 0.48 kJ/kg in samples with 45 % frozen meat addition at -6 °C (Berger et al., 2023). In their study, Berger et al. (2023) also found out, that the SME was higher, if the frozen meat was tempered to colder temperatures. Thus, the SME for grinding samples containing 45 % frozen meat at -12 °C to 2.4 mm particle size was 2.91 ± 0.39 kJ/kg. A typical grinding process with a mixture of 75 % pre-cooled (*T* = 1 °C) and 25 % frozen meat (*T* = -10 - -12 °C) content required around 1.2 kJ/kg for grinding from 13 mm to 2.4 mm using a standard 3 part cutting system. SME values during the grinding with different cutting sets are currently under investigation and have been shown to vary up to 2.5 kJ/kg [data under publication] which also accounts for the usage of frozen, re-fed material up to 20 % [data under publication]. Kamdem and Hardy (1995) described higher grinding energy in systems with higher screw speed (15 J/g at 60 rpm; 17.5 J/g at 120 rpm) and smaller drill hole diameter (7.5 J/g at 9 mm drill hole diameter; 15 J/g at 2.5 mm drill hole diameter).

From the above-mentioned studies, it can be summarized that the SME is a powerful parameter to assess the process stability and effectiveness. It was shown that SME exhibits correlations to ground meat product properties such as structure or functionality. It this therefor helpful to assess the SME in order to predict product properties or optimize the production process.

4.3. Size reduction

The efficiency of a meat grinder is rated on the output volume as this parameter is influenced by many raw material and process parameters. As an example, the speed and quality of conveying, the ability to increase pressure, or the efficiency and quality of cutting in the cutting set are important (Haack et al., 2003b). Usually, the size is reduced gradually as this increases the grinders' efficiency and results in better ground meat quality (Barbut, 2015; Haack et al., 2003a).

Barbut (2015) includes many parameters in the consideration of meat quality. He explains that, in addition to processing, conditions during breeding and slaughtering of the animals are also decisive. The quality of meat can be defined in many ways, as important factors he mentions water holding capacity, fat holding capacity, color, but also texture, odor and taste in the sensory evaluation. In their study, Haack et al. (2003a) investigate the relationship between machine parameters and meat quality of the end product. Among other things, they examine the function of the perforated discs, the processes and time sequences during cutting, and the function of the knives. They conclude that quality and machine characteristics are closely related. For example, better product qualities are achieved when less pressure is applied to the meat, less heating takes place, or the perforated disc and knives are optimally adjusted to each other and the raw materials.

The depth of penetration of the meat into the holes of the hole plate has an influence on the ground meat quality, depending on the size of the holes, the support surface, the type of meat and its quality. The more accurate the cut during grinding, the lower the drip loss, the better the ground meat quality. To sum, this means that the less energy the meat has to expend, the better the ground meat quality (Haack et al. 2003a).

The first step of size reduction takes place in the pre-cutting, hereby grinding the meat to 13 – 19 mm particle size is followed by the final reduction to 2.4 – 3 mm particle size (Haack et al., 2003a; Rust and Knipe, 2004). Precise knowledge about the alteration and influence of meats' quality during processing and storage is of great importance for producers (Grau and Hamm, 1957). According to Grau and Hamm (1957), meat quality is determined in particular by water retention during preparation and processing as other quality-determining characteristics, such as the taste, texture, and color, are directly related to the hydration of the muscle proteins. Proteins are most important for, e.g., the water-binding and consistency of meat products but are affected by various extrinsic and intrinsic factors, such as the processing and formulation (Xiong and Kenney, 1999).

Particle size reduction in the meat grinder is based on the deformation of the meat under pressure in the hole plates of the cutting set and a subsequent rotatory shear cut (Krickmeier, 2015). Pressure increases throughout the conveying as the material is pressed towards the hole plates, a surface with only restricted openings. This pressure gradient generates backpressure (Dobraszczyk et al., 2005; Morales Alvarez, 2020). As pressure is highest at the end of the screw in front of the hole plates the meat is extruded into the drill holes and is deformed into meat cones (Haack et al., 2003d). When the pressure exceeds a certain raw material-dependent limit, the fibrous structure ruptures. This is the case when the applied external forces are higher than the intrinsic, reversible visco-elasticity range of the material. This deformation is irreversible and causes a disintegration of the fibrous, anisotropic structure (Haack et al., 2003b, 2003d).

From our unpublished data, further interesting results were retrieved: increasing the free surface area (cm^2) or the free volume (cm^3) using oblique perforated hole plates does not alter any quality parameter, such as results of drip loss, cooking loss, raw ground meat firmness, sensory testing of cooked meat balls, or the ANIC. However, using thick hole plates does alter the ANIC and ground meat quality slightly as results of firmness and sensory testing vary.

To summarize, the setting of operational parameters influences processing efficiency and product properties. Thus, it is of the highest importance to know about the relationship between the process, structure, and function of ground meat and adjust the parameters accordingly. In meat grinding mainly raw material and process-related parameters are important.

5. Raw material

5.1. Meat

Upon grinding, the meat changes from a highly anisotropic structure in a whole meat piece to a mixture of randomly distributed intact and non-intact muscle cells and muscle fiber bundles (Honikel, 2014; Raudsepp et al., 2017; Tornberg, 2005), as indicated in the following **Figure I-2**.

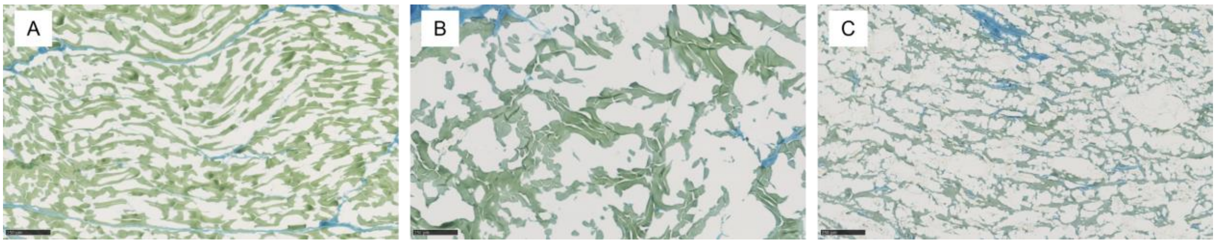

Figure I-2: Microstructure of (A) whole beef muscle, (B) beef hamburger, (C) beef batter in histological images (Calleja-Luglo staining, 20-fold magnification). Green structures represent muscle cells, blue structures fat, or connective tissue.

Thereby, proteins are solubilized which acts as a binder after pressure or heat treatment (Ranken, 2000). With longer mechanical treatment and higher intensity, the amount of solubilized proteins increases having an impact on the functionality of the product (Xiong and Kenney, 1999). The processing quality of slow-twitch (red) and fast-twitch (white) muscle groups differ largely due to variations in the functional properties of the myofibrillar protein, which are associated with the fiber type, water retention, and texture of processed muscles differ. The fiber types differ according to their rheological and biochemical characteristics and therewith their microstructure. To acquire a uniform quality, the temperature, ionic strength, pH, as well as other factors need to be considered in dependence on the fiber type or muscle section, since they differ in physiologic behavior and appearance depending on the amount of myoglobin contained in the muscle fibers (Xiong, 1994). The results of different studies are not always comparable as the methods applied vary (Honikel and Hamm, 1994). In contrast to a whole muscle with well-defined anisotropic structures, ground beef consists of 50 to 70 % randomly distributed more or less intact meat fibers and fiber bundles (Tornberg, 2005).

The following **Table I-3** summarizes the influence of different raw material properties on the structure and functionality of ground meat.

Table I-3: Influence of raw material properties on the structure and functionality of the ground meat.

Parameter	Influences…	Explanation	Source
Meat temperature	Processability: Conveying/ residence time Particle size of ground meat Energy consumption & input	Altered viscoelastic properties cause different interactions of material and housing/ screw → elasticity module is temperature dependent Increased cutting force at colder temperature requires more energy for size reduction and cause higher pressure → higher SME at colder temperatures Meat above freezing point (-2°C) resulted in bigger particles compared to meat below freezing point	Berger et al. (2023); Dobraszczy et al. (1987); Haack et al. (2003d); Haack et al. (2007); Haack and Sielaff (2005); Krickmeier (2015); Roth et al. (1999); Sukenko et al. (2017); Wild et al. (1991); Zhao and Sebranek (1997)
	Microbial stability	Cooler raw material temperature counteracts frictional heat during grinding and thus microbial growth	Honikel (2014)
	Visual appearance	Colder material temperature promotes clean and clear cut of meat and fat without smearing	Krickmeier (2015); Wild et al. (1991)
	Physicochemical & functional properties	Colder processing temperatures increase cooking loss & drip loss and reduce hardness (as Warner-Bratzler shear force)	Chesney et al. (1978)

Composition (fat content)	Nutritional values Quality perception of consumer	FAT: Sensory (flavor, juiciness) and textural (tenderness, dryness) characteristics affected by fat content Fat content correlates with consumer acceptance Lipids contribute to formation of volatile compounds (e.g., aldehydes, ketones, hydrocarbons, free fatty acids) upon heating and therefore determine typical meat flavor Fat content correlates with pressure acting on material in cutting set (higher fat → higher pressure) WATER: Ability to hold water determines the juiciness PROTEINS: Provides fibrous structure Determine ability to hold water, thus influencing the texture	Brewer (2012); Cross et al. (1980); Haack et al. (2003b); Roth et al. (1999)
Meat source/ origin (pork – beef)	Nutritional values Material properties	The pressure within the hole plate is higher in beef than in pork Raw material specific strength properties strongly determine the comminution process and the resulting forces acting on the meat	

Pre-cutting size	Conveying and product quality	Inhomogeneous particle sizes cause fluctuating conveying and product quality Too small particles are less effective in transferring energy Best energy transfer and conveying: solid material with medium sized particles Using optimal product particle size: • guarantees optimal conveying and comminution • increases the homogeneity of the raw material • reduces the required time in the following mixing step	Barbut (2015); Haack et al. (2003a, 2003d); Lazou (2022)
Particle size of end product	Products quality and functionality	Protein extractability correlated to particle size Texture correlates with particle size Viscoelastic properties correlate with particle size	Berger et al. (2023); Berger, Gibis, et al. (2022); Berger, Witte, et al. (2022); Oppen et al. (2022); Sukenko et al. (2017); Tomasevic et al. (2023); Witte et al. (2022)

Claus and Sørheim (2006) investigated the effect of using pre-rigor beef in the production of "patties" (containing also starch, water, and salt) and found that the functionalities are greater than for post-rigor beef in terms of the cooking yield, fat stabilization, protein solubility, and textural strength of the cooked patty. Moreover, the chilling of beef before production would be unnecessary. By comparing comminution methods (chopping *vs*. grinding), Berry (1980) found that the cooking loss of hamburgers from USDA Choice was different than from USDA Cutter-Canner and that USDA Choice received higher sensorial ratings in all tested categories indicating that the composition influences the product. Usually, reliable information about the composition of meat is obtained using optical, e.g., color, or mechanical, e.g., shear force, measurements. Multi-image analysis can give information about the composition, e.g., nuclear magnetic resonance or near-infrared spectroscopy.

5.2. Fat

When ground meat products contain pork, the major fat (trims) shall be hard fat from ham, back fat, shoulder, or jowl as softer fats, such as belly trimmings, can cause a soft texture (Pegg and Boles, 2004). Moreover, overmixing can result in a rubbery texture with possible fat smearing, which is why colder temperatures help minimize fat melting and smearing (Rust and Knipe, 2004). The primary source of flavor compounds in cooked meat results from volatiles, which are partly resulting from lipids, such as unsaturated fatty acids. The volatiles resulting from heated unsaturated fatty acids can produce characteristic odors of the different meat species (Brewer, 2012). Lipids contribute to meat flavor directly or indirectly as reaction products by compounds like free fatty acids, aldehydes, and ketones (Mottram, 1998; Rowe, 2002) and thus are an essential contributor to meat quality (Brienne et al., 2001). Moreover, the fat binding capacity is important for ground meat as it improves the flavor carry-over, retention, and mouthfeel. Thus, increasing fat binding and absorption, decreasing cooking loss, and maintaining dimensional stability are important for the quality of ground meat (Zayas, 1997).

In ground meat, the lipids can result from subcutaneous, intra- and intermuscular, intramyocellular fat, and structural phospholipids, whereby the intramuscular fat is the major contributor to volatile compounds (Brewer, 2012). Increasing the fat concentration from 11 to 22 % in lean ground beef patties also increases some flavor compounds (2-butanone, 2-pentanone, 3-hydroxy-2-butanone) (El-Magoli et al., 1995). Thus, fat replacers affect the flavor as they reduce the original fat flavor and entail their flavor. Moreover, fat replacers can enhance or delay the release of some volatile compounds (Chevance et al., 2000).

Using time-temperature indicators (TTI), the off-flavor development during the storage of ground beef can be detected since the deteriorated quality is assessed by microbial growth. This was shown for defrosted samples at storage temperatures of 5, 10, 15, and 25 °C about a sensorial evaluation of ten trained panelists and the measurement of volatile basic nitrogen and titratable acidity of the microbial TTI system (Y.-A. Kim et al., 2012).

5.3. pH-value and composition

The pH-value and the content of moisture, fat, and protein are usually determined when analyzing the ground meat quality (Berger et al., 2023; Berger, Gibis, et al., 2022; Berger, Witte, et al., 2022; Tomasevic et al., 2023; Witte et al., 2022). The pH-value can be determined directly in the meat mass (Tomasevic et al., 2023; Witte et al., 2022) or homogenized in distilled water (Jayasingh et al., 2002).

The composition of the meat strongly determined its textural perception. The texture in a sensorial perceived way includes several kinesthetic characteristics as the texture is perceived (1) before testing regarding the particle size and oiliness/fattiness, (2) during testing/chewing regarding juiciness and tenderness, as well as (3) after testing regarding the mouth coating and possible residues. Even if the meat is ground, it has several associated kinesthetic characteristics: fibers, connective tissue, fat, and/or moisture exudation. With an amount of 75 % water is a major component in the muscle and is arranged around the polar molecules as well as between the cellular material with restricted possible movements (Brewer, 2011). Thus, the water-holding capability of meat, which is mainly determined by contractile proteins, is an integral characteristic of its texture and the reason why decreasing pH and increasing temperature can increase drip and cooking loss, thereby reducing the juiciness and acceptability of ground beef (Brewer and Novakofski, 1999; Offer, 1988). Analyzing the juiciness in a sensory evaluation is another, however, less standardized way to measure the water-holding capacity of meat (Honikel and Hamm, 1994).

When cooked ground meat was sensory analyzed, it was found that the hardness increased with increasing temperature. Moreover, the storage modulus of ground meat increases steeply from 50 to 65 °C, and the phase angle decreases from 35 °C onwards with a plateau at 65 to 70 °C indicating that a spatial arrangement of the fibers is important for the texture. The structure of ground meat is no longer anisotropic as the muscle fiber and bundles, which are more or less disintegrated, are randomly distributed in the batter (Tornberg, 2005). The extracted protein in the batter of hamburger forms a gel, when the temperature increases from 45 to 65 °C, whereas the product gets denser when cooked > 65 °C as more water is lost and the connective tissue contracts. Due to these structural changes, the elasticity increases and the sensorial perceived toughness enhances (Tornberg, 2005). Since the protein functionality in frozen meat is better preserved when meat is frozen fast, ground beef patties made from fast frozen meat are juicier and more tender (Nusbaum et al., 1983). This can be attributed to the development of small ice crystals resulting in less protein denaturation when freezing fast.

6. Assessment of the ground meat quality

6.1. Old but up-to-date assessment of ground meat: water-holding / water binding

The water-binding capacity (WBC) describes the water binding of a muscle under defined conditions (Trout, 1988), whereas the water-holding capacity (WHC) describes the ability of a meat system to hold its and/or added moisture (Honikel and Hamm, 1994). Both, the WBC and WHC, are studied intensively, especially the WBC due to their economic relevance as it affects the texture and flavor of the meat (Trout, 1988). This can be attributed to changes in meats' cellular constituents associated with water molecules upon meat processing, such as grinding (Honikel and Hamm, 1994). As an example, dimensional shrinkage can serve as an indicator of cooking loss and WHC and is determined by measuring the dimension of the hamburger before and after cooking (Troy et al., 1999). Witte et al. (2022) showed that using a filter paper press and determining the cooking loss of ground pork can show the same tendencies, whereas Berger, Gibis, et al. (2022) showed in their study that no correlation exists for ground beef.

In 1957 several methods to determine the water-binding capacity, as well as the quantification of the different binding forms, were described (Grau and Hamm, 1957). To measure the loosely and unbound water, a force, e.g., compression, centrifugation, or gravity, is applied (Trout, 1988). However, as rigor progresses, moisture can be forced from myofibrils and myofibrillar proteins to extra myofibrillar spaces, possibly resulting in drip loss. This is influenced directly by the ionic strength, pH, and oxidation, which affect the proteolysis of key cytoskeletal proteins (Huff-Lonergan and Lonergan, 2005).

Generally, a higher cooking loss at temperatures between 50 to 65 °C corresponds with the largest alteration in the volume of the muscle cells. Comparing the cooking loss of ground meat, formed as hamburgers, and intact muscles, it is similar at temperatures from 45 to 80 °C. During cooking, the physical and thermal properties of ground beef change (Pan and Singh, 2001): the volume decreases significantly with increasing temperature and holding time (30.5 % at 75 °C and 20 min) and the water and fat losses increase with increasing temperature and holding time (30 % and 40 % at 75 °C, respectively). The measurement of 3 mm ground beef hamburgers containing 23.8 % fat was conducted in a water bath at temperatures of 40, 50, 60, 70, and 75 °C and holding times of 2, 10, and 20 min. (Pan and Singh, 2001). Dreeling et al. (2000) showed that the cooking method significantly affects the moisture content as deep- fat fried and grilled hamburgers resulted in the highest cooking loss in comparison to roasted, griddled, and fried hamburgers, which also affects the sensorial perception and firmness. However, griddling resulted in the most acceptable low-fat burgers (Dreeling et al., 2000). Cooking loss was highest for hamburgers with a high-fat content (30 %), whereas hamburgers with 5 to 10 % fat were redder, firmer, and less juicy, but also flavorful with a less oily coating of the mouth. The fat content of the hamburger did not affect their height and width. However, the sensorial differences due to the fat content need to be improved to obtain for low-fat hamburgers to gain palatability scores similar to high-fat hamburgers (Troutt et al., 1992).

To sum up, slight changes are recognizable in the WHC as it is a sensitive indicator for the charge and structure of the muscle proteins. The WHC of pork is better than that of beef resulting in a higher drip loss of retail cuts of beef than of pork (Zayas, 1997). The drip loss increases with an increasing amount of frozen meat (Berger et al., 2023; Tomasevic et al., 2023), but not significantly with increasing processing steps (Berger, Witte, et al., 2022). The drip loss decreases with an increasing amount of beef batter (Berger, Gibis, et al., 2022), but not significantly with an increasing amount of pork batter added to the ground meat (Witte et al., 2022). A pre-cooking as well as an increase in processing steps contributes also to a higher total cooking loss (Berry et al., 1981).

6.2. Microbiology

The tremendously increased surface of ground meat reduces the shelf-life since microorganisms and oxygen are homogeneously distributed, whereas the meat cut is almost sterile in the interior. An immediate chilling at 0 to 2 °C inhibits or at least retards further growth of microorganisms (Honikel, 2014). In the European Union, raw ground meat is defined as boneless meat ground into fragments and containing less than 1 % salt (Lautenschlaeger and Upmann, 2017). Adding more than 1 % salt would alter the fibrillar structure recognizably, which can be required for, e.g., hamburger processing (Honikel, 2014).

Several studies exist showing the microbiological evaluation of retail ground meat and/or differences to traditional preparation, mostly retrieved from different regions, e.g. Duitschaever et al. (1973); Field et al. (1977); Foster et al. (1977); Jahan et al. (2015); Joshi and Joshi (2010); Kammenou et al. (2003); Shoup and Oblinger (1976); Sumner (1978); Westhoff and Feldstein (1976). Nortjé et al. (1989) found in their microbiological survey about aerobic and psychrotrophic counts, *Enterobacteriaceae*, lactobacilli, and several *Pseudomonadaceae* on five cuts of fresh meat that the chilling regime will not preserve the inherent meat quality and underlined the significance of the initial microbial population. To this, one supermarket receiving carcasses with significantly lower microbial surface counts and one supermarket with a more efficient overall sanitation program were evaluated. A more stringent hygiene contributed to generally lower microbial counts and resulting in meat with an extended shelf life. The ground meat, produced from the carcasses, had the highest mean aerobic total microbial counts due to increased surface exposure and excessive handling during production. Thus, the aerobic total counts could presumably function as a suitable indicator to monitor the sanitary condition of ground beef. However, besides the sanitary conditions of the raw material and the production, also the securing of the cooling chain and the sanitary of the equipment and personnel surfaces affect the microbiological quality of the product (Nortje et al., 1990; Nortjé et al., 1989).

Rao and Ramesh (1988) checked the shelf-life of ground meat at higher temperatures to mimic tropical conditions by analyzing the microbiological quality of *Staphylococcus aureus*, coliforms, *Enterococci*, psychrotrophs, and total plate counts. The isolation and identification of bacteria associated with fresh meat spoilage revealed *Micrococcus*, *Escherichia*, and *Staphylococcus aureus*, whereby the last two are mesophilic microorganisms developed due to higher storage temperatures. Ground meat produced in local retail shops had significantly higher and varied microbial counts and, thus, a shorter shelf-life leading to economic losses than ground meat processed under hygienic conditions in modern abattoirs (Rao and Ramesh, 1988).

With the previously described use of TTIs utilizing, e.g., lactic acid bacterial strains as indicators in MAP of ground beef, the products' microbial quality and safety can be ensured (Ellouze and Augustin, 2010; Y.-A. Kim et al., 2012; Vaikousi et al., 2009). Using an electronic nose Winquist et al. (1993) showed that the origin of ground meat (beef *vs*. pork) can be differentiated as well as the storage time predicted. Due to the occurrence of different gaseous components by microorganisms in dependence on the species and storage time, the electronic nose could be used as an estimation for the quality of ground meat.

7. Integrated lines - Industrial scale equipment

7.1. Vacuum filler grinder

In contrast to the free conveying meat grinders, the filler grinder is based on forced conveying using the positive displacement principle, thus having a pre-defined product volume flow (Irmscher et al., 2016; Irmscher et al., 2015; Weiss et al., 2010). Following this, the pressure in the system might be almost constant throughout the grinding at the same raw material and system parameters. Changes in raw material properties would not affect the volume flow but would lead to altered pressure, motor torque, and thus mechanical load applied to the meat. The rotational speed of the knives, i.e., the cutting speed, can be adjusted independently at some devices (depending on the manufacturer and the used system) (Fürgut and Schreiber, 2022; Irmscher et al., 2015).

Similar to the free conveying meat grinders the cutting set composition mainly affects the efficiency and the size reduction process, the volume flow rate, and the energy consumption of the vacuum filler grinder process (Irmscher et al., 2013; Irmscher et al., 2016).

Nowadays vacuum filler grinders are mainly used for the production of industrial pre-packaged self-serviced ground meat in angel-hair-shape in Germany. The advantage is, that formation of an angel-hair-shape using "eye-shape" hole plates (**Figure I-3**) and portioning into packages can be directly connected to the cutting set, where the meat is ground to the final particle size (Fürgut and Schreiber, 2022). Synchronizing those process steps increase the line efficiency, the direct portioning also avoids the transport of the material into another machine, which reduces the load on the material (Büchele, 2009). It was reported, that up to 170 kg/min ground meat can be produced with the vacuum-filler-grinder system (Irmscher et al., 2016).

Figure I-3: Photograph of an "eye shape" hole plate.

7.2. Production line for ground meat production

In ground meat production lines, grinders are used to process both fresh and frozen meat without changing the screw or cutting set. **Figure I-4** shows a typical ground meat production line. The operating principle is highly variable regarding the available rotating blades and different-sized hole plate combinations (Weiss et al., 2010). In combination with a separator that uses special cutting knives, bones, cartilage, tendons, and other solid particles can be separated from the meat mass (Haack and Schnäckel, 2008).

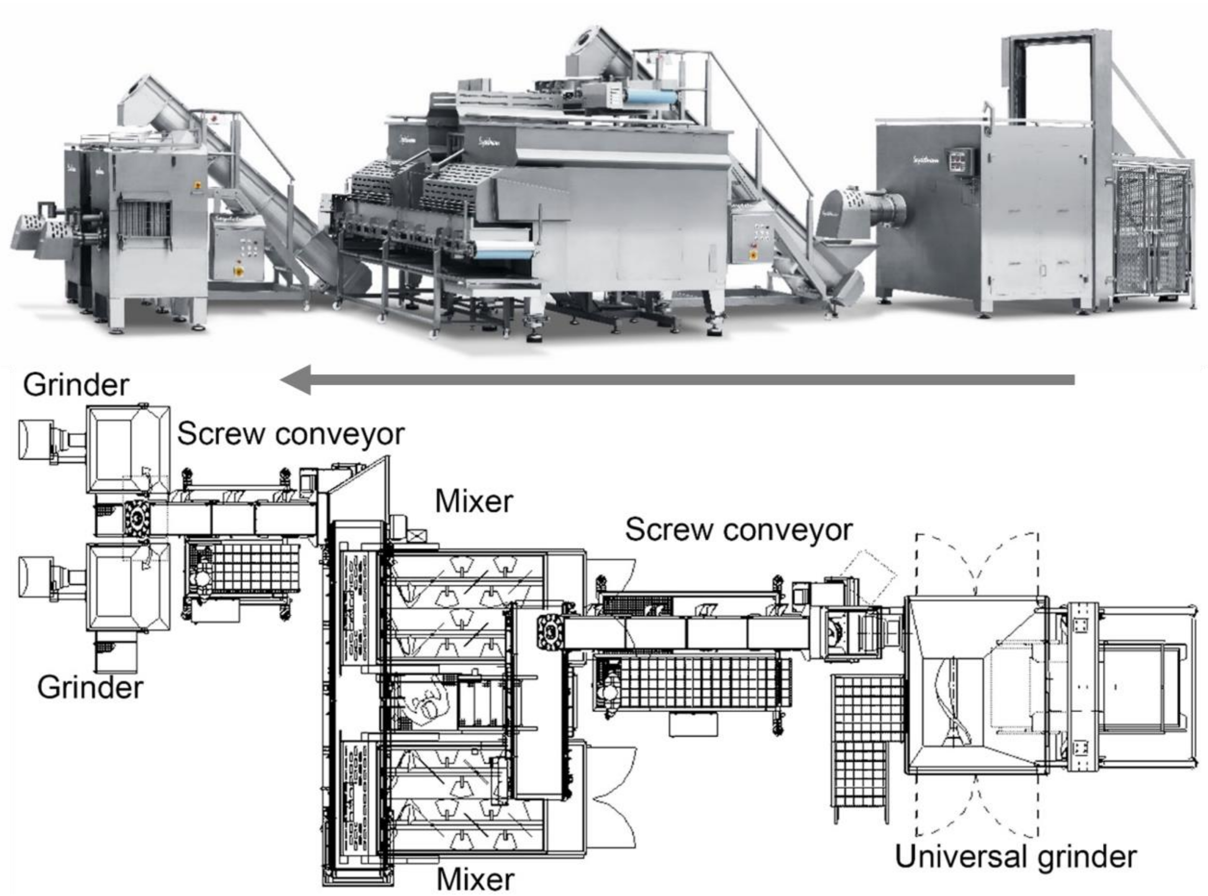

Figure I-4: Production line for ground meat and hamburgers (with kind permission of Maschinenfabrik Seydelmann KG).

Ground meat production usually involves pre-grinding, mixing, and final grinding of meat (Berger, Witte, et al., 2022). The grinder is either loaded with the integrated hydraulic feed loading device or with an angled conveyor belt. For standardization of lean meat and fat, the material is first coarsely cut and then pre-ground to particle sizes of 13 – 16 mm (Nollet and Toldra, 2006; Weiss et al., 2010). The pre-ground meat is conveyed by a screw or belt conveyor into a special grinder with a mixing function to obtain a homogeneous coarsely ground meat that is cooled to the ideal processing temperature of 0 °C to a maximum of 2 °C to ensure microbial safety (Honikel, 2014; Tomasevic et al., 2023). For the final mixing process, screws with paddles and ribbons are used in the discharge direction to obtain a homogeneous coarsely ground lean and fatty meat (Berger et al., 2023; Witte et al., 2022). The screw is located at the bottom of the hopper and discharge flap. After the mixing and cooling process, the pre-ground meat is finally ground through a hole plate with hole sizes of 2 – 3 mm and then discharged (Berger, Witte, et al., 2022).

7.3. Production line for hamburger production

After the production of the ground meat, it is possible to directly integrate a forming process. The ground meat can be mixed in this mixer grinder in most cases without, but also with spices, and can be formed without discharge in a connected forming unit directly after the grinding process (Berger, Witte, et al., 2022). In this forming unit, the ground meat can be continuously formed into different shapes such as discs, spheres, or sticks, resulting in different products, e.g., hamburgers, meatballs, or cevapcici. In most cases, the forming process starts with a positive displacement pump conveying the ground meat mass to the forming units by using low pressure to avoid over-processing of the meat structure (Berger, Witte, et al., 2022). In addition, interfacing with metal detectors or X-ray inspection systems is possible to detect bones and foreign objects (Einarsdóttir et al., 2016) or determine the fat content (Brienne et al., 2001; Christensen and Larsen, 2014). Another advantage is the possibility of a direct connection to a depositing unit that fills and/or divides the ground meat or hamburger directly into plastic boxes of the linked packaging system, improving meat safety and shelf life (McMillin, 2017; Weiss et al., 2010).

7.4. Inline measurements, control techniques, and cleaning systems

In modern industrial lines for ground meat production, control techniques with inline measurements are important to maintain the consistent quality of the ground meat. The basis of a fully automated process is high-performance in-line measurements and automatic control techniques that can control the composition of the raw material (Seaton, 2022; Seydelmann, 2013) and can quickly adjust the desired parameters of the prepared ground meat. Another quality attribute is adulteration during grinding. According to Mohammed et al. (2014) adulteration can be controlled, e.g., when included in the HACCP scheme, using effective cleaning-in-place schedules and having separate production lines. The authors suggest developing a sensory quality evaluation method as beef has an identifiable aroma and color (Cassens, 1994), whereas computer vision technology could mitigate errors of sensory analysis (Jackman et al., 2011).

The quality of ground meat and thus the safety of the product is depending on the hygienic conditions (Rao and Ramesh, 1988). An example is airborne contamination due to air movements during production, however, absolute environmental control is complex and, hence, almost impossible (Masotti et al., 2019). Feasible and cost-effective solutions for air disinfection of selected areas are, e.g., UV irradiation, ozonation, and chemical aerosolization. With these techniques, the settling of microorganisms on frequently touched surfaces can be reduced to prevent the risk of spreading (Masotti et al., 2019).

Besides microbiological issues, also the exact dosing of the product is important in terms of correct labeling. The actual weight of the meat is determined by a special weighing unit of the mixer, usually under a vacuum. This facilitates successive dosing of different ingredients to obtain the exact composition of the meat. In continuous production lines, it is also very important to maintain a constant product flow to prevent the machines from running dry. For this, the machine automatically stops or activates the filling systems to refill the hopper initiated by laser measurements. The in-line measurement of the material by near-infrared (NIR) or X-ray results in the determination of fat and foreign matter such as hard plastic, bone, or metal pieces (Brienne et al., 2001; Christensen and Larsen, 2014; Hansen et al., 2003). There are different X-ray systems such as the single-energy X-ray, which is used to determine the composition of the meat product by analyzing the differential X-ray absorption between lean and fat (Brienne et al., 2001; Damez and Clerjon, 2008). The dual-energy X-ray with two energy ranges (approx. 50 – 70 keV and 100 – 120 keV) provides a fast and continuously accurate determination of the composition (Clarke, 2014), and analysis of fat via low energy X-ray is also reported (Brienne et al., 2001).

NIR scanning of the surface of the meat provides continuous, real-time measurements of the fat and moisture content of the ground meat (Wold et al., 2011). Depending on the application, the measurements are based on reflection or/and transmission. The in-line system can be directly integrated into the production flow (e.g., installation on a conveyor after the grinder) (Seydelmann, 2013). In 1996, the first in-line application of this technique was published for the determination of fat, moisture, and protein contents in ground beef (Isaksson et al., 1996) using a diffuse NIR instrument on a conveyor fixed at the outlet of the meat grinder and multiple linear regression as the calibration method (Huang et al., 2008). These measurements are often integrated into recipe control in an automatic production line (Huang et al., 2008). Similar to NIR, an integrated conveyor located directly below the grinder outlet transports all material to the analysis zone. Both systems (NIR and X-ray) result in a noticeable increase in quality and standardization. To prevent differences in the composition of the product, the compositions of the incoming lean and fatty raw material streams are measured in-line. With the help of different conveyor systems, these are automatically added according to their composition to achieve the specified values of the end product (Seydelmann, 2013; Weiss et al., 2010). Material can be transported between the different processing steps by trolleys. However, using screw conveyors, conveyor belts, and pumps for transportation requires less personnel and time (Seaton, 2022; Seydelmann, 2013).

Automated cleaning systems in manufacturing are essential to guarantee the hygiene and the shelf life of ground meat. Automated processes are used to ensure improved quality such as hygiene, safety, and production efficiency, e.g., continuous process lines that include pre-cutting, cutting, grinding, and mixing under vacuum.

An effective cleaning system is essential for these automatic production lines to minimize the amount of labor required for cleaning. To maintain consistent cleaning quality, machines such as screw or belt conveyors and storage hoppers are most often covered and can be equipped with clean-in-place and/or sterilize-in-place (CIP/SIP) capabilities at temperatures up to 130 °C (Moerman et al., 2014). The main problems of fully automated production lines are the possibility of inefficient cleaning and the formation of microbiological biofilms that are difficult to reach with cleaning materials (Moerman et al., 2014; Van Houdt and Michiels, 2010). In particular, steam sterilization up to 130 °C and CIP can improve the safety of production lines (Moerman et al., 2014). Fat and protein residues are the main causes of contamination, fouling, and biofilm formation in the meat processing industry. These often accumulate to high levels during the production process (Allen and Wang, 2014). Chemical cleaning provides chemical energy to disperse and suspend contaminations in an aqueous solution. However, additional mechanical energy may be required through scrubbing, pressure spraying, or turbulent flow in pipes, vessels, etc. that are cleaned using a CIP system (Allen and Wang, 2014). In grinders, the CIP nozzles can be equipped with a main reservoir accessible for cleaning, with the cleaning nozzles located between the seal and the support of the screw drive. When the seal wears out, the material entering can be cleaned out. Both detergents and water (approx. 82 °C) as well as disinfectants with a short contact time (30 – 60 s) can be pumped out (Mohammed et al., 2014).

The control unit can control both the feed and the cleaning device with individually controllable in-line measurements, as well as control the cleaning devices in a targeted or time-controlled manner. Product identification and batch traceability are also important, which can be facilitated by barcode, key chip, or radio-frequency identification (RFID) and monitored by the controller

(Mohammed et al., 2014; Seydelmann, 2013). All of these in-line measurements, product identification for traceability, and cleaning equipment presented result in improved hygiene, shelf life, and quality of well-standardized meat products.

7.5. Packaging for ground meat and meat products measurements, control techniques, and cleaning systems

Packaging for ground meat and meat products is important to the shelf life of this product group. Ground meats are packaged in overwrap air-permeable, modified atmosphere (MAP), or vacuum packages (McMillin, 2017). For ground meats, foam trays are often used for air-permeable wrapping, and in most cases, plastic trays are used for MAP (McMillin, 2017). The gas composition of MAPs for ground meat is typically 65 – 80 % oxygen, 15 – 30 % carbon dioxide, and approximately 10 % nitrogen (Kropf and Mancini, 2014). The use of a small amount of carbon monoxide (0.4 %), which enhances the cherry red color by forming carboxymyoglobin, is not permitted in the European Union but permitted in many other countries (Kropf and Mancini, 2014). High oxygen MAP (80 %) packaged in the oxygen-impermeable film was found to be effective in maintaining a desirable red meat color during 10 days of refrigerated storage; however, after 6 or 10 days the flavor of samples in high-oxygen were evaluated as less desirable and the levels of thiobarbituric acid reactive substances increased compared to the control (Jayasingh et al., 2002).

Active and smart packaging can be achieved through the use of indicators, sensors, barcodes, and RFID systems. RFID systems can be implemented with potential benefits to the ground meat production, distribution, and retail chain (Ahmed et al., 2018; Zuo et al., 2022). The RFID tags provide wireless systems to monitor the packages via computer systems and reader devices (Ahmed et al., 2018). TTIs can also be integrated into RFID tags (Ahmed et al., 2018; Miscioscia et al., 2020). Maintaining appropriate storage temperatures should provide significant benefits in terms of the safety and shelf life of the products. There are active RFIDs, operating in the 433MHz range, and passive RFIDs, mostly operating between 860 and 960MHz, used for temperature monitoring in addition to supply chain management and logistics (Zuo et al., 2022). RFID is priced at approximately $0.09 to $0.18 (Janeczek et al., 2019).

Sensors as indicators provide information about the changes that occur in the food product or its environment through visual or other changes. Generally, three types of TTIs were developed based on diffusion, enzyme, and polymer sensors, which are distinguished by physical, chemical, or enzymatic reaction methods (Ahmed et al., 2018; Chun et al., 2013; Kerry et al., 2006). Chun et al. (2013) used an enzymatic TTI (lipases) to investigate the quality of pork patties based on color changes (from green to red) caused by lipolysis during a specific time and temperature. TTIs using lactic acid bacterial strains, e.g., *L. sakei*, were established as indicators of microbial quality and safety in MAP of ground beef (Ellouze and Augustin, 2010; Y.-A. Kim et al., 2012; Vaikousi et al., 2009). In another study, a microbial TTI based on the formation of violacein by *Janthinobacterium sp.* as a function of temperature and growth medium properties was used (Mataragas et al., 2019). In addition, a microbial TTI was utilized to predict the off-flavor and quality of ground beef during storage (Y.-A. Kim et al., 2012). Often the working principle is based on decreased pH caused by bacterial growth (M. J. Kim et al., 2012).

8. Conclusion

Nowadays, the process engineering working principle, the transportation method, and the forces acting on the product during grinding are known and well-described. The relationship between the process, raw material, and quality and functionality of ground meat and ground meat products was intensively investigated by our research group. From this point of view, both, ground meat and ground meat products, remain an open and interesting topic for future research focus.

In recent decades, the production of ground meat changed while consumer expectations remained constant. Furthermore, the analytics for quality assessment of ground meat and hamburgers was not specifically developed for ground meat, but for meat products in general – decades ago. However, a detailed description of the interaction of these novel process designs, including inter alia mixing, grinding, transportation, and portioning, with the ground meat structure and functionality could rarely be found in the literature.

The results of our research project and this review revealed some important factors in ground meat processing. As an example, higher frozen meat contents were found to increase the non-intact muscles cells without influencing product characteristics, e. g., water holding capacity. Reworking ground meat showed neither an influence on the amount of non-intact cells (ANIC), nor on quality parameters such as cooking loss, firmness, or sensory firmness. Yet unpublished data indicate that mixing of up to 15 min leads to an approx. threefold increase of the ANIC compared to the base value. The effects on functionality and physico-chemical properties, however, were small [data under publication]. Considering the amount of non-intact cells (ANIC) in an international perspective, it seems to be only a German regulatory analysis.

However, our statements hold true beyond borders. The histochemical structure analysis is used to specifically assess the ANIC and can indicate the mechanical process load of the ground meat. Interestingly, in any of our published and not-published results, we could not find any clear correlation between the ANIC and the quality parameters. At the moment, we assume that some of the quality parameters, which we attribute to the ground meat quality, are counter affected by proteins or minerals from the inner cell, such as the water holding capacity.

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Contribution of authors

All authors contribute in equal parts to the manuscript.

CRediT authorship contribution statement

Lisa M. Berger: Data curation, Project administration, Visualization, Writing - original draft. Franziska Witte: Data curation, Project administration, Visualization, Writing - original draft. Monika Gibis: Funding acquisition, Project administration, Supervision, Writing – review & editing. Nino Terjung: Funding acquisition, Project administration, Supervision, Writing - review & editing. Igor Tomasevic: Writing - review & editing. Volker Heinz: Resources, Supervision, Funding acquisition. Jochen Weiss: Resources, Supervision, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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First section: Structure-Function Relationship in Ground Meat

II. Chapter:

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 ≥ 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 x 5 x 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 over night 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 a 5-wing pendulum knife (TC90820, turbocut Joo 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 **Figure II-1**.

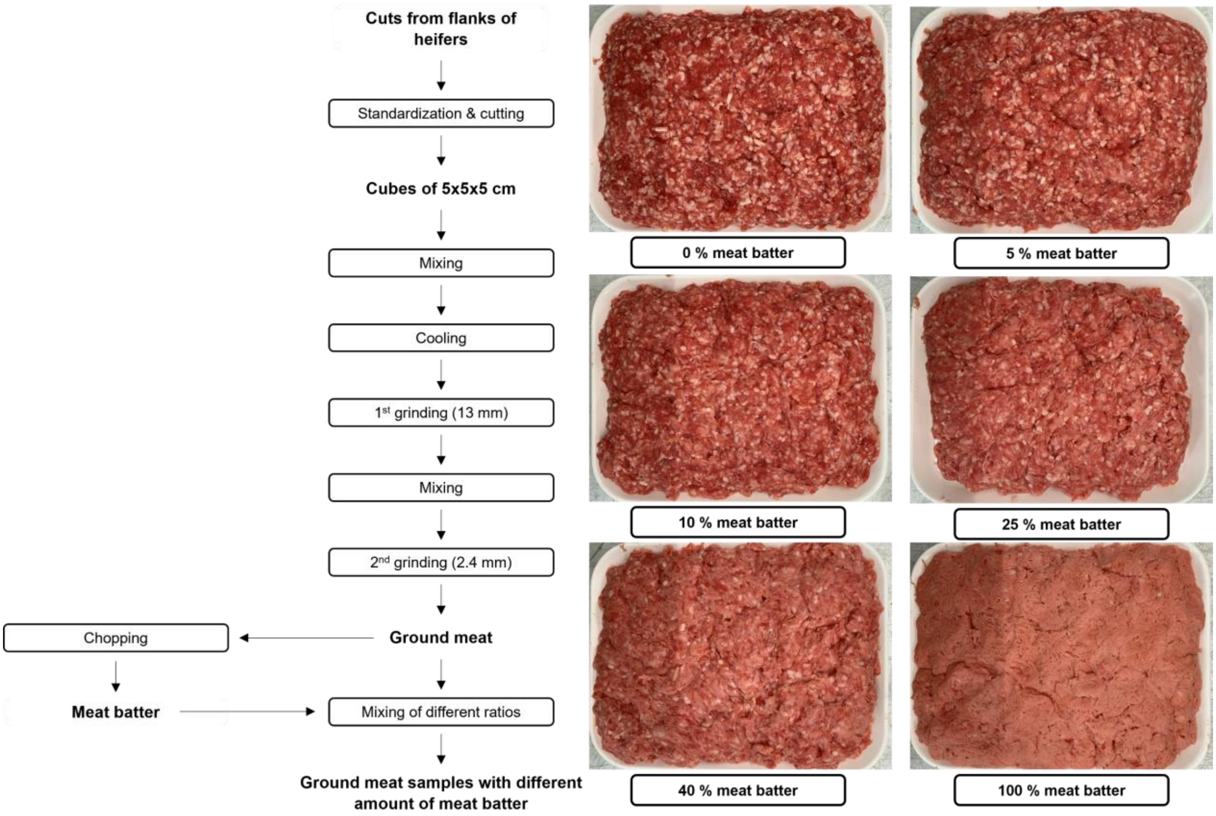

Figure II-1: Flow chart of the ground beef sample manufacturing and photographs of the samples containing 0 – 100 % meat batter

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.

Methods

Determination of 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 picroindigo carmine (CALLEJA) coloring agent and transferred into high-resolution images (Labor Kneissler, Burglengenfeld, 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. II-1 [27]:

$$\hat{p}_u \cong \hat{p} - \left[\frac{1}{2n} - 2.3263 \cdot \sqrt{\frac{\hat{p} \cdot \hat{q}}{n}}\right] \qquad \text{II-1}$$

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

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

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

$$f(x_{meat\ batter}) = ANIC_0 + k \cdot x_{meat\ batter} \qquad \text{II-2}$$

With $ANIC_0$ being the y-aches intercept, k being a slope factor and $x_{meat\ batter}$ 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 pH 7 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 (NunclonTM 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. II-3 according to Trout [30].

$$MetMb\ \left(\frac{mg}{mL}\right) = \left(1.395 - \frac{A_{572} - A_{700}}{A_{525} - A_{700}}\right) \cdot 100 \qquad \text{II-3}$$

with A_λ = absorbance at λ 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. II-4:

$$Drip\ loss\ (\%) = \frac{m_{before\ centrifugation} - m_{after\ centrifugation}}{m_{before\ centrifugation}} \cdot 100 \qquad \text{II-4}$$

With $m_{before\ centrifugation}$ = weight of meat sample before centrifugation (g) and $m_{after\ centrifugation}$ = 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. II-5:

$$Cooking\ loss\ (\%) = \frac{m_{before\ cooking} - m_{after\ cooking}}{m_{before\ cooking}} \cdot 100 \qquad \text{II-5}$$

With $m_{before\ cooking}$ = weight of meat sample before cooking [g] and $m_{after\ cooking}$ = 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 **Figure II-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 II-1**). Thereby the ANIC increases from 20.61 ± 1.75 Vol% in the base material ($x_{meat\ batter}$ = 0 %) to 97.38 ± 1.25 Vol% in the meat batter ($x_{meat\ batter}$ = 100 %). **Figure II-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 $f(x_{meat\ batter}) = 1.542 + 18.648 \cdot x_{meat\ batter}$ (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.

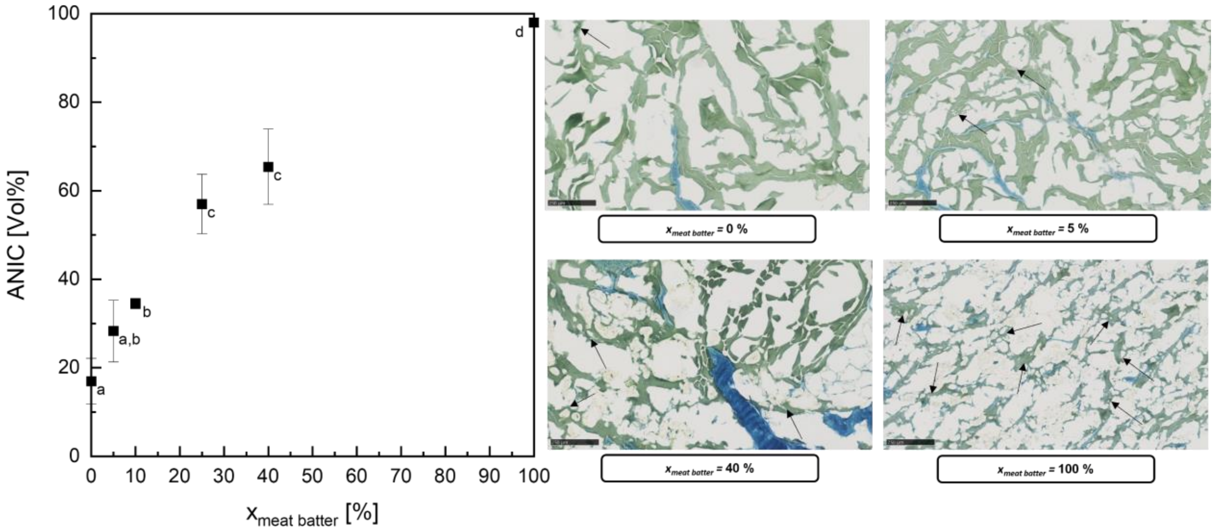

Figure II-2: Amount of non-intact cells (ANIC) in ground beef samples as a function of amount of added meat batter $x_{meat\ batter}$ (0 %, 5 %, 10 %, 25 %, 40 %, 100 %) and representative details of the histological images (Calleja-Luglo staining, 20-fold magnification). Data points with different letters are significantly different ($p < 0.05$). Green structures represent muscle cells, blue structures fat, or connective tissue. Nonintact cells are exemplarily pointed out with an arrow

It is known that ANIC$_0$ 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.

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

		$x_{meat\ batter}$ (%)	ANIC (Vol%)	*SPC* (%)	*MetMb* (mg/mL)	*DL* (%)	Firmness (N)	*CL* (%)
$x_{meat\ batter}$ (%)	*r*	1	0.947**	-0.573**	0.924**	-0.834**	-0.499**	-0.275
	p		0	0	0	0	0	0.110
ANIC (Vol%)	*r*	0.947**	1	-0.670**	0.870**	-0.749**	-0.389*	-0.260
	p	0		0	0	0	0.019	0.132
SPC (%)	*r*	-0.573**	-0.670**	1	-0.526**	0.472**	0.049	0.500**
	p	0	0		0.001	0.004	0.779	0.002
MetMb (mg/mL)	*r*	0.924**	0.870**	-0.526**	1	-0.787**	-0.441**	-0.289
	p	0	0	0.001		0	0.008	0.098
DL (%)	*r*	-0.834**	-0.749**	0.472**	-0.787**	1	0.533**	0.268
	p	0	0	0.004	0		0.001	0.120
Firmness (N)	*r*	-0.499**	-0.389*	0.049	-0.441**	0.533**	1	0.247
	p	0	0.019	0.779	0.008	0.001		0.153
CL (%)	*r*	-0.275	-0.260	0.500**	-0.289	0.268	0.247	1
	p	0.110	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

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

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

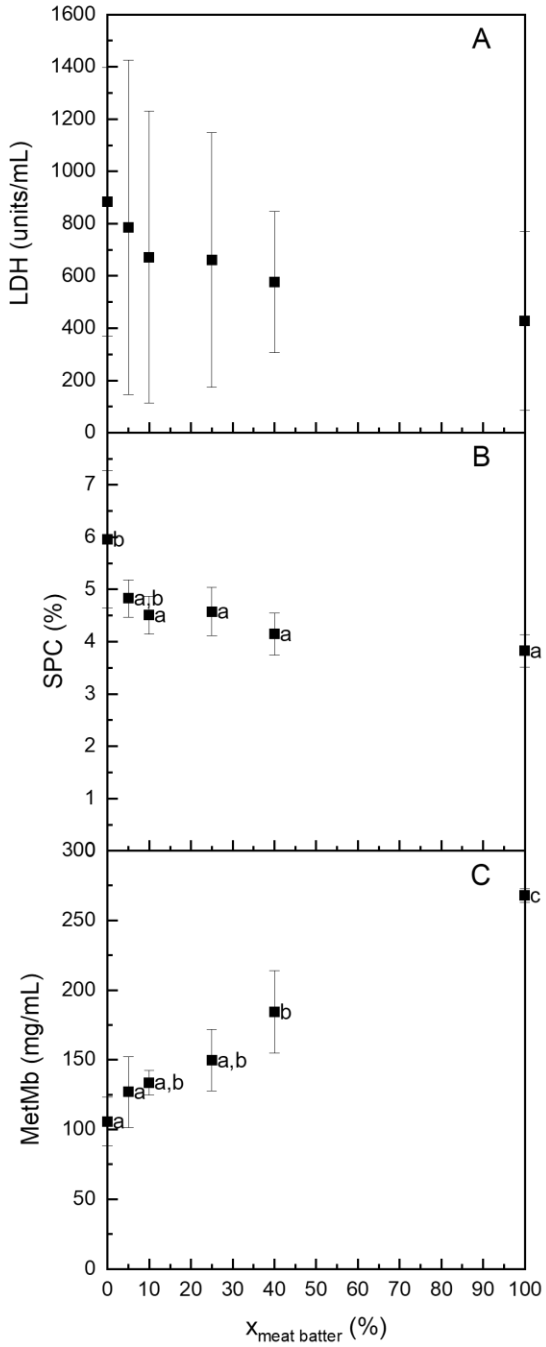

Figure II-3: Characterization of the material properties **A** Lactate dehydrogenase activity (LDH), **B** Soluble protein content (SPC) and **C** Metmyoglobin content (MetMb) of the ground beef samples extracts as a function of the amount of added meat batter $x_{meat\ batter}$. Data points with different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

Lactate dehydrogenase (LDH)

The LDH of the extracts ranged from 884.07 ± 514.50 units/mL ($x_{meat\ batter}$ = 0 %) to 427.75 ± 342.78 units/mL ($x_{meat\ batter}$ = 100 %) (**Figure II-3A**). Although homogeneous sample material was ensured, measurement data strongly fluctuated. Due to high standard deviations and non-normal distributed data (**Figure II-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.

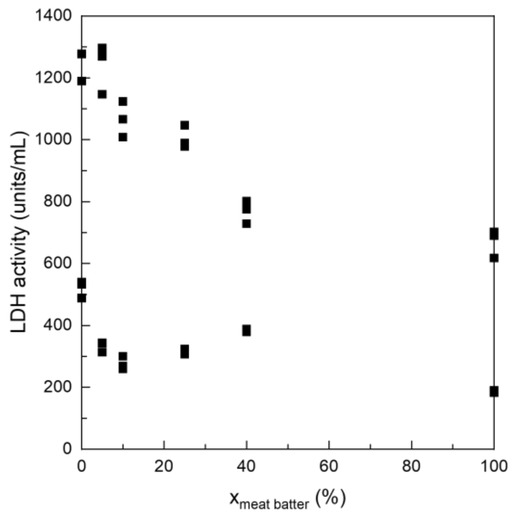

Figure II-4: All data points of the Lactate dehydrogenase activity (LDH) determination in the ground beef samples extracts as a function of the amount of added meat batter $x_{meat\ batter}$ (0 %, 5 %, 10 %, 25 %, 40 %,100 %)

Soluble protein content (SPC)

Increasing the amount of meat batter slightly reduces the SPC from 5.96 ± 1.31 % ($x_{meat\ batter}$ = 0 %) to 3.83 ± 0.32 % ($x_{meat\ batter}$ = 100 %) (**Figure II-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 II-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.

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 ($x_{meat\ batter} = 100$ %) (**Figure II-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 II-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.

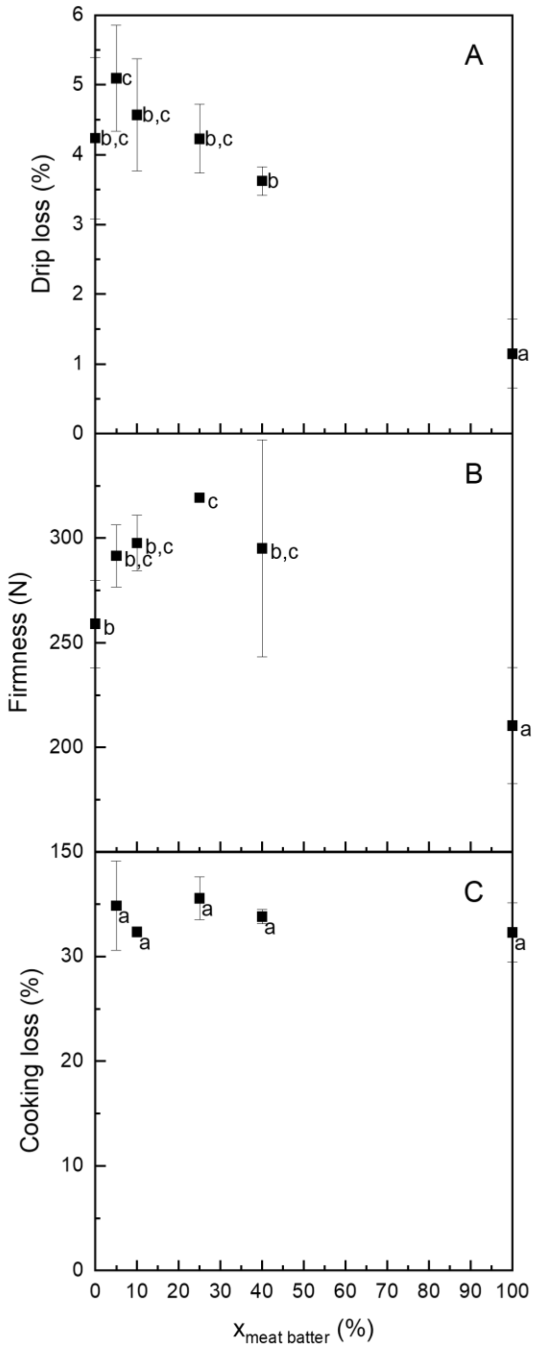

Figure II-5: Characterization of the quality parameters **A** Drip loss (DL), **B** Firmness and **C** Cooking loss (CL) of the ground beef samples as a function of the amount of added meat batter $x_{meat\ batter}$ (0 %, 5 %, 10 %, 25 %, 40 %, 100 %). Data points with different letter letters are significantly different (p < 0.05)

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 (**Figure II-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 II-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 (**Figure II-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 %) (**Figure II-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 (**Figure II-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 (**Figure II-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 II-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 II-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 (**Figure II-6**).

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

The base material properties ($x_{meat\ batter}$ = 0 %, $ANIC_0$ = 20.61 ± 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 ($x_{meat\ batter}$ = 100 %)

In contrast, the meat batter ($x_{meat\ batter}$ = 100 %, $ANIC_{100}$ = 97.38 ± 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 (ANIC$_{Δ}$)

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.

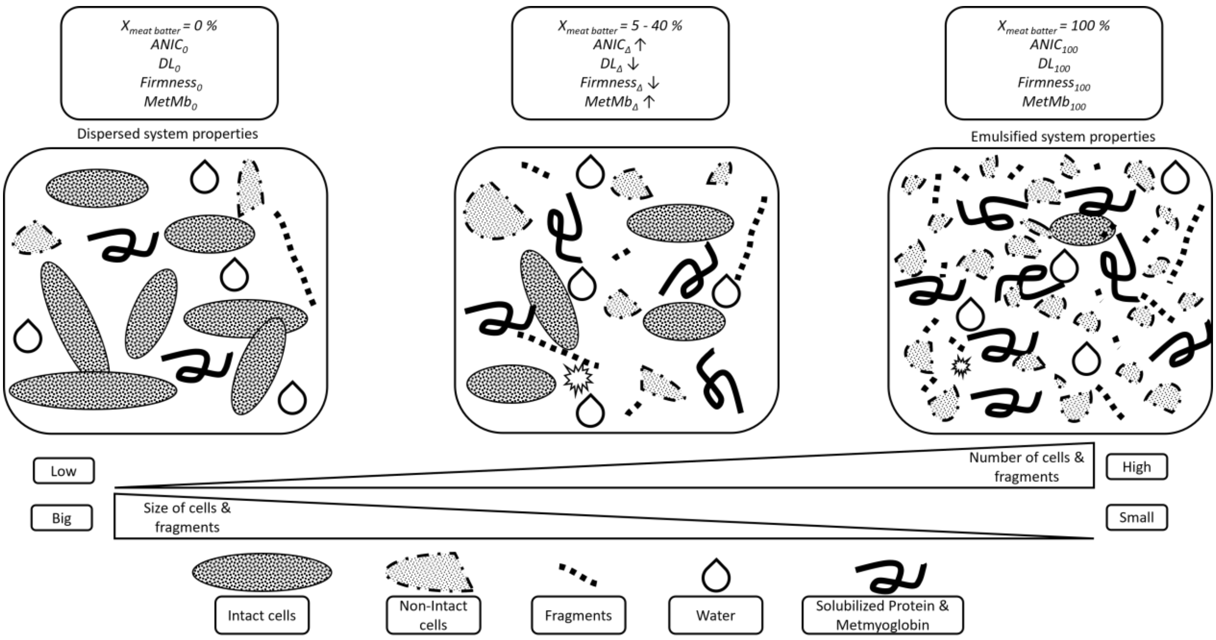

Figure II-6: Proposed mechanism of interaction of ground beef and added meat batter ($x_{meat\ batter}$) and their influence on the amount of nonintact cells (ANIC), material the structural properties (Lactate dehydrogenase activity (LDH), Soluble protein content (SPC), Metmyoglobin content (MetMb)) & and the quality parameters (Drip loss (DL), Firmness, Cooking loss (CL))

Conclusion

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 ($x_{meat\ batter}$ = 0 % vs. $x_{meat\ batter}$ = 100). A more precise differentiation in the practically occurring range (up to ca. 35 Vol% ANIC) was not possible.

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Conflict of Interest

The authors declare no conflict of interest.

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Second section: Raw Material-Function Relationship in Ground Meat

III. Chapter:

Effect of Manufacturing and Frozen Meat Temperatures on Structural and Functional Properties of Hamburgers

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Highlights

- Changes in frozen meat content caused a more pronounced effect than changes in frozen meat temperature
- Increased frozen meat content decreased the batch processing temperature
- Colder batch processing temperature caused higher energy input in samples during grinding
- Hamburgers' structural, functional and quality parameters were affected by temperature

Abstract

Varying percentages of frozen meat are normally used in the production of hamburgers. The influence of the frozen meat content and temperatures on the structural and functional properties of the hamburgers, process parameters and batch processing temperatures were assessed. The batch processing temperatures were much lower when a higher content of frozen meat was used (r = -0.695 at -6 °C frozen meat temperature, r = -0.690 at -12 °C frozen meat temperature; $p < 0.01$), resulting in higher specific mechanical energy input (r = 0.643 at -6 °C frozen meat temperature, r = 0.778 at -12 °C frozen meat temperature; $p < 0.01$), indicating higher stress on the meat during processing. The influence of the frozen meat content was higher for most parameters than the frozen meat temperature. The findings indicate that the frozen meat content can influence the quality and should be carefully optimized for the production process.

Keywords: Ground beef, meat structure, meat functionality, meat processing behavior, frozen meat, process design

Abbreviations:

Abbreviation	Meaning
ANIC	Amount of non-intact cells
ANOVA	Analysis of variance
BPT	Batch processing temperature
DL	Drip loss
FMC	Frozen meat content
FMT	Frozen meat temperature
Mb	Myoglobin
MetMb	Metmyoglobin
SME	Specific mechanical energy input

Introduction

The raw material and process conditions are two of the most important parameters affecting the properties of ground meat and hamburgers (Dobraszczy et al., 1987; Schnäckel et al., 2011). They determine the microbiological safety of not only products and processability but also structural and functional properties. Thereby, properties of the raw material, such as the temperature, strongly influence the interaction of the meat with the meat grinder and, thus, the processability and the mechanical load during grinding (Krickmeier, 2015). Frozen meat is used in the manufacturing of hamburgers to counteract frictional heat. It ensures low batch temperatures of a maximum of 2 °C (Honikel, 2014), which are important for microbial safety and the preservation of a clear and clean cut (Krickmeier, 2015; Wild et al., 1991).

In addition to microbial stability, the meat temperature also determines the processability of the meat. Krickmeier (2015) reported, that meat acts as the element for transferring the energy between the screw and housing of the grinder, which is the basis for moving material in a freely conveying meat grinder. The continuous push of raw material generates the energy for the comminution and the penetration into the boreholes of the cutting system (Haack et al., 2007; Haack and Sielaff, 2005). The interactions change with the changing properties of the raw material, such as the viscoelastic behavior or cutting resistance (Brown et al., 2005). Thus, the properties of the raw material are responsible for the extent of energy consumption and load generated during grinding.

Brown et al. (2005) and Schnäckel et al. (2011), *inter alia*, stated a fundamental relationship between meat temperature and cutting forces. In addition to reducing the batch temperature, a decrease in the temperature of the raw material leads to an increase in its cutting and friction forces. By decreasing the raw material temperature below -12 °C, the meat loses its viscoelastic cutting behavior. As a crystalline structure is built, it becomes brittler, which changes the physical properties of the meat (Dobraszczy et al., 1987; King, 1999). Pressures within the cutting set are increased at lower temperatures, escalating the mechanical load acting on the meat (Wild et al., 1991). Due to its altered elastic properties in the frozen state, the meat can only partially migrate into the drills of the end-hole plate. Thus, the muscle fibers are destroyed on the edges of the bore hole due to high pressure (Schnäckel et al., 2012). This is why process parameters, such as pressure in the cutting set or torque of the screw, might be used to draw a conclusion about the mechanical load of the meat during processing and the subsequent changes in material characteristics.

Freezing always results in the formation of ice crystals in the meat matrix, which influences its structural, functional and quality parameters (Ballin and Lametsch, 2008). The freezing temperature determines whether intracellular (at T = -10 °C), intercellular (at $T \leq$ -33 °C) or both forms (at T = -22 °C) of ice crystals predominate, causing the highest damage when both crystal forms are present (Rahelić et al., 1985). Pronounced cell damage causes, for example, increased histologically determined cell disintegration, increased drip loss, increased enzyme activity or altered sensorial properties (Ballin and Lametsch, 2008).

Although research on the effect of freezing on meat and its processability have been conducted, the influence of different temperatures and varying percentages of frozen meat in ground meat manufacturing and its correlation on the batch processing temperature and their various effects have not yet been investigated. This is a major issue during ground meat and hamburger production and mainly defines their quality, therefore, respective analyses were conducted in this study. It is assumed that an increased frozen meat content and colder frozen meat temperatures decreases the batch processing temperature. Based on that, it was hypothesized that the specific mechanical energy input (SME) during grinding would increase due to the altered properties of the raw material. Thus, higher amounts of non-intact cells (ANIC) and significant changes in other physicochemical and functional properties of the hamburgers were assumed.

Materials and methods

Materials and sample preparation

Samples with varying amounts of frozen material at different temperatures were produced according to the production scheme (**Figure III-1**). Details of all equipment used are summarized in **Table III-1**, and photographic images are depicted in **Appendix III-1**. The beef was purchased from MEGA (MEGA – Das Fachzentrum für die Metzgerei und Gastronomie eG, Stuttgart, Germany). The cuts from flanks of heifers (*M. transversus abdominis, M. obliquus externus abdominis, M. obliquus internus abdominis*; Prändl et al. (1988)) were precut manually into pieces of approximately 5 x 5 x 5 cm, the fat content was visually adjusted to 20 % and stored at T = 1 °C overnight. The chilled meat was mixed at 32 rpm for each 30 s with a clockwise and counterclockwise rotation in a horizontal paddle mixer and, firstly, ground to 13 mm particle size using a meat grinder equipped with a 3-folded grinding system (pre-cutter with plastic spacer, 4-wing knife, 13 mm inclined end-hole plate). Seven batches each of 18 kg of the pre-ground meat were cooled with liquid nitrogen to temperatures of -6 and -12 °C using the paddle mixer with a closed lid at 32 rpm. Batches containing 15, 30, 45 and 60 % frozen material at both temperatures were prepared by mixing with chilled (T = 1 °C) meat at 13 mm particle size in the paddle mixer at 32 rpm for 30 s in each direction. One batch was prepared without the addition of frozen meat as a reference.

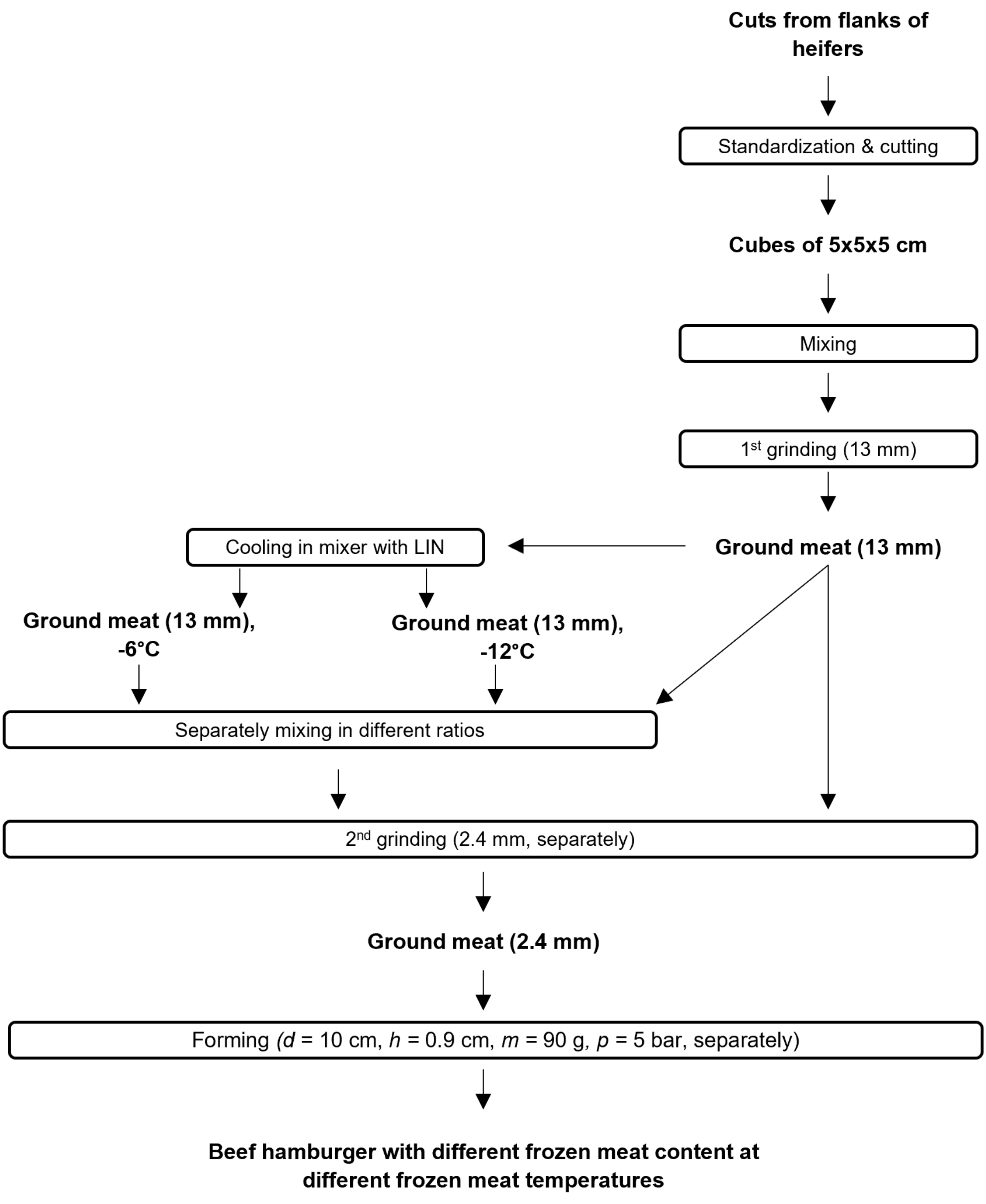

Figure III-1: Flow chart of hamburger manufacturing of the samples containing varying percentages of frozen material at different temperatures. LIN = liquid nitrogen.

Table III-1: Details on equipment used for patty manufacturing.

Description	Model	Manufacturer
Horizontal paddle mixer	Paddle mixer type RC-40	Equipamientos Cárnicos, S.L., Mainca, Barcelona, Spain
Meat grinder	Forschungsautomatenwolf Typ AE 130	Maschinenfabrik Seydelmann KG, Stuttgart, Germany
Precutting device	2031809T	Maschinenfabrik Seydelmann KG, Aalen, Germany
4-wing knife	TC93094	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany
13 mm inclined end-hole plate	TC3090278.1	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany
Plastic spacer for meat grinder	-	Maschinenfabrik Seydelmann KG, Stuttgart, Germany
Counteractive metal disc springs	-	Maschinenfabrik Seydelmann KG, Stuttgart, Germany
5-wing pendulum knife	90820/1211134	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany
2.4 mm inclined perforated end-hole plate	TC3093457.1	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany
Hamburger press	MH-100 (modified)	Equipamientos Cárnicos, S.L. (Mainca), Barcelona, Spain

Each batch was separately ground in the second grinding step to a particle size of 2.4 mm using the meat grinder equipped with a hanging 3-folded grinding system (two counteractive metal disc springs, 5-wing pendulum knife, 2.4 mm inclined end-hole plate). Using a modified hamburger press, the ground meat was then formed into patties of m = 90 g weight, h = 0.9 cm height, and d = 10 cm diameter by applying p = 5 bar forming pressure. The grinding speeds in both grinding steps were adjusted to 20 rpm at the feeding screw and 187 rpm at the grinding screw. The experiment was performed in triplicate.

Methods

Chemical composition of hamburgers

Prior to the water, fat and protein determination, one hamburger per batch was homogenized for 30 s in a blender (Blixer® 2, Robot-Coupe, Montceau-en-Bourgogne, France). The water content was determined from the meat mass by the sea-sand method (§64 LFGB L 06.00-3 (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2006)), the fat content by Soxhlet extraction (Büchi 810, Büchi Laboratoriums-Technik AG, Flawil, Switzerland) from the residues of the water determination (§64 LFGB L 06.00-6 (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2006)) and the protein content by the Dumas' combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) (§64 LFGB method L 06.00-20 (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2006)) using a nitrogen to protein conversion factor of 6.25 (Mariotti et al., 2008).

Histochemical analyses

The amount of non-intact cells (*ANIC*) in the hamburgers was histochemically assessed according to the method described previously by Berger, Gibis, et al. (2022) and Berger, Witte, et al. (2022), following the official guidelines mentioned in § 64 LFGB (L 06.00-13) (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2006). In short, thin cryo-cut cross-sections of the hamburgers were assessed for non-intact cells by the point-count method. The ANIC were then calculated. At least $n = 6$ cross-sections per sample were analyzed, wherefrom two cross-sections each were averaged. The mean and the standard deviation of the three repetitions were calculated.

Determination of drip loss

The hamburgers' drip loss was determined by a centrifugation method according to the procedure described previously by Berger et al. (2022a,b). In short, a 10 g sample was centrifuged for 20 min at 5 °C and 16,000 rpm (Centrifuge Z32HK, Hermle Labortechnik GmbH, Wehingen, Germany). The drip loss was determined by differential weighing.

Determination of firmness by forward extrusion

The firmness of the ground meat was determined using the forward extrusion method described previously by Berger et al. (2022a,b). In short, ground meat was pressed through a cylinder (d = 50 mm) with a 7.5 mm hole opening using a texture measurement device (Instron, Model 3365, Instron Engineering Corporation Ltd, Massachusetts, USA), thereby recording the force required.

Determination of hardness by Warner-Bratzler shear cell

Frozen hamburgers were grilled on an electric dual slide contact grill (XPE-24 model, Garland Commercial Ranges Ltd., Mississauga, Ont., Canada) for 130 s with an upper plate temperature of 218 °C and a lower plate temperature of 177 °C until a core temperature of 72 °C was reached. The hamburgers were cooled to room temperature, and strips of 1.5 cm in width were manually cut from the center. The grilled hamburgers' hardness was determined using a texture analyzer (Model 3365, Instron Engineering Corporation Ltd., Massachusetts, USA) equipped with a V-shaped Warner-Bratzler guillotine and a crosshead speed of 250 mm/min. The maximal shear force (N) was recorded (n = 18 per sample).

Sensory evaluation

The sensory evaluation was performed in terms of gustatory and optical properties of the fried hamburgers using a 10-point rating scale, where a score of 5 points represents the compared reference standard ("Reference"), which was evaluated as standard in pretests by sensory experts. Prior to the gustatory evaluation, grilled and cooled (T = 7 °C) hamburgers were cut into quarters using a standardized cutting template. Reheating was performed using a microwave oven, as pre-liminary test showed, that textural (e.g., hardness, juiciness) and sensory perception were not affected by the reheating in the microwave (data not shown).

Following the insights of James et al. (2002) the reheating process was highly standardized to obtain consistent and comparable results and the reliability of the method was tested (see supplementary data). For reheating, one hamburger was placed on a melamine plate (white melamine plate 190 x 145 mm, WACA-Kunststoffwarenfabrik, Halver, Germany), covered with another plate of the same type and reheated in a microwave (model HF15M541, Siemens Aktiengesellschaft, Munich, Germany) under standardized conditions (P = 800 W; t = 45 s) to a core temperature of T = 70 ± 2 °C. The core temperature of the hamburger was checked before sensorial analysis. The parameters hardness, juiciness, texture and overall acceptability of the hamburgers were assessed. Regarding optical assessment, the hamburgers were cut cross-sectionally with a slicer (Bizerba VS8A, Wilhelm Kraut GmbH & Co. KG, Balingen, Germany), and the inner structure was evaluated in terms of the coarseness of the particles, amount of batter-like substance and overall acceptability. Each sample was evaluated by at least n = 20 panelists.

Preparation of meat solution extracted

In preparation for further analyses, extracted meat solutions from all samples were produced following to the procedure of Berger, Witte, et al. (2022) and Berger, Gibis, et al. (2022). Accordingly, meat was diluted 1:10 with 10 mM potassium phosphate buffer (pH 7, T = 2 °C), filtered and stored cooled in brown glass bottles until further usage.

Determination of myoglobin (Mb) and metmyoglobin (MetMb) content

The Mb and MetMb content of the respective extracted meat solution were determined photometrically, following the previously described method of Berger, Gibis, et al. (2022) (MetMb determination) and Berger, Witte, et al. (2022) (Mb determination) to assess the oxidative changes of the pigments.

Process control parameters

The grinding screw torque was recorded during grinding by the operating software of the grinder (S7-To-Excel Tool – Expert – for Windows, Träger Industry Components, Weiden, Germany) for all batches containing 0 – 45 % frozen material. The idle torque of the grinding screw was determined previously to be $M = 6.9 \pm 1.1$ Nm (data not shown). The temperature of each batch ($m \geq 10$ kg) was determined manually before grinding at different positions of the sample ($n \geq 5$) using a thermometer (testo 926, Testo SE & Co. KGaA, Tittisee-Neustadt, Germany). The mass flow was determined by weighing the output of ground meat within 15 s ($n \geq 3$).

Determination of specific mechanic energy input (SME)

The SME (J/kg) as the degree of shear strain during grinding was calculated according to the following equation III-1 and III-2 (Fang et al., 2013; Godavarti and Karwe, 1997; Villmow et al., 2010).

$$SME = \frac{P}{\dot{m}} \qquad \text{III-1}$$

$$P = (M - M_{empty}) \cdot n_{rad} \qquad \text{III-2}$$

With P = average power during grinding process (W), M = average grinding screw torque during grinding (Nm), M_{empty} = idling torque of grinding screw (Nm), $\dot{m} = \frac{m}{t}$ being the mass flow (kg/s), $n_{rad} = \frac{2\pi \cdot n_{grinding\,screw}}{60}$ being the radial rotational speed of grinding screw (s^{-1}) and $n_{grinding\,screw}$ = rotational speed of grinding screw (s^{-1}). The average torque during grinding is determined as the torque during the plateau phase of grinding.

Statistical analyses

Three independent experiments (biological replicates) with at least three analytical replicates (technical replicates) were done. All results are given as mean ± standard deviation or mean ± standard error (torque profiles), both calculated by MS Excel (Microsoft, Redmond, WA, USA). Plots were prepared by OriginPro 2020 (OriginLab Corporation, North Hampton, MA, USA), and statistical analyses by SPSS (IBM SPSS Statistics 25, IBM Deutschland GmbH, Ehningen, Germany).

The normal distribution of data and variance homogeneity were tested by Shapiro-Wilk and Levene tests, respectively. A two-factorial analysis of variance (two-way ANOVA) was performed by applying the Tukey post hoc test (confidence interval of 95 % (p = 0.05)) for the parameters drip loss, firmness, Warner-Bratzler shear force, Mb and MetMb content. Data were transformed with LN-transformation before two-way ANOVA (Field, 2009), if necessary, to ensure variance homogeneity. A Pearson correlation analysis was performed to assess the linear correlation between two normally distributed variables.

Results and discussion

Chemical composition

The hamburgers of the study were composed of 62.8 ± 1.9 % water, 17.3 ± 2.5 % fat, 19.159 ± 1.014 % protein and 1.03 ± 0.03 % ash, resulting in a sum parameter of 100.37 %. The production of hamburgers followed the German guidelines for meat and meat products and contained only beef (Bundesanstalt für Landwirtschaft und Ernährung, 2015). The hamburgers were produced without the addition of salt and spices.

Structural changes

The ANIC and the influence on the batch processing temperatures of the meat mass before grinding were determined to check the influence of frozen meat content and temperature on the structural changes of the beef during grinding. The manually determined batch processing temperatures are depicted in **Figure III-2**, which decrease at a higher frozen meat content.

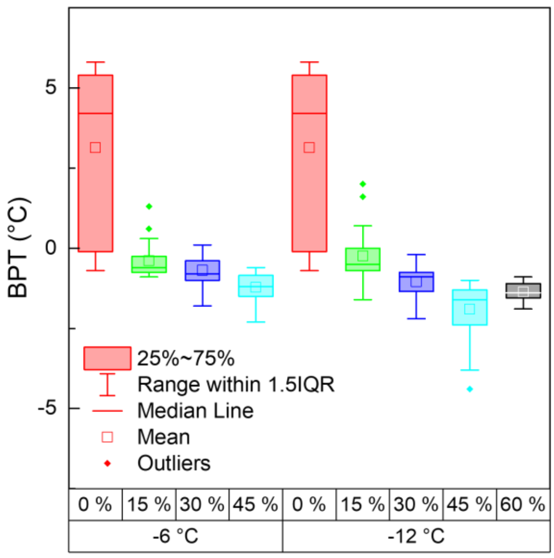

Figure III-2: Influence of frozen meat content (0, 15, 30, 45, and 60 %) and frozen meat temperature (-6 and -12 °C) on batch processing temperatures (BPT) during hamburger production.

Figure III-3 shows the influence of an increasing frozen meat content in the hamburgers on the ANIC and the batch processing temperature for both frozen meat temperatures of -6 °C (**Figure III-3A**) and -12 °C (**Figure III-3C**). At -6 °C frozen meat temperature, the batch processing temperatures decreased from 2.80 ± 2.98 °C at 0 % frozen meat content to -1.21 ± 0.74 °C at 45 % frozen meat content (**Figure III-3A**). At -12 °C frozen meat temperature, it decreased to -1.36 ± 1.03 °C at 60 % frozen meat content (**Figure III-3C**). With increasing frozen meat content, the ANIC remained constant between 25.93 and 28.54 Vol.% (T = -6 °C), whereas it increased from 26.84 ± 2.99 Vol.% at 0 % frozen meat content to 32.07 ± 2.46 Vol.% at 60 % frozen meat content (T = -12 °C). Photographic images of the ground meat samples are depicted in **Appendix III-1**.

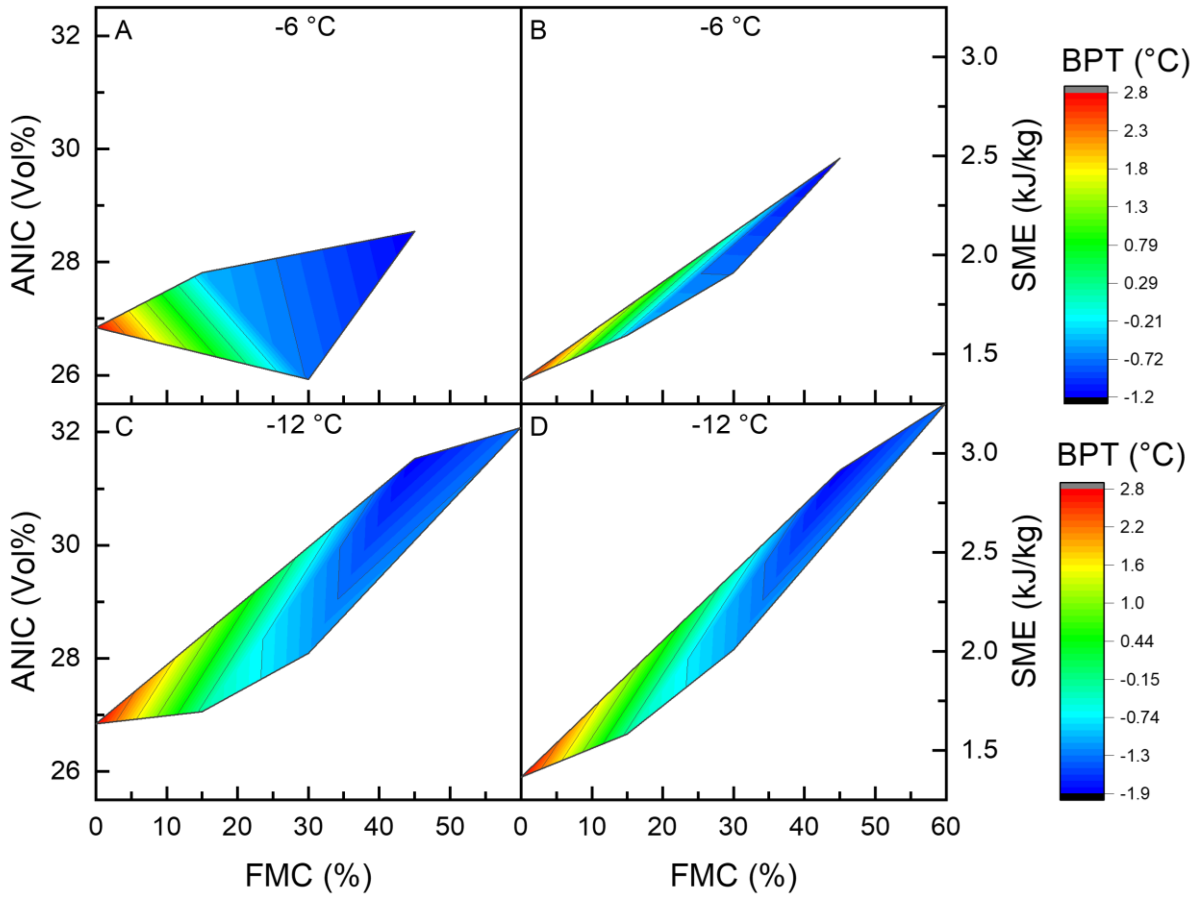

Figure III-3: Influence of frozen meat content (FMC; 0, 15, 30, 45, and 60 %) on batch processing temperature (BPT), amount of non-intact cells (ANIC) (A, C), and specific mechanical energy input (SME) (B, D) at frozen meat temperature of -6 °C (A, B) and -12 °C (C, D) during hamburger production.

Table III-2: Pearson correlation coefficients *r* of frozen meat content (FMC), amount of non-intact cells (ANIC), specific mechanical energy input (SME) and batch processing temperature (BPT) at different frozen meat temperatures (FMT) of -6 and -12 °C.

		FMT = -6 °C				FMT = -12 °C			
		FMC (%)	ANIC (Vol.%)	SME (kJ/kg)	BPT (°C)	FMC (%)	ANIC (Vol.%)	SME (kJ/kg)	BPT (°C)
FMC (%)	*r*	1	0.096	0.643[b]	-0.695[b]	1	0.613[b]	0.778[b]	-0.690[b]
ANIC (Vol.%)	*r*	0.096	1	-0.118	0.074	0.613[b]	1	0.622[b]	-0.302[a]
SME (kJ/kg)	*r*	0.643[b]	-0.118	1	-0.440[b]	0.778[b]	0.622[b]	1	-0.527[b]
BPT (°C)	*r*	-0.695[b]	0.074	-0.440[b]	1	-0.690[b]	-0.302[a]	-0.527[b]	1

[a]The correlation is significant at a level of 0.05

[b]The correlation is significant at a level of 0.01

A Pearson correlation analysis revealed no statistically significant correlation between the frozen meat content and ANIC (**Table III-2**) at a frozen meat temperature of -6 °C ($r = 0.096$, $p = 0.578$), but a highly significant, positive correlation at a frozen meat temperature of -12 °C ($r = 0.613$, $p \leq 0.001$). Furthermore, a highly significant negative correlation between the frozen meat content and batch processing temperatures were found for both frozen meat temperatures (-6 °C: $r = -0.695$, $p \leq 0.001$; -12 °C: $r = -0.690$, $p \leq 0.001$, **Table III-2**), confirming the initial hypothesis of a reduced batch processing temperature at a higher frozen meat content. The nonsignificant correlation between the frozen meat content and ANIC might be attributed to the high variance of the ANIC data determined (**Appendix III-2**). This underlines the previous statement of Berger, Gibis, et al. (2022) that the histochemical determination of cell disintegration is not sensitive enough to characterize small, process-related product parameters properly. As the batch processing temperature is reduced at both frozen meat temperatures, it is assumed that the frozen meat content influences the batch processing temperature more strongly than the frozen meat temperature. However, the use of colder material leads to a greater batch processing temperature reduction (e.g. at 45 % frozen meat content: -1.22 ± 0.15 °C at T = -6°C and -1.90 ± 0.82 °C at T = -12 °C, **Figure III-3**), which alters the structure and the processability of the meat (Brown et al., 2005; Dobraszczy et al., 1987; King, 1999; Schnäckel et al., 2011). Comparing the enthalpy changes based on the reduced specific heat capacities of frozen meat with the changes based on reduced meat temperatures underlined the initial hypothesis. The enthalpy of the systems is reduced by a factor 2.1 due to reduced specific heat energy in frozen ($c_p \approx$ 1.67 kJ/kgK) (Marella and Muthukumarappan, 2013) compared to unfrozen meat ($c_p \approx$ 3.44 kJ/kgK) (Fellows, 2009), whereas the effect of the temperature reduction from -6 to -12 °C results in a nearly constant enthalpy (factor 1.02).

Changes in mechanical process control parameters

The SME and the grinding screw torque during the second grinding were determined to check the influence of the frozen meat content and temperature on the mechanical process control parameters and to determine the stress that the meat undergoes during processing.

The SME increased from 1.36 ± 0.06 kJ/kg at 0 % frozen meat content to 2.49 ± 0.48 kJ/kg at 45 % frozen meat content (T = -6 °C) (**Figure III-3B**) and to 3.25 ± 0.12 kJ/kg at 60 % frozen meat content (T = -12 °C) (**Figure III-3D**). It was reported that the freezing temperature of meat depends strongly on the freezing behavior of the free water fraction and the water, bound due to the hydration of biomolecules (van der Sman and Boer, 2005). Among other things, the size of those water fractions and the amount and molecular weight of solutes affect the glass transition temperature of the meat product and, thus, its freezing temperature (Brake and Fennema, 1999). Glass transition temperatures of meat were generally reported between -14 and -20 °C (Brake and Fennema, 1999). Based on this theory, it can be assumed that larger water fractions are frozen at colder freezing temperatures, resulting in a greater firmness of the meat. Consequently, meat at a frozen meat temperature of -12 °C contained more frozen water compared to that at a frozen meat temperature of 6 °C, which would explain the higher SME at colder frozen meat temperatures. The stronger SME increase at colder frozen meat temperatures might, therefore, be attributed to the higher cutting resistance of frozen meat, which would require more energy for comminution (Dobraszczy et al., 1987). These results are in agreement with the study of Dobraszczy et al. (1987), who showed that the work required to fracture increased strongly when the meat temperature was reduced from -5 to -15 °C. The higher SME can also be attributed to the colder batch processing temperature at higher frozen meat content described previously (**Figure III-3B** and **D**).

Figure III-4 shows the grinding screw torque during the second grinding. Similar to the SME, the torque increased with increasing frozen meat content from 24.72 ± 1.61 Nm at 0 % frozen meat content to 45.95 ± 4.87 kJ/kg at 45 % frozen meat content (T = -6 °C) (**Figure III-4A**) and to 53.43 ± 11.07 kJ/kg at 45 % frozen meat content (T = -12 °C) (**Figure III-4B**). As torque is a measure of the energy that the motor must apply to process the material (Voisey and deMan, 1970), a higher torque indicates a stronger load in the processed meat. This confirms the initial hypothesis of a higher load at higher frozen meat content. The increase is more pronounced at colder frozen meat temperatures, due to the higher cutting resistance mentioned previously. In addition, the grinding screw torques fluctuate more at higher amounts of frozen material, which can be attributed to an inhomogeneous material composition. This might lead, in addition, to an uneven stress distribution on the meat masses during processing.

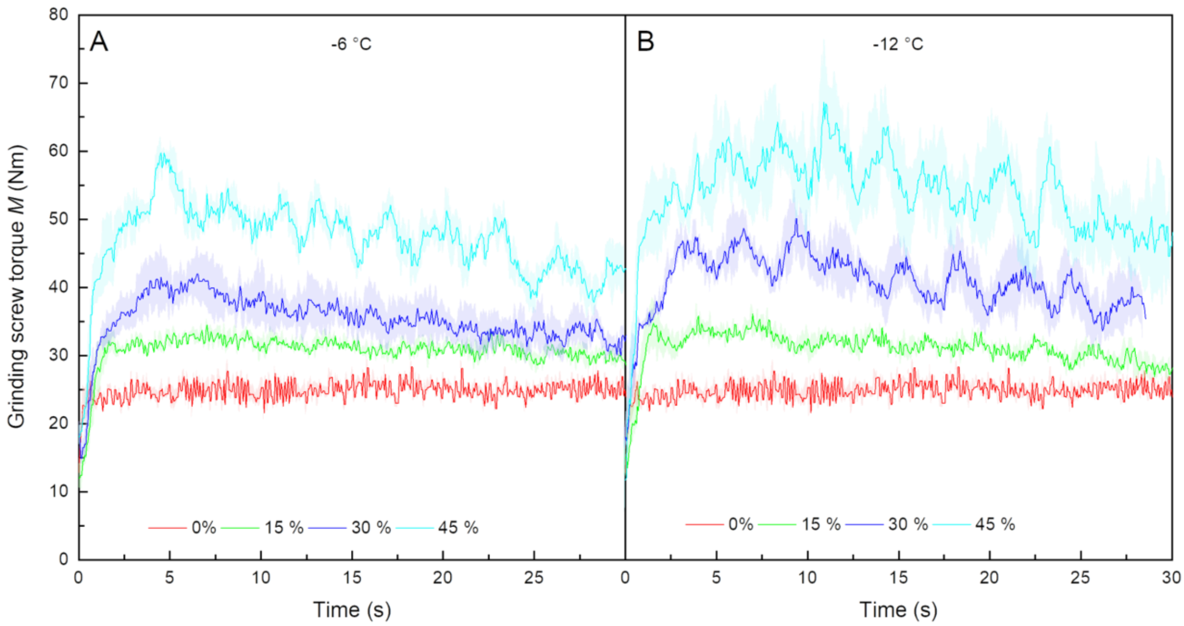

Figure III-4: Grinding screw torque *M* during the second grinding of meat masses with different frozen meat content of 0 – 45 % at frozen meat temperatures of -6 and -12 °C.

Influence on physicochemical and functional properties of hamburgers

Although the ANIC was only slightly affected by changes in frozen meat content and batch processing temperature (**Figure III-3**, **Table III-2**), the effects of increasing the frozen meat content on other physicochemical and functional parameters were more pronounced. Therefore, the hamburgers drip loss, firmness and hardness as well as the extracted meat solutions' MetMb and Mb content were determined.

The Pearson correlation analysis revealed a significant increase in the drip loss of hamburgers with a higher frozen meat content at a frozen meat temperature of -6 °C (r = 0.361, $p \leq 0.05$); at a frozen meat temperature of -12 °C the correlation was not significant (**Figure III-5A**). The two-factorial ANOVA also detected an influence of the frozen meat content ($p \leq 0.001$) on the drip loss, but no influence of the frozen meat temperature (p = 0.770). This indicates that hamburgers produced with a higher frozen meat content retain the moisture of the meat less effectively, which is in accordance with previous reports (Ballin and Lametsch, 2008). In addition, it was reported that moisture loss was enhanced by the broken cell structure due to ice crystal formation during freezing and was expected to cause quality and microbiological problems (Ballin and Lametsch, 2008; Rahelić et al., 1985).

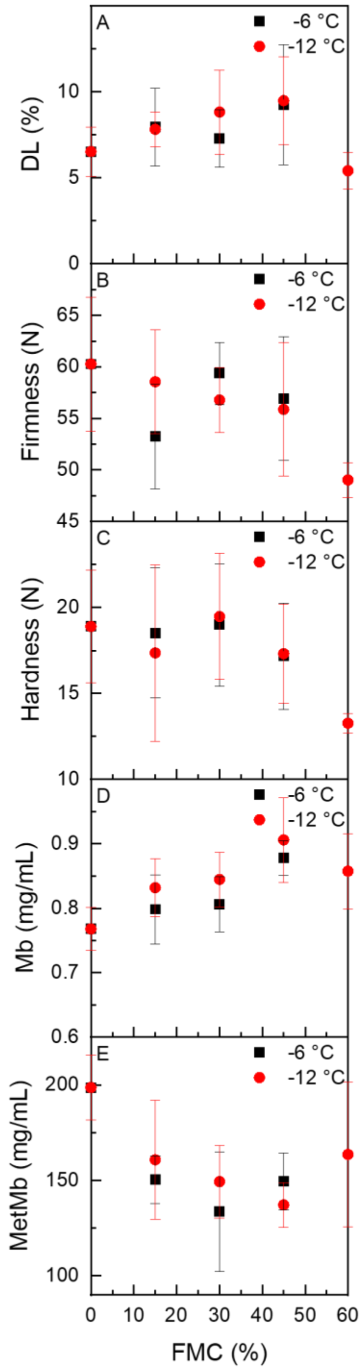

Figure III-5: Characterization of the physicochemical and functional properties (A) drip loss (DL), (B) firmness, (C) hardness, (D) myoglobin content (Mb), and (E) metmyoglobin content (MetMb) as a function of frozen meat contents (FMC) from 0 to 60 % and different frozen meat temperatures of -6 and -12 °C.

The firmness of the ground meat was significantly reduced by a higher frozen meat content at a frozen meat temperature of -12 °C (*r* = 0.532, $p \leq 0.001$), whereas a frozen meat temperature of -6 °C did not influence the firmness (**Figure III-5B**). This was supported by the two-factorial ANOVA, which revealed an influence of the frozen meat content ($p \leq 0.001$) but no influence of the frozen meat temperature (*p* = 0.802). The reduction in the sample's firmness at a higher frozen meat content might be attributed to the higher mechanical load acting on the meat during processing (SME, see **Figure III-3** and the higher ANIC (**Figure III-3**). Samples with a higher frozen meat content, thus, had less intact meat structures, for example, muscle fibers, but might contain a mixture of cell fragments and solubilized substances. The intermolecular interactions could be altered depending on the composition, so that the morphological structure would be modified, resulting in lower firmness (Berger, Gibis, et al., 2022). These results are in accordance with the previous findings of Berger, Gibis, et al. (2022), who found a reduced firmness when the amount of less intact substances incorporated into ground meat exceeds 25 %.

Similar to the firmness in the raw meat, the hardness, the force necessary to cut the fried hamburgers, was also significantly reduced with the increasing frozen meat content at both frozen meat temperatures (frozen meat temperature -6 °C: *r* = -0.178, $p \leq 0.001$; frozen meat temperature -12 °C: *r* = -0.380, $p \leq 0.001$) (**Figure III-5C**). The reduction was stronger at colder frozen meat temperatures due to the morphological changes at higher frozen meat content described previously and the resulting changes in intermolecular interaction and network formation after heat denaturation of the meat proteins.

The Mb and MetMb content of the extracted meat solutions were determined as a measure for chemical changes, such as oxidation. As expected, the Mb content was significantly increased with the increasing frozen meat content for both temperatures (frozen meat temperature -6 °C: *r* = 0.591, $p \leq 0.001$; frozen meat temperature -12 °C: *r* = 0.532, $p \leq 0.001$) (**Figure III-5D**), whereas the MetMb was significantly reduced (frozen meat temperature -6 °C: $r = -0.544$, $p \leq 0.001$; frozen meat temperature -12 °C: $r = -0.408$, $p \leq 0.001$) (**Figure III-5E**). Mb is an intracellular compound, thus, it is released upon intense processing and at higher ANIC (Trout, 1989; Tyszkiewicz et al., 1997). MetMb is an oxidative product of the Mb (Renerre et al., 1996). Intense processing also increased the amount of oxygen incorporated into the ground meat (McNeill et al., 1988), therefore, causing the oxidation of Mb to MetMb. It can, thus, be concluded that higher percentages of frozen meat content also intensify oxidation processes in the hamburgers, which can additionally negatively influence the quality of the hamburgers.

A sensory and optical evaluation was conducted to determine the influence on the quality perception, by evaluating the hardness, juiciness and texture, as well as the coarseness, amount of batter-like substance and the overall acceptance of the hamburgers. The results are depicted in **Figure III-6** for optical and sensorial assessment at both temperatures. **Figure III-6A** and **B** show that neither the increased frozen meat content nor the frozen meat temperature influenced the sensorial perception of the hamburgers significantly ($p \leq 0.05$). The optical appearance of the hamburgers was only slightly affected at higher frozen meat contents of 45 % (**Figure III-6C** and **D**). At -6 °C frozen meat temperature, the hamburgers with 45 % frozen meat content were rated significantly coarser and with less batter-like substance ($p \leq 0.05$). At -12 °C frozen meat temperature, the hamburgers with 0 % frozen meat content were rated as finer and containing more batter-like substance than the other samples ($p \leq 0.05$). Those results underline the previous findings of an increased ANIC and, thus, a finer appearance at higher frozen meat contents (**Figure III-3**). However, the optical overall acceptance was not affected by the changes in frozen meat content or temperature ($p \leq 0.05$).

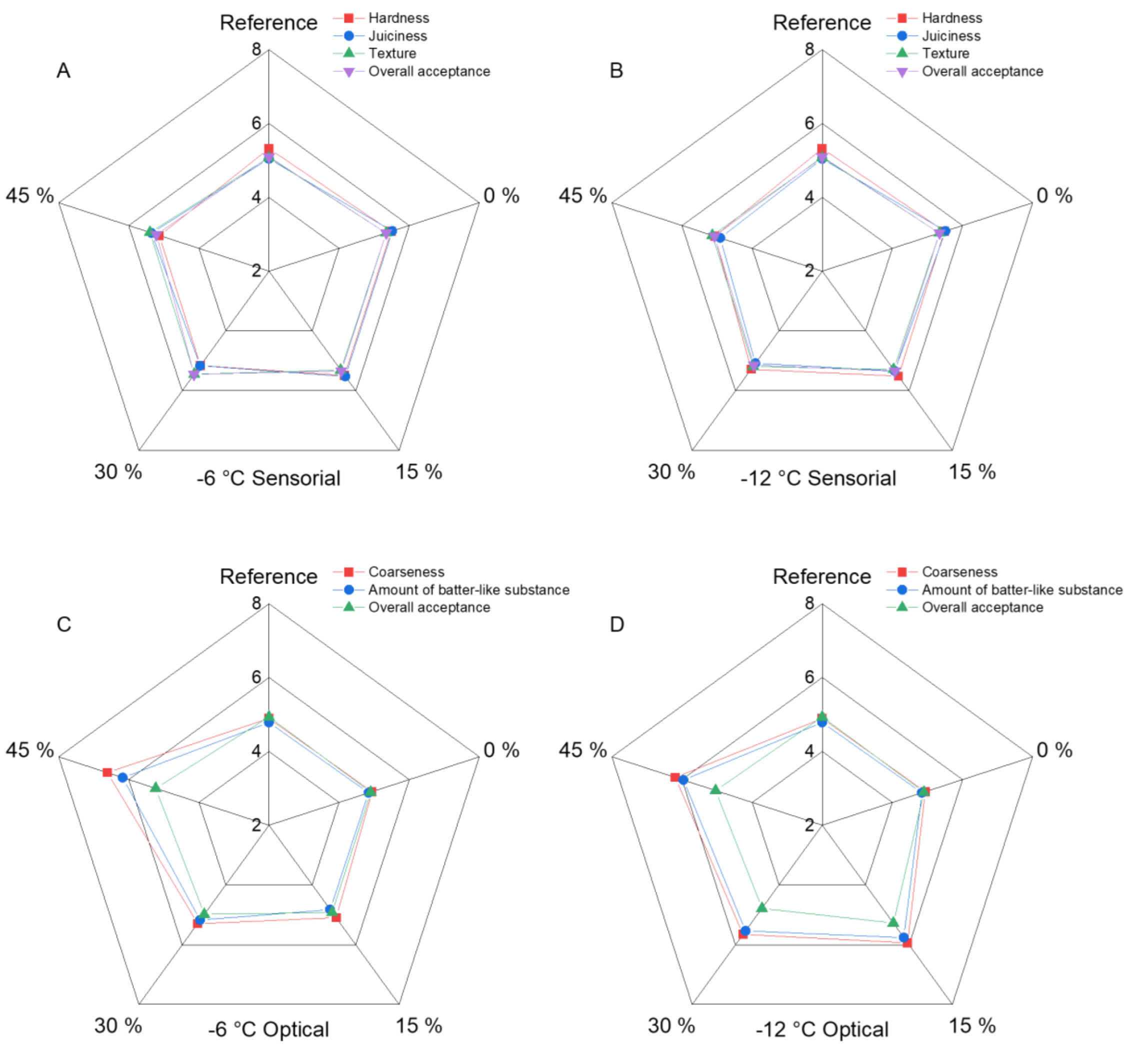

Figure III-6: Sensory evaluation of hamburgers in terms of hardness, juiciness, texture and overall acceptance with different frozen meat content (0 – 45 %) at frozen meat temperatures of (A) -6 °C and (B) -12 °C. Optical assessment of hamburgers halves in terms of coarseness, amount of batter-like substance and overall acceptance of hamburgers with different frozen meat content (0 - 45 %) at frozen meat temperature of (C) -6 °C and (D) -12 °C. Before each sensorial and optical evaluation, a reference (sensory score = 5) was provided to train the panelist. Sensory score 0 = harder/ dryer/ compacter/ worse/ finer/ more batter-like substance and 10 = softer/ juicer/ looser/ better/ coarser/ less batter-like substance.

Mechanistic consideration

The changes in structural, physicochemical and functional properties described previously, as well as the enhanced energy input and increased load during grinding can be attributed to structural changes in the meat upon freezing, as described earlier by Brown et al. (2005).

When lowering the meat temperature T_{meat} of the raw material, the properties of the meat were altered, having a great influence on its processability. **Figure III-7** summarizes the effect of different meat temperatures on the processability of the meat. Its viscoelastic properties changed from elastic to plastic, thus, strongly increasing the cutting resistance of the meat (Krickmeier, 2015). Thereby, the comminution behavior was shifted from a smooth cutting, where intramuscular substances acted as a lubricant between cutting edges of the knives and meat, to a brittle fracturing and splintering of the meat particles, ending up in more damaged cells (Krickmeier, 2015). Thus, more energy was required to comminute the meat at lower temperatures, as underlined in the higher SME (**Figure III-3**) and the higher grinding screw torque at a higher frozen meat content (**Figure III-4**).

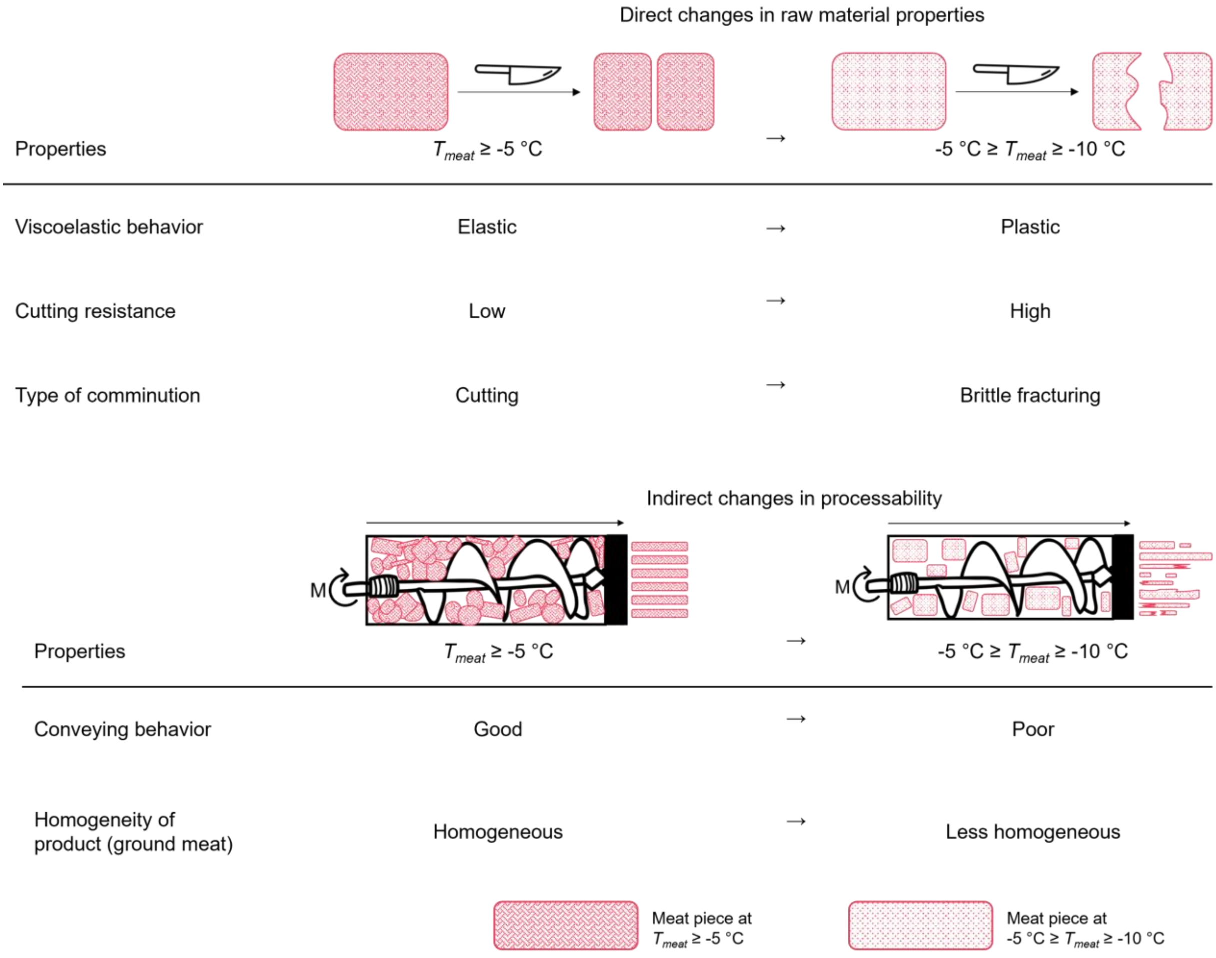

Figure III-7: Mechanical representation of direct and indirect changes in meat processing during grinding caused by different meat temperatures (T_{meat}).

The processability was altered due to the raw material changes upon freezing. The interaction of the meat and the grinder casing was reduced at lower temperatures, mainly due to its reduced elastic properties. As the interaction of the meat and the grinder were the basis of conveying in a free-flowing grinder, the conveying behavior was poorer at lower temperatures, resulting in a longer residence time of the meat in the grinder, as described in previous studies (Brown et al., 2005; Haack et al., 2007; Haack and Sielaff, 2005). Furthermore, the ground meat strands were irregular, more inhomogeneous and often smaller at lower meat temperatures (see **Appendix III-1**), caused by the brittle fracturing and the increased load acting on the meat during processing. Those inhomogeneities might be the reason for the altered physicochemical and functional properties of the samples shown in **Figure III-5**.

Conclusions

It was shown that the increasing frozen meat content caused a pronounced decrease in the batch processing temperature and, thus, an increase in the SME during the main grinding due to altered material properties. As initially assumed, structural and functional changes were found, influencing the quality of the product. The usage of a colder frozen meat temperature slightly increased the effects described, but not to the same extent as the frozen meat content, indicating a minor influence on the parameters analyzed in this study. It can be concluded from the current results that the frozen meat content is an important process parameter and can, therefore, be used for process control and adjustment in industrial hamburger production.

For the pilot plant scale meat grinder used in this study a maximum SME reduction of 1.89 kJ/kg caused by changes in the raw material led to a yearly cost savings of approx. 59 €. This is based on an assumed electricity price of 0.45 €/kWh and an average production time of 8 h/d for 250 d/a (= 2000 h/a) with a production volume of 1000 kg/d. Those numbers indicate that the influence on energy and cost savings during production are less significant, whereas the impact on the material properties is of higher importance. Considering that industrial meat grinders are larger than the pilot plant scale equipment used in this study (typically by a factor of 100), this would lead to higher energy and cost savings, which are however still not decisive.

Authors contribution

Lisa M. Berger, Kurt Herrmann: conceptualization; Lisa M. Berger, Carsten Böckle: data curation; Lisa M. Berger: formal analysis; Kurt Herrmann, Nino Terjung, Monika Gibis, Jochen Weiss: funding acquisition; Lisa M. Berger, Carsten Böckle, Kurt Herrmann: investigation; Lisa M. Berger: methodology; Lisa M. Berger, Kurt Herrmann, Nino Terjung, Monika Gibis, Jochen Weiss: project administration; Kurt Herrmann, Lisa M. Berger: resources; Lisa M. Berger: Software; Monika Gibis, Jochen Weiss, Kurt Herrmann: supervision; Lisa M. Berger, Monika Gibis: validation; Lisa M. Berger: visualization; Lisa M. Berger: writing - original draft; and Lisa M. Berger, Monika Gibis, Jochen Weiss: writing - review and editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Most data are included in the article, supplemental materials are available upon request.

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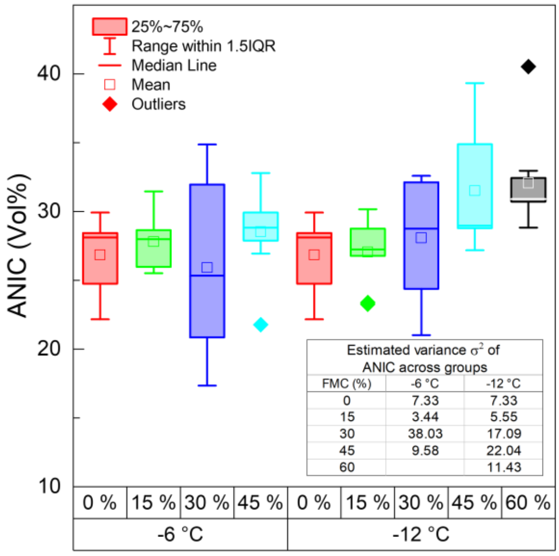
Appendix

Appendix III-1: Description and product photos of ground meat samples (2.4 mm) with different frozen meat content (FMC; 0, 15, 30, 45, and 60 %) of frozen meat at different frozen meat temperatures (FMT; -6 or -12 °C).

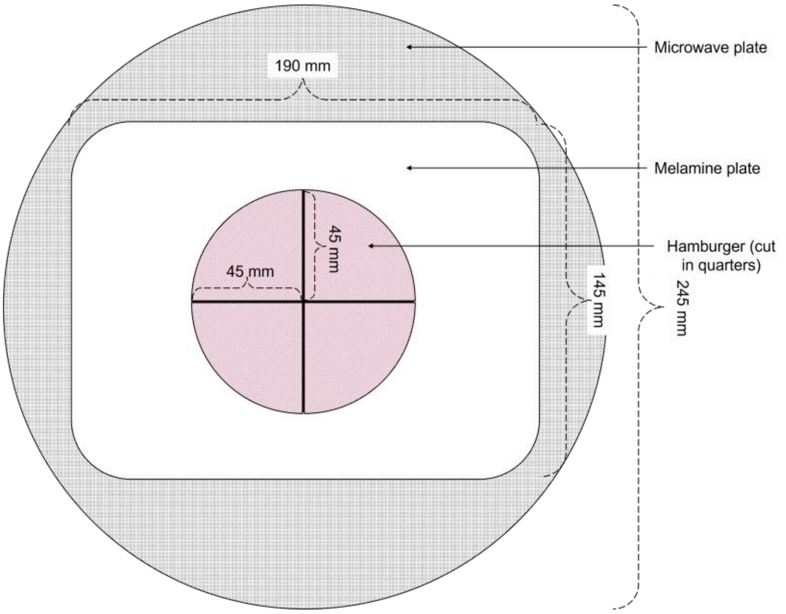
FMC (%)	FMT = -6 °C	FMT = -12 °C
0		
15		
30		
45		

60	n.d.	



Appendix III-2: Data distribution of amount of non-intact cells (ANIC) as a boxplot for samples with different frozen meat temperature (-6 or -12 °C) and frozen meat content (FMC) from 0 – 60 %; table in the lower right corner shows the variance of the data set within one group.

Supplementary data



Supplemental Data III-1: Reheating setup of the hamburgers in the microwave (P = 800 W, t = 45 s, T_{core} = 70 ± 2 °C)

Supplemental Data III-2: Influence of frozen meat content (0, 15, 30, 45, 60 %) and frozen meat temperature (-6 °C, -12 °C) on mean and standard deviation of batch processing temperatures (BPT) during hamburger production.

	BPT (°C)	
FMC (%)	*FMT* = -6 °C	*FMT* = -12 °C
0	2.80 ± 2.98	
15	-0.45 ± 0.47	-0.28 ± 0.75
30	-0.73 ± 0.38	-1.11 ± 0.25
45	-1.22 ± 0.15	-1.90 ± 0.82
60	n.d.	-1.36 ± 0.00

Supplemental Data III-3: Reproducibility of reheating in the microwave. Reheating test material (350 g water in cylindrical, borosilicate beaker (Borosilicate Glass 3.3 400 mL 213-1173, VWR, Darmstadt, Germany) with metallic spoon) at two different temperatures (ice water $T \approx 1.6$ °C, cold water $T \approx 16$ °C) was heated for $t = 1$ min at $P = 800$ W in a microwave (model HF15M541, Siemens Aktiengesellschaft, Munich, Germany), stirred and temperature was measured (testo 108, Testo SE & Co. KGaA, Tittisee-Neustadt, Germany). The actual power consumption and the actual current were recorded (Secutest S2 N+, Gossen Metrawatt GmbH, Nürnberg, Germany). Mean and standard deviation were calculated for reproducibility

	Ice Water $T \approx 1.6$ °C			Cold Water $T \approx 16$ °C		
Replication	ΔT_{water} (K)	P_{output} (W)	I_{output} (A)	ΔT_{water} (K)	P_{output} (W)	I_{output} (A)
1	22.1	1271.25	5.90	20.7	1281.00	5.96
2	21.2	1253.25	5.81	21.1	1276.50	5.86
3	21.3	1237.75	5.71	21.1	1255.75	5.80
4	21.0	1230.00	5.66	20.6	1247.50	5.76
5	20.7	1220.75	5.62	20.3	1241.50	5.72
Reproducibility (mean ± standard deviation)	21.26 ± 0.52	1242.60 ± 19.96	5.74 ± 0.11	20.76 ± 0.34	1260.45 ± 17.53	5.82 ± 0.09

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IV. Chapter:

Effect of Re-fed Meat in Beef Hamburger Production

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Abstract

Background

In food production, re-feeding material into the manufacturing process is common practice to meet ecological and economical requirements. In production of hamburgers, the products with external imperfections are re-fed in a frozen, coarsely crushed state.

Results

In this study, the influence of the addition of frozen, pre-crushed hamburgers (re-fed meat) during the manufacturing process on the structural, physico-chemical, functional, and quality attributes of the hamburgers was assessed. The recording of process control parameters showed no changes among the samples. It was further found that most of the studied parameters remained nearly unaffected by the addition of re-fed meat (RFM) up to 20 %. Neither the specific mechanical energy input during grinding, the histologically determined amount of non-intact cells, nor the sensory characterization of the samples differed strongly upon the addition of re-fed meat.

Conclusion

The results indicate that it is technologically feasible to re-feed unimpaired, high-quality material due to ecological and economic reasons and still maintain high product quality. However, to ensure product safety, microbiological and hygienic standards must be maintained and controlled during processing.

Keywords: Re-processed meat, ground meat processing, frozen meat, structural analysis, process analysis, upcycling

Introduction

Hamburgers are produced by grinding beef meat to approximately 2.4 – 3 mm particle size and forming it under pressure to obtain flat and round patties of different sizes. According to the German guidelines for meat and meat products, beef hamburgers are mainly produced from lean, connective tissue-reduced beef meat usually without the addition of other ingredients or spices (1). Typically, they contain around 20 % fat, as this is beneficial for sensory perception (2, 3). They further define, that the amount of non-intact cells (ANIC) in beef hamburgers is determined via histological analysis and limited to 20 Vol.% to ensure consistent quality (1, 4).

In ground meat production, proportions of frozen meat are used to maintain a low product temperature and to ensure microbial stability and processability (5-9). In industrial meat processing, some products show external deviations such as weight or size variations (8, 10). Since these products are still unimpaired, high-quality meat products are returned to the process in small quantities up to 2 %, due to ecological and economic reasons (10). In meat grinders, the raw material transfers energy between material, grinding screw, and housing and thus enables the conveying (5). Changes in raw material properties, such as temperature, composition, and pre-cutting size, are therefore responsible for energy consumption and force generation during the grinding (11, 12). It is known, that the increased cutting resistance in frozen material increases the cutting and friction forces during grinding (13, 14), due to changed viscoelastic properties (6, 13, 14).

According to the German guidelines for meat and meat products (1, Section I, No. 2.18) the re-use of meat in meat products is allowed, if guidelines are adhered to, generally meaning that the product must not differ in analytical a sensorial perception. Structural changes of meat in mechanically deboned meat were studied before (15) and changes in the histochemical assessed product structure and sensory perception were reported. In their study, Upmann, Hölscher, et al. (10) found, that the incorporation of rework in boiled sausage manufacturing has no negative impact on the quality of the product. Upon rework addition up to 3.6 %, the color, hardness, and sensory perception remained comparable.

In contrast to the present study, Upmann, Hölscher, et al. (10) used cooked sausages with an elastic behavior. However, different results were hypothesized for re-feeding frozen hamburgers. Thus, the study aimed to check the influence of the usage of re-fed meat (RFM) in hamburger production. To describe the changes holistically, process-related parameters, structural, functional, and quality parameters were determined for different proportions of

RFM. It was assumed, that higher proportions of frozen, RFM might increase the load during grinding, as frozen material exhibits higher cutting resistance (5). In addition, a higher amount of non-intact cells was assumed, due to the double-processing of parts of the meat. Thus, the structural, functional, and quality parameters were expected to deviate accordingly, as previously described by Berger, Gibis, et al. (16).

Materials and Methods

Materials

The beef was purchased from MEGA (MEGA – Das Fachzentrum für die Metzgerei und Gastronomie eG, Stuttgart, Germany). Cuts from the flank of heifers were visually standardized by removing excessive material and adjusting 20 % fat content and manually precut into cubes of approximately 5 x 5 x 5 cm. 25 % of the meat cubes were frozen ($T \approx$ -18 °C) overnight, and 75 % were chilled ($T \approx$ 1 °C) to ensure microbiological stability during processing. Prior to the first grinding step, the frozen and chilled meat were mixed in a horizontal mixer (Paddle mixer type RC-40, Mainca, Barcelona, Spain) at 32 rpm for 30 s clockwise and 30 s counterclockwise rotation. A meat grinder (Fleischwolf model AE 130, Maschinenfabrik Seydelmann KG, Aalen, Germany) equipped with a 3-part grinding system (pre cutter with plastic spacer, 4-wing knife (TC93094), 13 mm inclined perforated end plate (3090278.1) (turbocut Jopp GmbH, Bad Neustadt, Germany)) was used for the first grinding step to a particle size of 13 mm. To obtain the RFM, hamburgers were produced according to the standard production process (**Figure IV-1**) without the addition of RFM and stored at -18 °C for one week before precutting the frozen hamburger with a 3-part grinding system to 16 mm particle size (pre cutter with plastic spacer, 4-wing knife (TC93094), 16 mm inclined perforated disc (3090279) (turbocut Jopp GmbH, Bad Neustadt, Germany)). The settings at the grinder were fixed in all grinding steps with rotational speed of 20 rpm at the feeding screw and 187 rpm at the grinding screw.

Samples with 0 %, 2.5 %, 5 %, 10 %, 15 %, and 20 % RFM content were produced by separated mixing (32 rpm for 30 s in each direction) of ground meat (13 mm) and ground RMF (16 mm), temperatures of approx. -0.5 °C were adjusted before grinding. Samples were separately ground to 2.4 mm particle size with a hanging 3-part grinding system (plastic spacer, 4-wing knife (TC93094), 2.4 mm inclined perforated end plate (3093457.1) (turbocut Jopp GmbH, Bad Neustadt, Germany)) at a feeding screw speed of 20 rpm and grinding screw speed of 187 rpm. The mixed samples were then separately formed using a modified hamburger press (MH-100 (modified), Equipamientos Cárnicos, S.L. (Mainca), Barcelona, Spain) into hamburgers of m = 90 g weight, h = 0.9 cm height, and d = 10 cm diameter by applying p = 5 bar forming pressure. The production scheme is summarized in **Figure IV-1**.

Samples were stored in sealed polyethylene bags at chilled conditions ($T \approx$ 1 °C) or vacuum-packed (C400, Multivac, Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany) in a frozen state ($T \approx$ -18 °C) until further analyses. Three independent experiments (= biological replicates) per sample variation with a batch size of 10 kg were conducted.

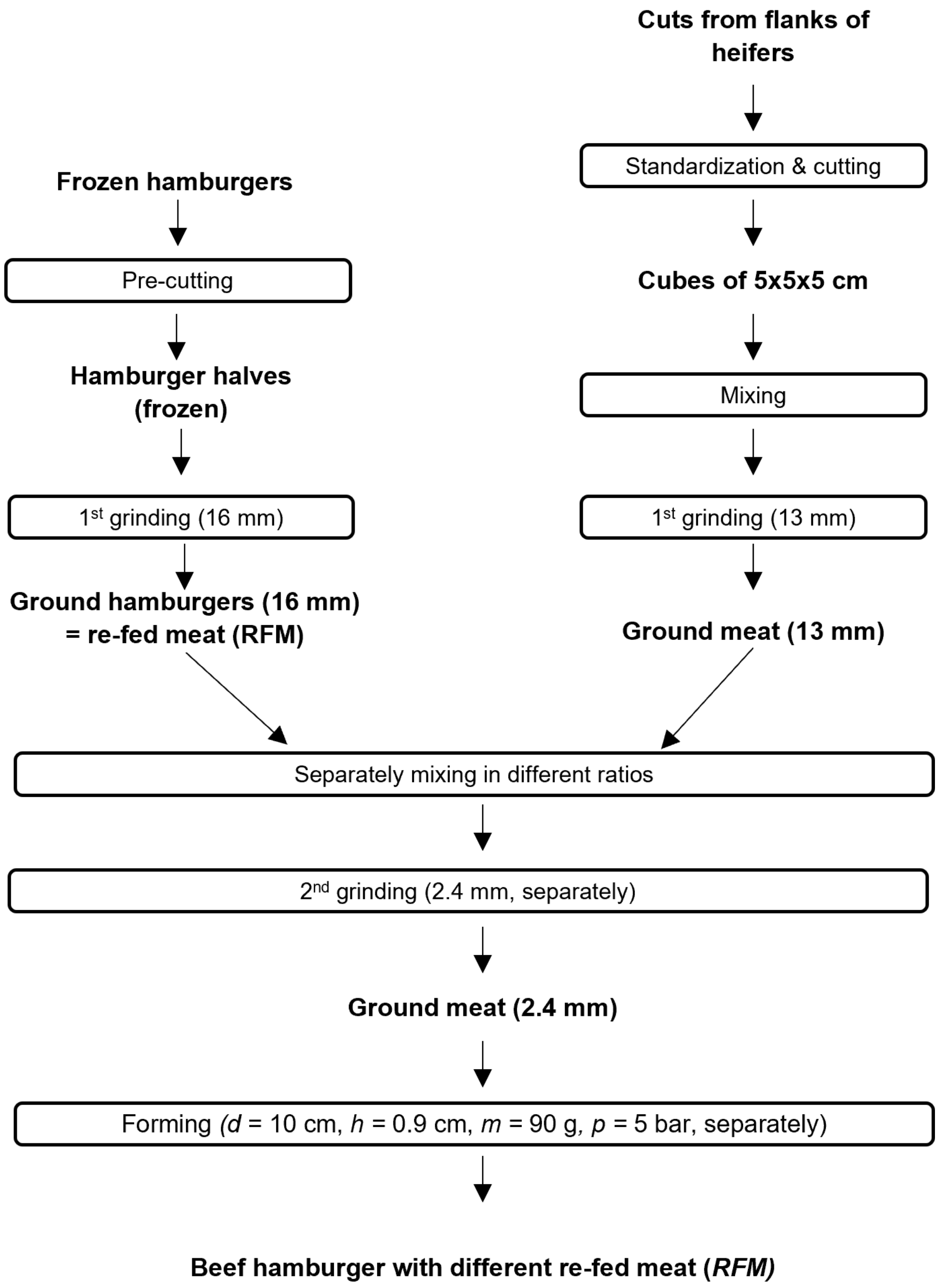

Figure IV-1: Flow chart of hamburger manufacturing of the samples containing different shares of re-fed meat (RFM).

Methods

Chemical composition

The determination of the chemical composition was performed as previously described by Berger, Gibis, et al. (16). In short, one hamburger per batch was homogenized for 30 s in a blender (Blixer® 2, Robot-Coupe, Montceau-en-Bourgogne, France) before the water, fat, and protein determination. The moisture content of the meat mass was determined by the sea-sand method (§64 LFGB method L 06.00-3) (17), the fat content by Soxhlet extraction (Büchi 810, Büchi Laboratoriums-Technik AG, Flawil, Switzerland) (17) from the residues of the moisture determination (§64 LFGB method L 06.00-6) (17), and the protein content by the Dumas' combustion method (§64 LFGB method L 06.00-20, Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) (17) using nitrogen to protein conversion factor of 6.25 (18).

Histochemical analyses

The amount of non-intact cells (ANIC) in the hamburgers were histochemically assessed according to the previously described method by Berger, Gibis, et al. (2022) and Berger, Witte, et al. (2022) following the official guidelines mentioned in § 64 LFGB (L 06.00-13) (17). In short, thin cryo-cut cross sections of the hamburgers were assessed for non-intact cells by the point-count method. Following, the ANIC was calculated. At least n = 6 cross-sections per sample were analyzed, wherefrom two cross-sections each were averaged. The mean and the standard deviation of the three repetitions were calculated.

Determination of Drip Loss

The hamburgers drip loss was determined via differential weighing before and after centrifugation of 10 g sample for 20 min at 5 °C and 16,000 rpm (Centrifuge Z32HK, Hermle Labortechnik GmbH, Wehingen, Germany) as previously described by Berger, Gibis, et al. (16).

Determination of Firmness by Forward Extrusion

The firmness of the ground meat was determined by the forward extrusion method recently described by Berger, Gibis, et al. (16) by pressing ground meat through a cylinder (d = 50 mm) with a 7.5 mm hole opening using a texture measurement device (Instron, Model 3365, Instron Engineering Corporation Ltd, Massachusetts, USA) thereby recording the required force.

Preparation of Extracted Meat Solution

Extracted meat solutions of all samples were produced according to the procedure of Berger, Witte, et al. (19) by diluting meat 1:10 with 10 mM potassium phosphate buffer (pH 7, T = 2 °C), and subsequent filtration. Extracts were stored cooled in brown glass bottles until further usage.

Determination of Soluble Protein Content

To quantify the amount of soluble protein in the extracted meat solution, rapid nitrogen analysis according to Dumas' combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) was applied using nitrogen to protein conversion factor of 6.25 Mariotti, Tomé, et al. (18).

Determination of Myoglobin (Mb) and Metmyoblobin (MetMb) content

The oxidative changes of the pigments were assessed by photometrical determination of the myoglobin (Mb) and metmyoglobin (MetMb) content in the respective extracted meat solution, following the previously described method of Berger, Gibis, et al. (2022) (MetMb determination) and Berger, Witte, et al. (2022) (Mb determination).

Determination of Cooking Loss

The cooking loss of the hamburgers was assessed by differential weighing of the hamburgers before and after frying. Frozen hamburgers were grilled on an electric dual slide contact grill (XPE-24 model, Garland Commercial Ranges Ltd., Mississauga, Ont., Canada) for 130 s with an upper plate temperature of 218 °C and a lower plate temperature of 177 °C until a core temperature of 80 °C.

Determination of Hardness

The fried hamburgers were cooled down to room temperature, and stripes of 1.5 cm in width were manually cut from the center. The hardness of the grilled hamburger was determined using a texture analyzer (Model 3365, Instron Engineering Corporation Ltd., Massachusetts, USA) equipped with a V-shaped Warner-Bratzler guillotine (crosshead speed = 250 mm/min).

Sensory evaluation

The sensory evaluation was performed in terms of gustatory and optical properties of the fried hamburgers using a 10-point rating scale, where a score of 5 points represents the compared reference standard ("Ref."). The gustatory determination was performed by reheating cooled samples with a microwave (model HF15M541, Siemens Aktiengesellschaft, Munich, Germany) to a core temperature of T = 80 °C and the hardness, juiciness, texture, and overall acceptability of the hamburgers were assessed. For optical assessment, the hamburgers were cut cross-sectional with a slicer (Bizerba VS8A, Wilhelm Kraut GmbH & Co. KG, Balingen, Germany), and the inner structure was evaluated in terms of the coarseness of the particles, amount of batter-like substance, and overall acceptability. At least 18 panelists of different genders, ages and backgrounds participated in each repetition, so that the sensory test was diversified, and errors could be reduced. Informed consent was obtained from each panelist prior to their participation in the study.

Process Control Parameters

The grinding screw torque during second grinding was separately recorded by the grinder software (S7-To-Excel Tool - Expert - for Windows, Träger Industry Components, Weiden, Germany) for all batches. The idle torque of the grinding screw was previously determined to be M = 6.9 ± 1.1 Nm (data not shown). For each batch, the temperature was manually measured before grinding (testo 926, Testo SE & Co. KGaA, Tittisee-Neustadt, Germany ($n \geq 5$) and the mass flow ($n \geq 3$) was manually determined by weighing the amount of ground meat within 15 s.

Determination of Specific mechanical energy input (SME)

The specific mechanical energy input was determined following the equation IV-1 and IV-2 (20-22) and is used as a measure for the stress acting on the sample during second grinding.

$$SME = \frac{P}{\dot{m}} \qquad \text{IV-1}$$

$$P = (M - M_{empty}) \cdot n_{rad} \qquad \text{IV-2}$$

With P = average power during grinding process (W), M = average grinding screw torque during grinding (Nm), M_{empty} = idling torque of grinding screw (Nm), $\dot{m} = \frac{m}{t}$ being the mass flow (kg/s), $n_{rad} = \frac{2\pi \cdot n_{grinding\ screw}}{60}$ being the radial rotational speed of grinding screw (s^{-1}), $n_{grinding\ screw}$ = rotational speed of grinding screw (s^{-1}). The average torque during grinding is determined as the torque during the plateau phase of grinding.

Statistical Analyses

Three independent experiments (biological replicates) with at least three analytical replicates (technical replicates) were performed. Results are reported as mean ± standard deviation or mean ± standard error (torque profiles), both calculated using MS Excel (Microsoft, Redmond, WA, USA). Statistical analyses were conducted by SPSS (IBM SPSS Statistics 25, IBM Deutschland GmbH, Ehningen, Germany) and plots were prepared by OriginPro 2020 (OriginLab Corporation, North Hampton, MA, USA). Normal distribution of data was tested by Shapiro-Wilk, variance homogeneity by Levene test. If data show normality and variance homogeneity, a significance analysis by univariant ANOVA (analysis of variance) was performed, applying the Tukey posthoc test (confidence interval of 95 % ($\alpha = 0.05$)). The Welch-ANOVA (analysis of variance) was conducted for data without variance homogeneity using the Games-Howell posthoc (confidence interval of 95 % ($\alpha = 0.05$)). Small letters attached to the mean value of the sample indicate statistical significance.

Results and Discussion

Chemical composition

To ensure constant raw material properties, the chemical composition of the meat of all three replications were chemically analyzed. The hamburger contained 62.2 ± 0.6 % moisture, 18.1 ± 1.1 % fat, 19.560 ± 0.325 % protein, and 0.87 ± 0.03 % ash, thus the samples were comparable in terms of chemical composition. The hamburgers were produced following the German guidelines for meat and meat products, and no salt, spices, or other ingredients were added (1).

Structural changes

The amount of non-intact cell (ANIC) was determined histochemically to check the influence of different amounts of RFM during manufacturing on the hamburger structure. **Figure IV-2** shows the influence of increasing RFM on the hamburgers ANIC. The ANIC of the samples remained nearly constant between 26.68 ± 2.61 Vol.% (0 % RFM) and 28.92 ± 5.69 Vol.% (20 % RFM). As indicated, no statistical difference was found between the samples. These findings stood in contrast with the initially hypothesized increase of ANIC with increasing RFM. Although the variance of the data (**Figure IV-2**) limits the accuracy somewhat, conclusions can be drawn. Originally, an accumulation of the grinding effects and therefore an increase of the ANIC was assumed when already produced, frozen hamburgers are re-fed to the process. **Figure IV-2** however, illustrates that this is not the case in this study. This can be attributed to the already small particle size of 2.4 mm of the RFM from the original production, and possible temperature equilibrium effects, shifting the characteristics of the material from plastic to elastic (5).

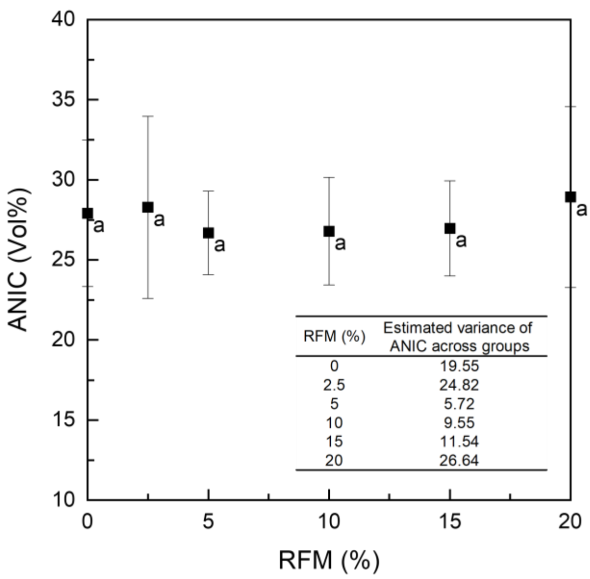

Figure IV-2: Amount of non-intact cells in beef hamburgers as a function of different amounts of re-fed meat (RFM). Data points with different superscript letters are significantly different ($p \leq 0.05$).

Changes in process control parameters

To check the influence of an increased RFM content and to characterize the mechanical load acting on the meat during second grinding, the specific mechanical energy input (SME) was calculated for each sample and the torque during second grinding was recorded. **Figure IV-3** displays the SME as a function of increasing RFM. The SME ranges from 2.10 ± 0.67 kJ/kg at 20 % RFM to 2.41 ± 0.83 kJ/kg at 2.5 % RFM. Statistical analysis revealed only small differences among the samples. The results indicate that a higher proportion of RFM did not increase the load acting on the meat during grinding as expected. The slight fluctuations between the SME of the samples can be attributed to the manual determination of the mass flow, which is a major factor in the calculation of the SME. The results contrast with the initial hypothesis but underline the insights of the structural analysis (**Figure IV-2**). As there were no changes in the load during grinding, the comparable structural properties are reasonable. The same accounts for the grinding screw torque, which is depicted in **Figure IV-3**. For all six samples, the grinding screw torque was at a comparable level of approximately 25 Nm without any major fluctuations being observed.

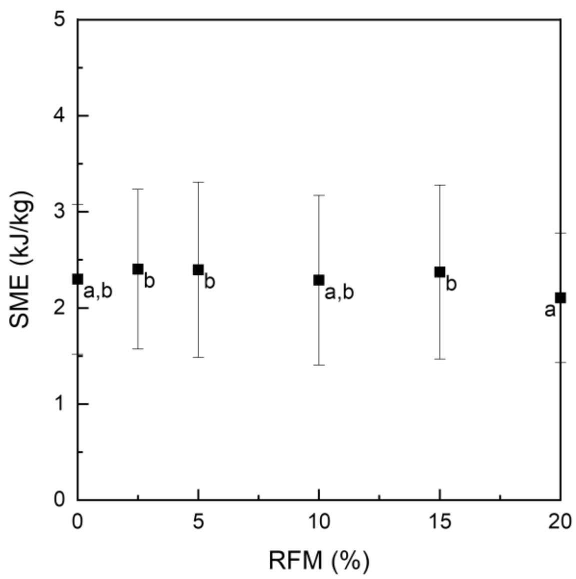

Figure IV-3: Specific mechanical energy input during 2nd grinding of samples containing different amounts of re-fed meat (RFM). Data points with different superscript letters are statistically significantly different ($p < 0.05$).

Influence on physico-chemical, functional, and quality properties of hamburgers

To check on possible changes in physico-chemical and functional properties of the hamburgers upon RFM increase, the raw samples drip loss (DL) and firmness, the extracted meat solutions soluble protein content (SPC), the myoglobin (Mb) and metmyoglobin (MetMb) content and the fried hamburgers cooking loss (CL), shrinkage and hardness were determined.

Table IV-1 summarizes the results of the analyses. It is shown that none of the determined characteristics significantly changed with increasing RFM content. These results are in accordance with the constant ANIC (**Figure IV-2**), mechanical load during grinding, specified as SME (**Figure IV-3**), and grinding screw torque (**Figure IV-4**).

Table IV-1: Characterization of physico-chemical and structural changes in hamburgers as a function of the amount of re-fed meat (RFM) in terms of drip loss (DL), firmness, soluble protein content (SPC), myoglobin (Mb) and metmyoglobin (MetMb) content, cooking loss (CL) and hardness. Superscript letters indicate statistical significance ($p < 0.05$).

	Raw hamburgers		Extracted meat solution			Fried hamburger	
RFM (%)	DL (%)	Firmness (N)	SPC (%)	Mb (mg/mL)	MetMb (mg/mL)	CL (%)	Hardness (N)
0	7.15 ± 0.37[a]	64.52 ± 15.96[a]	0.59 ± 0.06[a]	0.76 ± 0.02[a]	184.63 ± 22.77[b]	29.04 ± 3.69[a]	14.82 ± 3.29[a]
2.5	6.64 ± 2.27[a]	60.71 ± 9.29[a]	0.55 ± 0.02[a]	0.76 ± 0.01[a]	125.85 ± 32.47[a]	27.84 ± 3.12[a]	15.33 ± 1.88[a]
5	7.58 ± 2.12[a]	60.11 ± 13.42[a]	0.58 ± 0.06[a]	0.80 ± 0.02[a]	128.08 ± 19.22[a]	30.63 ± 0.76[a]	15.89 ± 4.56[a]
10	7.26 ± 2.16[a]	60.27 ± 8.95[a]	0.58 ± 0.07[a]	0.78 ± 0.04[a]	136.58 ± 45.79[a,b]	30.16 ± 0.82[a]	16.10 ± 7.25[a]
15	7.04 ± 1.24[a]	61.12 ± 8.09[a]	0.58 ± 0.04[a]	0.78 ± 0.02[a]	113.96 ± 15.55[a]	31.23 ± 1.46[a]	17.12 ± 6.79[a]
20	6.64 ± 1.79[a]	62.62 ± 9.90[a]	0.59 ± 0.07[a]	0.79 ± 0.04[a]	123.25 ± 22.97[a]	31.03 ± 0.78[a]	15.93 ± 5.64[a]

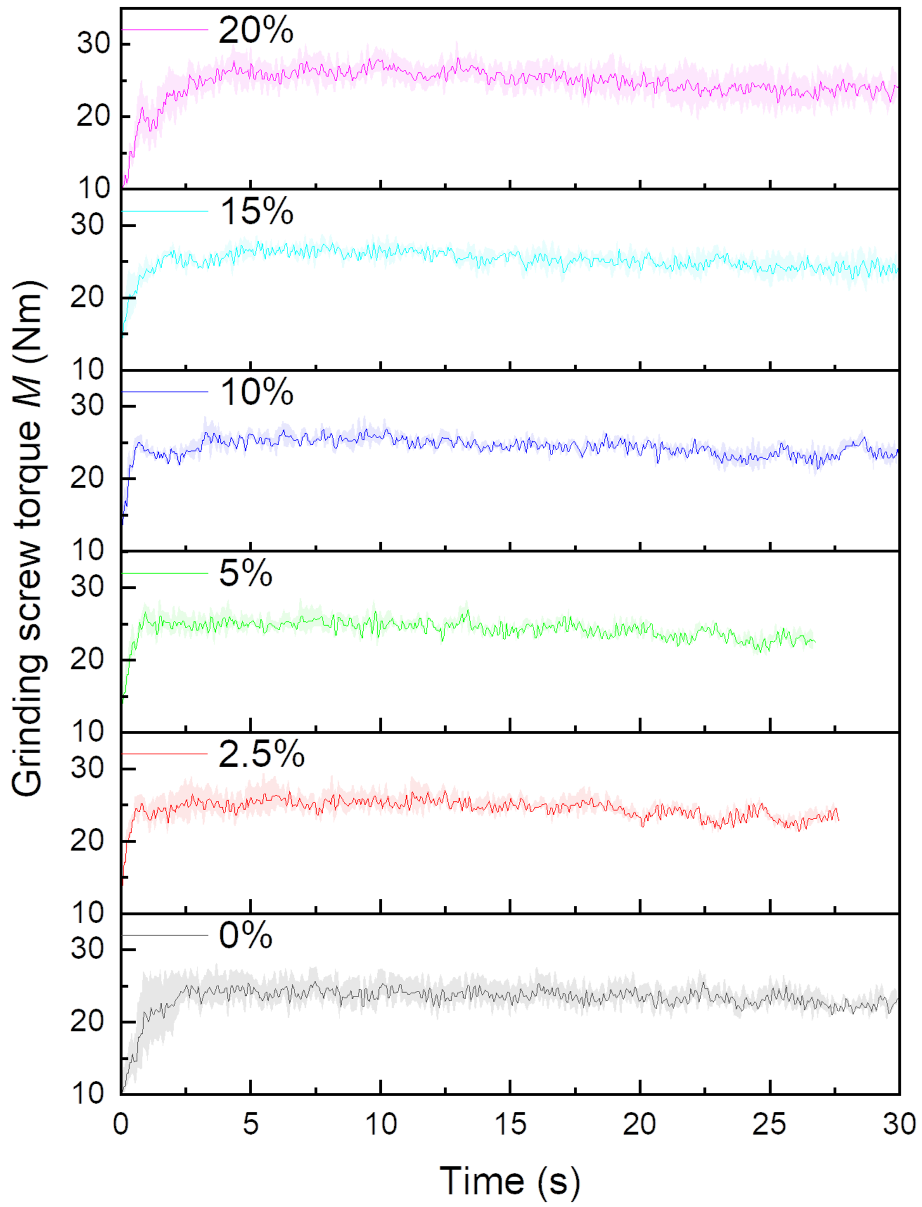

Figure IV-4: Grinding screw torque during second grinding of samples containing different amounts of re-fed meat (RFM).

Based on the literature on water holding capacity in meat, it was assumed, that the drip loss increased with an increasing amount of RFM, due to the higher amounts of freeze-damaged cell structures, which cause leakage of intracellular substance (23, 24). In contrast to the literature, no statistically significant influence on the DL was determined in the present study, as the DL remained nearly constant at around 6.64 – 7.58 % (**Table IV-1**) for all samples.

The firmness of the raw hamburgers was expected to increase with an increasing amount of RFM. As already described by Berger, Gibis, et al. (16), the firmness of a ground meat mass changes with increasing ANIC due to altered intramolecular interactions. As the ANIC, which describes the physical state of the muscle cells and thus their possibility to interact, remained constant in this study, also no changes in the firmness can be detected.

The parameters detected in the extracted meat solution also remained nearly constant, except for the MetMb, which showed a slight statistical deviation of the sample containing 0 % RFM. The SPC leveled around 0.55 – 0.59 %, the Mb content around 0.76 – 0.8 mg/mL, and the MetMb content around 113.96 – 184.63 mg/mL for all samples. The reason for the slightly increased MetMb content at 0 % RFM might be due to the absence of frozen material, which is known to limit oxidation reactions (25, 26). Further, the ground meat with 0 % RFM has a looser structure, which allows a more intense oxygen contact. Based on the initially expected increase in ANIC upon RFM, the parameters SPC, Mb, and MetMb were expected to rise similarly. Following the literature, increased ANIC leads to increased leakage of intracellular compounds (23, 27), which was expected to cause higher SPC, as well as enhance the oxidation of myoglobin to metmyoglobin. Here a pronounced effect was expected, as more oxygen might be introduced by the multi-processing of the meat, whereby oxidation reactions can take place more intensively (28). This effect was not detected in this study and can be explained by the constant SME during grinding (**Figure IV-3**).

The parameters cooking loss (CL) and hardness were determined in the fried hamburger, but no statistical differences were detected between the samples due to the above-mentioned reasons, although both parameters were expected to rise upon RFM addition (29). The CL of about 30 % (**Table IV-1**) is comparable to previously determined values of Berry (29) (36 – 39 %) and Offer, Knight, et al. (30) (up to 40 %). The hardness ranged around 16 N for all tested samples (**Table IV-1**). The measurements showed that in addition to the physico-chemical and functional properties, also the quality parameters of the hamburgers are unaffected by the addition of the RFM. The findings of the current study are in line with the findings of Upmann, Hölscher, et al. (10), who found no reduction in the enjoyment value with increased rework addition in cooked sausages.

The sensorial perception of hamburgers can be related to their quality and depend on the meat's cellular structure (5, 31). The parameters hardness, juiciness, texture, and overall acceptance fluctuated by about 1 point from the standard (**Figure IV-5A**). This indicates that the hamburgers gustatory assessment revealed no strong deviation of the samples containing RFM from the previously defined standard. Thus, the addition of up to 20 % RFM could not affect the gustatory quality of the hamburgers in this study. The same effects were detected in the optical assessment of the hamburger, as the coarseness, amount of batter-like structure, and overall acceptance were rated comparable to the standard hamburger (**Figure IV-5B**). Thus, also the optical quality of the hamburgers was not affected by an increased RMF content. Cardoso, Mendes, et al. (32) reported comparability of human sensory testing and mechanical texture analyses, which can be underlined by the results of this study. The mechanically determined hardness via the Warner-Bratzler method (**Table IV-1**) revealed comparable results to the sensory evaluated hardness (**Figure IV-5A**). The sensory results are also following the previously described effects on parameters such as ANIC and SME. If the stress acting on the meat during processing (SME) and thus the ANIC remains constant upon RFM increase, also the sensorial properties are not expected to change. It was reported that changes in the cell destruction influence the sensory perception of meat products (15). Due to constant ANIC, no changes in the sensory were expected in this experiment. This is in accordance with the findings of Upmann, Hölscher, et al. (10), who detected no reduction in the enjoyment value with increased rework addition in cooked sausages.

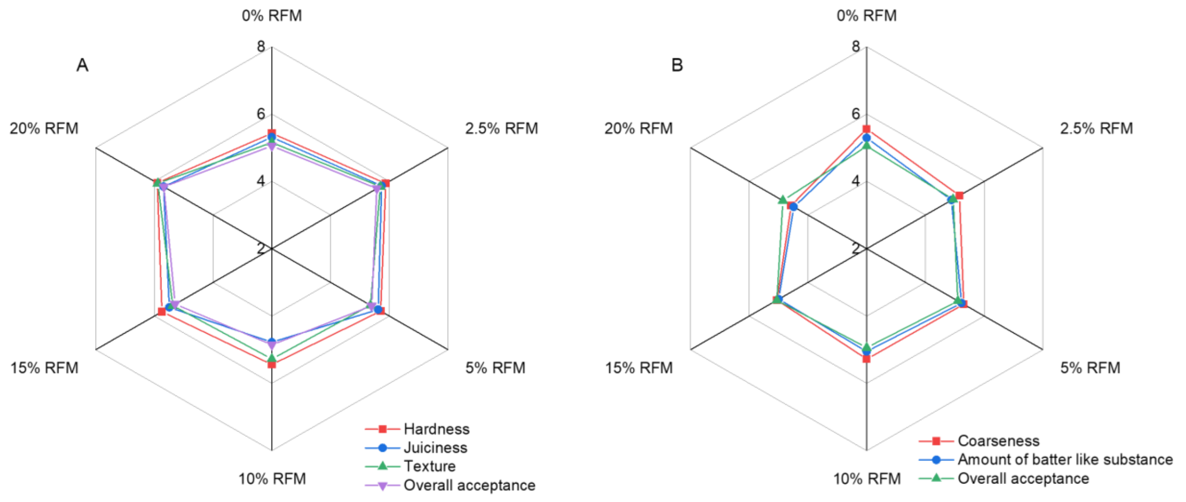

Figure IV-5: (A) Sensory evaluation in terms of hardness, juiciness, texture and overall acceptance and (B) optical assessment in terms of coarseness, amount of batter like substance and over all acceptance of hamburgers with different amounts of re-fed meat (RFM) in comparison to a reference (sensory score = 5). Sensory score 0 = harder/ dryer/ compacter/ worse/ finer/ more batter like substance and 10 = softer/ juicer/ looser/ better/ coarser/ less batter like substance.

Mechanistic consideration

The non-significant influence of the RFM addition on the product properties might be attributed to three main reasons:

(I) Formation of smaller fragments and mixed mass bulk properties: Due to their plastic properties, pre-grinding of the frozen RFM causes splintering and results in the formation of small fragments. After mixing with cooled, elastic material, these fragments are incorporated into the overall system. These fragments with plastic behaviour are thus suspended in the elastic mass and can move freely. It is therefore assumed, that if force is applied to the system, the firmer fragments split aside so that the force acting on the particles is absorbed by the total mass. Therefore, the frozen particles of the hamburgers are no longer subjected to so much stress during the grinding process.

(II) Pre-grinding particle size: Due to previous processing, RFM already has a particle size of about 2.4 mm and smaller. It is assumed, that they could pass through the end-hole plate without being subjected to greater stress since its diameter is smaller than that of the end-hole plate and the rest of the material.

Temperature equalization: Temperature equalization between the frozen and cooled material could leads to softening of the frozen particles, which would reduce the load during grinding (5) and lead to a more uniform grinding result.

Conclusion

There is no sensorial or qualitative disadvantage for the consumers when high-quality meat is fed-back into the production process of the hamburgers up to an amount of 20 %. Based on these findings, from a technological point of view, manufacturers can re-feed products, that were separated due to external deviations, without having to accept a loss in quality or functionality of the hamburgers. In any case, it is of the highest importance to take hygienic standards into account and ensure microbiological stability. Re-feeding unimpaired, high-quality material helps to meet ecological and economical requirements and contributes to more efficient and sustainable food production.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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Third section: Process-Function Relationship in Ground Meat

V. Chapter:
Influence of Processing Steps on Structural, Functional, and Quality Properties of Beef Hamburgers

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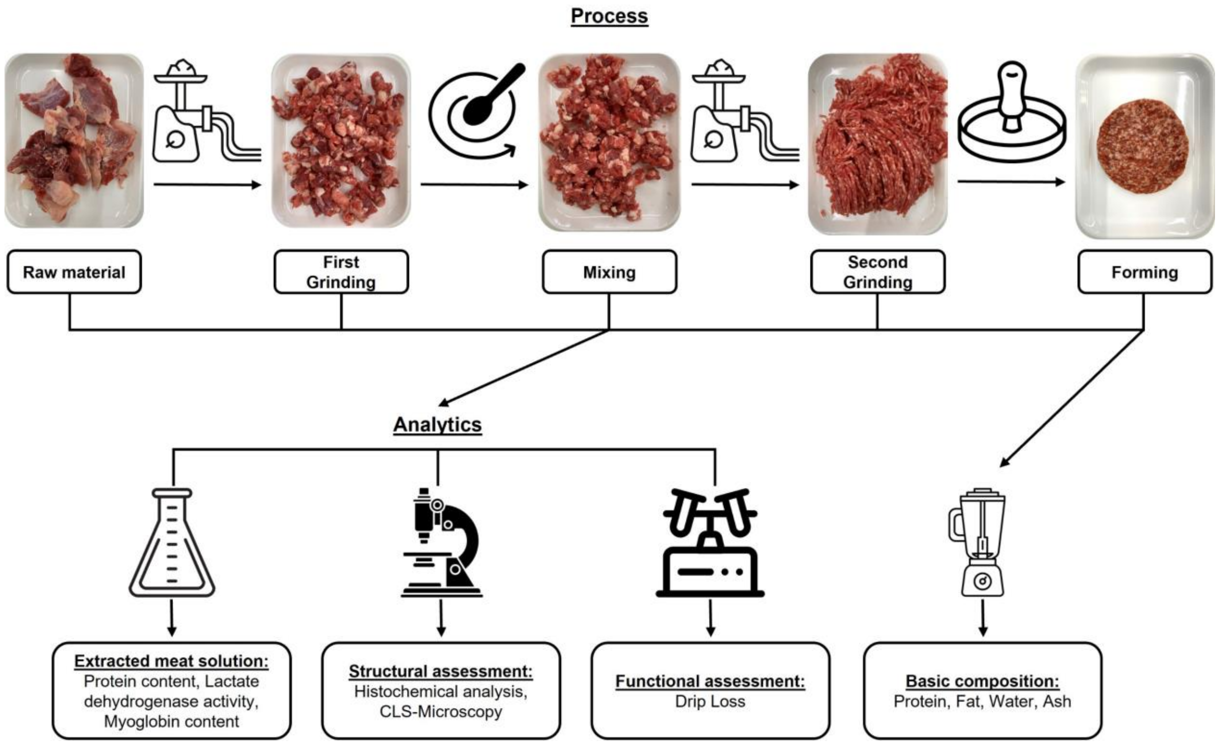
Abstract

In hamburger manufacturing, meat is subjected to four main processing steps (pre-grinding, mixing, grinding, and forming), whereby muscle fibers are disintegrated. In this study, the influence of these process steps was characterized by structural (amount of non-intact cells (ANIC), CLS-Microscopy), functional (drip loss) and qualitative (soluble protein content, lactate dehydrogenase (LDH) activity, myoglobin content (Mb)) parameters of the meat. Therefore, meat samples were analyzed after each process step. Histological analyses revealed an increased ANIC with progressive processing. Thereby, the first and second grinding steps caused the strongest increases (factors 2.43 and 2.69). Compara- ble results were found in the relative LDH activity (factor 2.20 and 1.62) and the Mb concentration (factor 2.24 and 1.33) of the extracted meat solution. The findings suggest that the disintegration of the meat structure increases with progressive processing, causing more vulnerable structures which result in increased leakage of intramuscular substances. Further, the type of stress acting on the meat determines the extent of the changes. The presented findings enable manufacturers to precisely adjust their process towards more gentle production parameters and thus, to meet the legal regulations.

Keywords: meat processing; quality characterization; techno-functional properties of beef hamburgers; structural modification; process characterization

Graphical Abstract



Introduction

The disintegration of muscle fibers during beef hamburger manufacturing affects their physicochemical properties. The term hamburger is protected by the guidelines of the German Food Code and must be prepared from 100 % beef [1], thereby not exceeding the legal limit of 20 Vol.% disintegrated muscle fibers [2]. Production of beef hamburgers usually involves four processing steps, namely pre-cutting during the first grinding, mixing, main grinding in the second grinding, and forming of the hamburgers [3].

Grinding is a mechanical way of comminution [4] and is widely used for size reduction of meat [5]. During grinding, the meat is continuously conveyed towards the cutting set [6], extruded through the hole plates, and cut by the rotating knife [5], whereby the pressures of 6 – 8 bars act on lean meat. Due to increased grinder efficiency and material homogeneity and better ground meat quality, the size reduction is done gradually [4,7]. In the pre- cutting (first grinding), meat is ground to 13 – 19 mm particle size [5], which is important to guarantee optimal conveying and comminution [4]. Mixing is performed to obtain an even distribution of meat and fat particles, often using paddle mixers with low energy input to ensure gentle handling [5]. In the main grinding step (second grinding), the meat is ground to the final particle size of approximately 3 mm [5]. Following, hamburgers are formed by filling the ground meat in a defined mold and applying pressure [8]. Meat is considered a plastic–elastic material that is able to store force acting on it to a certain degree [9,10]. During hamburger manufacturing, meat is exposed to three main mechanical forces. First, wall friction forces are acting on the meat during grinding and mixing [6]. Second, pressure acts on the meat during forming and as it is forced through the hole blades. This pressure is higher, the smaller the hole plate diameter is [4], whereby the structure of the meat is disintegrated. It was found that the pressure within the hole plate is higher in beef meat than in pork meat and is higher when the fat content of the meat is higher [4]. Third, the meat is exposed to shear force due to conveying and due to cutting of the meat strands by the rotating knife at the end of the hole plate [6]. In this stage, the structural and mechanical properties of the meat are altered due to size reduction [4], and muscle fiber structures are degraded [11].

A mechanical rupture of the muscle fiber opens its internal structure, thus making proteins and intramuscular substances available for extraction [12]. Thereby, the degree of muscle fiber disintegration depends on the applied force and stress, the type of raw material, and the used technology. Hence, mechanical treatments, such as mixing or forming under pressure, are key parameters in muscle fiber disintegration [2] and significantly change the meat structure. During grinding, the ordered fibrillar structures and connective tissue are disrupted by mechanical force [13], thus leading to an increased amount of non-intact muscle fibers (ANIC). The ANIC is evaluated by histological techniques [2], influences quality, functionality, and sensorial perception of beef hamburgers and is, thus, legally limited to 20 Vol.% in Germany [2,11]. Histological approaches are used to quantify muscle fiber disintegration in muscle tissue to identify, characterize, and evaluate changes in the muscle fiber structure and enable the assessment of the meat in terms of legal rules [2].

As muscle fiber structures are disintegrated during grinding, resulting in leakage of meat juice, the meats drip loss will increase and meat quality is reduced [6]. The ability of meat to retain water is described as water-holding capacity [14], which is closely linked to the meat quality and the sensorial perceptions of the consumer, as it depends on muscle fibrillar changes during processing [14]. Thus, the amount of released water depends on the extent of muscle fiber disintegration during processing.

Enzymatic methods are also based on the release of intramuscular fibrillar enzymes, which increases lactate dehydrogenase (LDH) activity [15]. Additionally, the determination of soluble protein and myoglobin content is based on the fragmentation of muscle fibers during grinding, thereby creating more open ends [13] where intramuscular fibrillar substances, such as proteins, leak out.

By now, it has not been determined which processing step in beef hamburger manufacturing influences the characteristics and properties of the meat the strongest. The aim of this study was, therefore, to identify the influence of the single process steps on structural, functional, and qualitative parameters of the meat. With the gained knowledge, hamburger manufacturers can identify the most influencing parameter and are, therefore, able to carefully design their processing steps and adjust the parameters to ensure a gentler production. It was hypothesized, that the process steps, including size reduction (such as grinding), might alter the properties of the meat the strongest as they accompany a stronger mechanical load [10].

Materials and Methods

Materials and Beef Hamburger Manufacturing

Hamburgers were produced, and samples were taken according to the production scheme (**Figure V-1**); details on all used equipment are summarized in **Table V-1**.

Cuts from flank of heifers, purchased at a local meat trading company (MEGA—Das Fachzentrum für die Metzgerei und Gastronomie eG, Stuttgart, Germany), were visually standardized four days after slaughtering and cut into cubes of 5 × 5 × 5 cm. Thereby, visible tendons were removed, and the fat content was adjusted to approximately 20 %.

Approximately 25 % of the meat was stored overnight to a core temperature of $T = -12$ °C and 75 % of the meat to a core temperature of $T = 1$ °C. The meat was mixed in a horizontal mixer for 30 s in a clockwise direction and 30 s in a counterclockwise direction at 32 rpm. The meat cubes were ground with a meat grinder equipped with a 3-part cutting system (precutting device, 4-wing knife, 13 mm inclined end-hole plate). The feeding screw rotated with a speed of 20 rpm and the grinder screw at 187 rpm. The meat was mixed again at 32 rpm for 15 s in a clockwise direction and 15 s in a counterclockwise direction and ground to a final particle size of 2.4 mm using a 3-part grinding system (plastic spacer, 5-bladed sickle knife, 2.4 mm inclined perforated end-hole plate) with a speed of 20 rpm at the feeding screw and 187 rpm at the grinder screw. The ground meat was then formed into hamburgers of $m = 90$ g weight, $h = 0.9$ cm height, and $d = 10$ cm diameter by applying $p = 5$ bar forming pressure with a modified hamburger press. Samples were taken after each processing step, namely, raw material, first grinding step, mixing, second grinding step, and forming (**Figure V-1**) and were then stored airtight at 1 °C until further analysis.

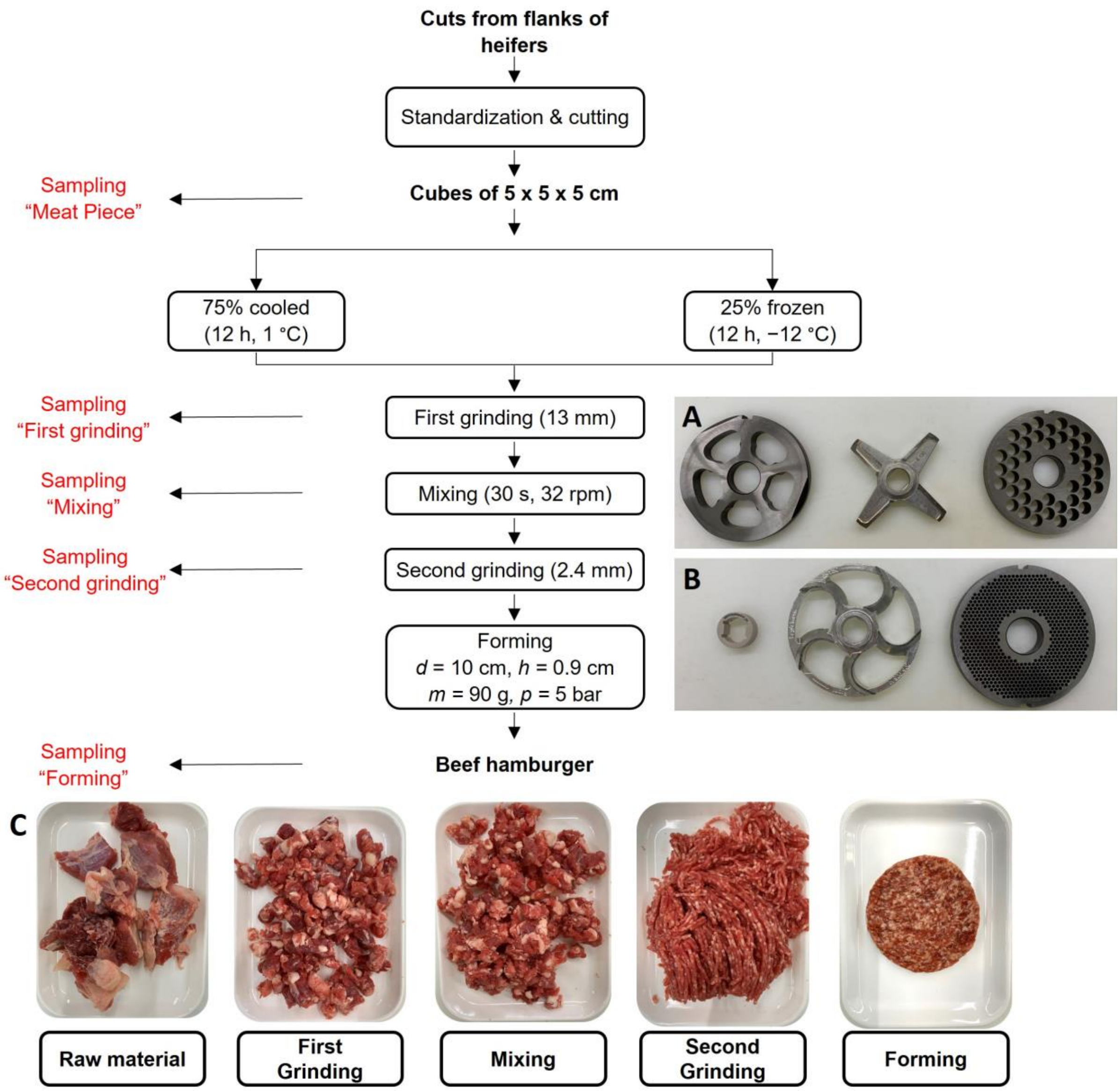


Figure V-1: Flow chart of the hamburger manufacturing with different points of sampling, cutting set composition of (**A**) first grinding step (3-part cutting system: pre-cutter, 4-bladed knife, 13 mm inclined perforated disc) and (**B**) second grinding step (3-part cutting set: spacer, 5-bladed sickle knife, 2.4 mm inclined perforated disc) and photographs of the samples (**C**), *n* = 3.

Table V-1: Details on equipment used for hamburger manufacturing.

Description	Model	Manufacturer
Horizontal mixer	Vacuum Paddle Mixer Type MVZ150	Asgo, Ermesinde, Portugal
Meat Grinder	Forschungsautomatenwolf Typ AE 130	Maschinenfabrik Seydelmann KG, Stuttgart, Germany
Precutting device	2031809T	Maschinenfabrik Seydelmann KG, Aalen, Germany
4-wing knife	TC93094	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany
13 mm inclined end-hole plate	TC3090278.1	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany
Plastic spacer for meat grinder	-	Maschinenfabrik Seydelmann KG, Stuttgart, Germany
5-bladed sickle knife	2067986W	Maschinenfabrik Seydelmann KG, Stuttgart, Germany
2.4 mm inclined perforated end-hole plate	TC3093457.1	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany
Hamburger press	MH-100 (modified)	Equipamientos Cárnicos, S.L. (Mainca), Barcelona, Spain

Methods

Chemical Composition of Beef Hamburgers

First, one hamburger was homogenized in a blender (Blixer® 2, M 02, robot-coupe®, Montceau-en-Bourgogne, France) for 30 s and analyzed for each repetition in triplicate.

The sea–sand method was used to determine the water content (§64 LFGB L 06.00-3 [16]). The fat content was determined by Soxhlet extraction (Büchi 810, Büchi Laboratoriums- Technik AG, Flawil, Switzerland), utilizing the leftovers of the water determination (§64 LFGB L 06.00-6). Dumas' combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) was used to assess the protein content (§64 LFGB method L 06.00-20) by applying a nitrogen to protein conversion factor of 6.25 [17].

Histochemical Analyses

To assess the amount of non-intact muscle fibers (ANIC) in the meat samples, histo- chemical analyses were performed, according to §64 LFGB method L 06.00-13 [16]. Cryo- cuts ($h = 5$ µm) were prepared, dyed with picro-indigo carmine (CALLEJA) staining, and transferred to high-resolution images of 27,881 dpi (Labor Kneissler, Burglengenfeld, Germany). The number of non-intact muscle fibers in the cryo-cut scans were detected by point-counting at 20-fold magnification (NDP.view 2.7.52, Hamamatsu Photonics K.K., Shizuoka, Japan), and the ANIC was calculated according to Equation V-1 [16]:

$$\hat{p}_u \cong \hat{p} - \left[\frac{1}{2n} - 2.3263 \cdot \sqrt{\frac{\hat{p} \cdot \hat{q}}{n}}\right] \quad \text{V-1}$$

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

With $\hat{p}_u$= lower limit, $\hat{p}_o$= upper limit, $\hat{p} = \frac{x}{n}$, $\hat{q} = \frac{n-x}{n}$, n = number of non-intact muscle fibers counted, x = number of total muscle fibers counted. Per sample at least six cross-sections were analyzed, whereby two cross-sections each were averaged. The mean and the standard deviation of the three repetitions were calculated.

Drip Loss (DL)

The drip loss of the meat samples was determined in triplicate by the centrifugation method [14]. In short, 10 g of meat were weighed in tubes (Nalgene 50 mL PP tubes, Nalgene Nunc International Corporation, Rochester, NY, USA) and centrifuged (20 min, 5 ◦C, 16,000 rpm) (Z32HK, Hermle Labortechnik GmbH, Wehingen, Germany). The excess meat juice was removed by placing the pellet on a paper tissue for 1 min. The percentage weight loss (drip loss) of the meat sample was calculated, as shown in Equation V-2:

$$Drip\ loss\ [\%] = \frac{m_{before\ centrifugation} - m_{after\ centrifugation}}{m_{before\ centrifugation}} \cdot 100\% \quad \text{V-2}$$

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

Confocal Laser Scanning Microscopy (CLSM)

A Nikon CLSM (Nikon D Eclipse C1, Nikon GmbH, Düsseldorf, Germany) equipped with a “Cobolt 06-MLD” laser was used to study the microstructure of the raw meat samples in the style of Irmscher [18]. A total of 20 µL of Calcofluor White solution (Sigma- Aldrich Chemie GmbH, Munich, Germany) for protein staining was applied onto a CLSM tray equipped with a cover glass. Round-shaped samples, taken from the raw samples with a special circular cutter, were placed in the tray and excited at 638 nm. Images of the representative areas were taken at 10-fold magnification (Plan-Apochromat Plan Fluor 4/0.13, Plan Fluor 10/0.30; Nikon GmbH, Düsseldorf, Germany) with the help of E- CZ1 software (NIS-Elements Confocal, Version 4.50, Nikon GmbH, Düsseldorf, Germany).

Preparation of Extracted Meat Solution (EMS)

As necessary for further analyses, the extracted meat solutions (EMSs) of the samples were prepared by diluting meat in the ratio of 1:10 with 10 mM potassium phosphate buffer pH 7 at 2 °C. The mixture was incubated (20 min, 7 °C, 85 rpm) (innova® 42R, New Brunswick Scientific/Eppendorf AG, Hamburg, Germany) and stored for 1 h at 7 °C for further extraction. The meat was separated by using folded filters (Rotilabo®-folded filters, type 113P, Carl Roth GmbH & Co. KG, Karlsruhe, Germany). Until further analyses, the EMSs were stored at 7 °C in brown glass bottles.

Soluble Protein Content (SPC)

Rapid nitrogen analysis, according to Dumas' combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany), with a nitrogen to protein conversion factor of 6.25 [17] was applied to quantify the amount of soluble protein in the EMS.

Lactate Dehydrogenase Activity (LDH)

The LDH activity of the EMS was determined by an enzyme detection kit (lactate dehydrogenase activity assay kit MAK066, Sigma-Aldrich Chemie GmbH, Munich, Germany) based on an NADH-dependent indicator reaction. A colored compound is formed that is photometrically quantifiable at 450 nm [19]. The EMS was first diluted 1:400 using 10 mM of a potassium–phosphate buffer adjusted to pH 7 and then 1:40,000 using the LDH sample buffer (part of the enzyme kit). Triplicates of each sample were carried out. The enzyme activity was calculated according to the manufacturer's instructions, the relative LDH activity was then calculated by dividing the respective enzyme activities by the activity of the meat piece.

Myoglobin Content (Mb)

The myoglobin content of the EMS was photometrically detected following the method of Trout [20]. In short, 100 µL of the previously prepared EMSs were transferred into a 96-well transparent plate (Nunclon Delta Surface, Thermo Fisher Scientific, Roskilde, Denmark) in triplicate for each sample. The absorption spectra of the EMSs were recorded from 300 nm – 800 nm in 1 nm steps at 25 °C (Biotek Synergy HT, Agilent Technologies, Inc., Santa Clara, CA, USA). The myoglobin content was then calculated according to Trout [20].

Statistical Analyses

Three independent experiments (biological replicates) with at least two to three analytical replicates (technical replicates) were performed. All results are shown as mean ± standard deviation or mean ± standard error (pressure profiles), calculated by MS Excel (Microsoft, Redmond, WA, USA) and plotted by OriginPro 2020 (OriginLab Corporation, North Hampton, MA, USA). Statistical analyses were done with SPSS (IBM SPSS Statistics 25, IBM Deutschland GmbH, Ehningen, Germany). The Shapiro–Wilk and Levene tests were used to test the normal distribution of data and variance homogeneity, respectively. All data were normally distributed. A significance analysis by univariant ANOVA (analysis of variance) was performed if the data showed variance homogeneity, applying the Tukey post hoc test (confidence interval of 95 % ($\alpha = 0.05$)). The Welch-ANOVA (analysis of variance) was conducted for data without variance homogeneity using the Games-Howell post hoc (confidence interval of 95 % ($\alpha = 0.05$)). Small letters attached to the mean values of the samples indicate statistical significance.

Results and Discussion

Chemical Composition

The hamburgers of this study were produced following the German guidelines for meat and meat products [1], where hamburgers typically contain beef meat only. Incorpo- ration of salt and spices into the hamburger patties are uncommon and not of industrial relevance. Usage of salt in the hamburger formulation would facilitate the solubilization of myofibrillar proteins and thus, influence product parameters, such as drip loss. The raw material used for the experiments was composed of 62.6 ± 0.4 % water, 19.0 ± 0.6 % fat, 18.765 ± 1.1719 % protein, and 0.87 ± 0.05 % ash, resulting in a sum parameter [21] of 101.25 %. The slightly increased sum parameter might be caused by the protein deter- mination by Dumas' method, which is based on the determination of the total nitrogen content of the sample. According to Berry and Abraham [22], a fat content of ca. 20 % is usually used for hamburger production. Keeton and Eddy [23] report that the protein and ash content of skeletal muscle tissue decreases with increased fat content, typically having approximately 18 % protein and 64 % water in meat with 20 % fat. Thus, the determined values are within the expected range.

Structural and Functional Properties

To check the influence of each process step on the ANIC, the meat samples were histologically analyzed for their degree of non-intact muscle fibers. During the first grinding step, the ANIC was approximately doubled (factor 2.4) from 3.79 ± 2.61 Vol.% in raw material to 9.21 ± 4.81 Vol.% after the first grinding, thereby reducing the volume of the particle by a factor of ca. 100 (**Figure V-2A**).

During the second grinding step, the volume of the particles was further reduced by a factor ca. 81, and the ANIC significantly increased about threefold (factor 2.7, **Figure V-2A**) from 8.87 ± 3.91 Vol.% after mixing to 23.86 ± 14.16 Vol.% after the second grinding. Both the mixing and the forming steps did not significantly change the ANIC ($p > 0.05$). Hence, the grinding steps have the biggest influence on muscle fiber disintegration, whereby the increase is higher in the second grinding compared to the first grinding. The higher standard deviations in **Figure V-2A** might be caused by variations in the raw material meat, as the displayed mean value is composed out of three biological replicates made from different meat cut at different days. It is known that parameters, such as physiological parameters of the animal [23], sex, age, or slaughtering conditions [24] influence the meat quality, wherefore changes between different batches are reasonable. Additionally, the histological determination of ANIC is based on a subjective, visual evaluation of the histological cryo- cut, thus making the determination suspectable to greater variations. The histological images (**Figure V-3**) depict the previously described changes in the meat structure, which are underlined by the CLSM images (**Figure V-3**). Naturally organized muscle fibers are present in the raw material after the first grinding and mixing, whereas the ordered structure is reduced after the second grinding and forming.

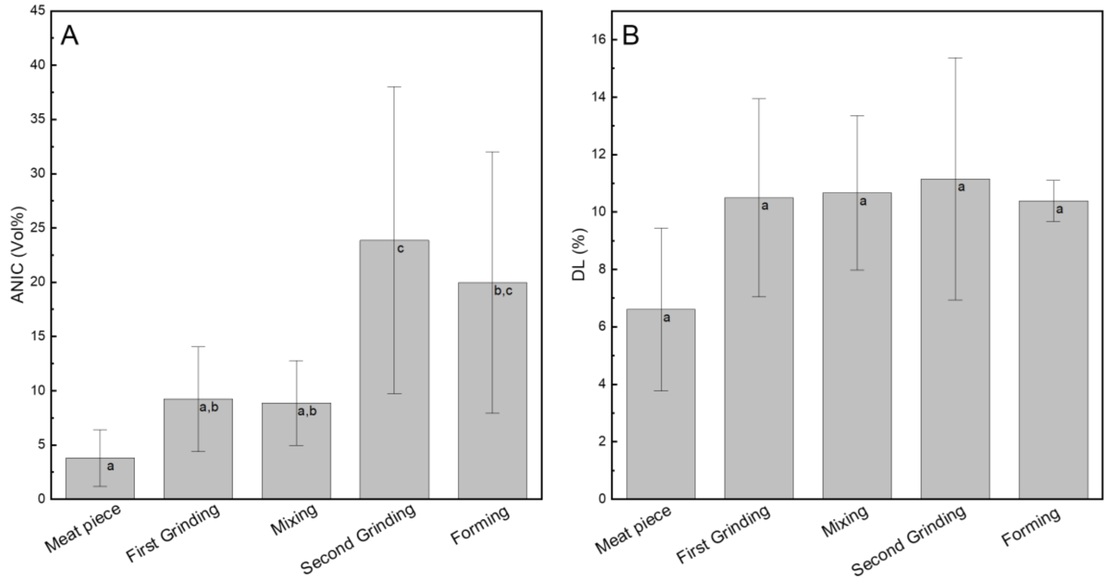


Figure V-2: Characterization of the structural and functional parameters: (**A**) amount of non-intact muscle fibers (ANIC) and (**B**) drip loss (DL) of the meat samples at different processing stages. Data points with different letters are significantly different ($p < 0.05$), $n = 3$.

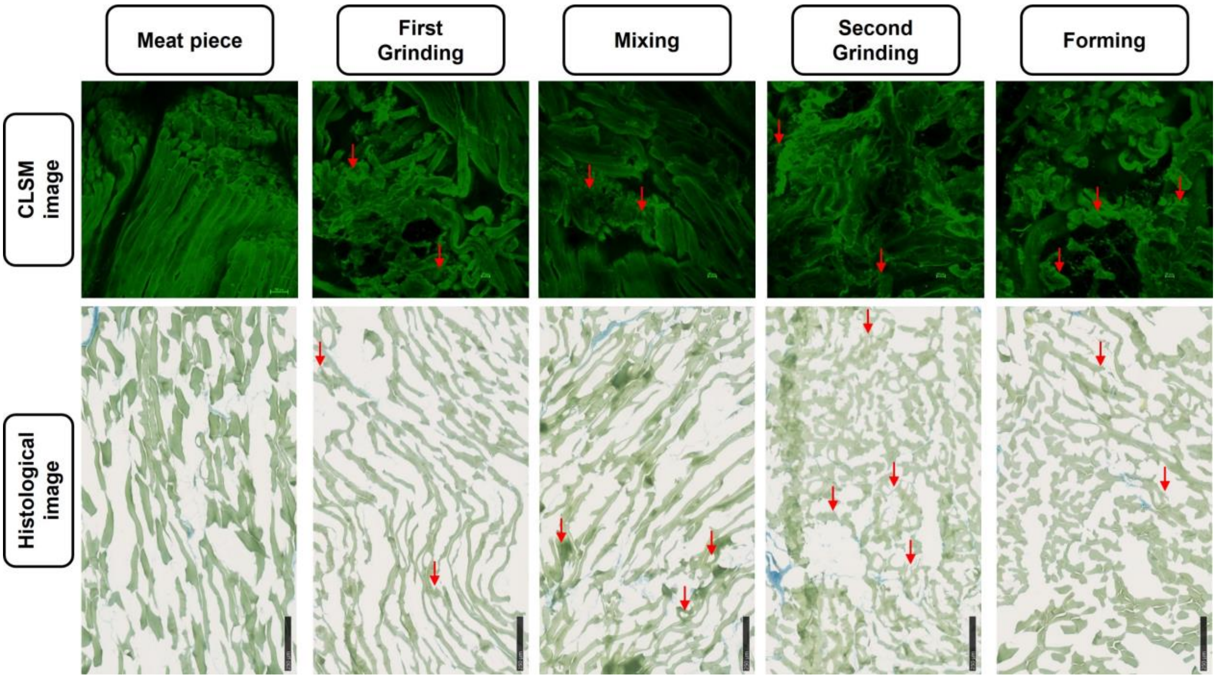


Figure V-3: Confocal laser scanning microscopy (CLSM) and histological images of the meat samples at different processing stages. Red arrows exemplarily indicate non-intact cells; images from the same processing step do not represent the identical samples section.

This outcome was expected, as grinding decreases the particle size through cutting and shearing the meat through end-hole plates, which disintegrates muscle structures [2]. The findings of Beneke [2] that mechanical treatment, such as mixing or forming, under pressure are key parameters in muscle fiber disintegration could not be verified in this study. This might be explained by quite gentle mixing and forming conditions in the present study. Increasing mixing time, speed, or higher formation pressure might cause stronger disintegration of meat muscle fibers [25,26].

To check the influence of each process step on the water-holding capacity, the samples were analyzed for their drip loss (DL). As muscle fiber disintegration is associated with increased DL [6], it is considered as a quality determining product parameter in this study. The centrifugation method was chosen over the sedimentation method, i.e., without applying an external force other than gravity (e.g., 24 h, 1 g) [14], as preliminary experiments revealed no DL on hamburgers over several days (data not shown). Hamm [27] described that all methods determining the free-water content can be used to determine the water- holding capacity of meat. Both traditional sedimentation, as well as accelerated methods applying centrifugal forces, are applicable. It is assumed that the sedimentation method might be more suitable for meat samples releasing free water easily, e.g., due to meat defects [21]. The DL increased slightly but not significantly from 6.61 ± 2.83 % in the meat piece to 10.50 ± 3.45 % after the first grinding (**Figure V-2B**). For all other process steps, the DL remains nearly constant at 10.38 to 11.15 %. As the volume reduction of the meat particles in the first grinding is stronger compared to the reduction in the second grinding, more.

muscle fibers are cut, and more intramuscular fibrillar substances might leak out. Thus, it is reasonable that the DL slightly increased during the first grinding step. In contrast to the present study, Tyszkiewicz et al. [12] found a more pronounced positive correlation between muscle fiber disintegration caused by rupture of myofibrils and water release from mechanically stressed pork meat. Additionally, they found that meat treated with a meat grinder lost more water compared to meat treated gentler with a meat activator or tenderizer. This underlines the hypothesis of a higher DL with increasing ANIC [12] but could not be fully confirmed in this study. Following the idea of Hughes et al. [28], that loss of moisture reduces the muscles' rigidity and structure, it is reasonable that the histologically determined ANIC increases (**Figure V-2A**) due to the grinding steps.

Changes in Chemical and Quality Properties

To check changes in the chemical and quality properties, EMS were analyzed for their relative LDH activity, their SPC, and Mb. As the aim of the study was to detect changes in the structural, functional, and chemical properties of samples with different mechanical treatment, the EMS were produced by a gentle extraction process without homogenization. A homogenization would mechanically disrupt the muscle fiber structure completely; this structural damage would superpose the process related changes and would, thus, rule out the differentiation between the samples.

LDH doubled (factor 2.20) during the first grinding step and further increased during the second grinding (**Figure V-4A**). Only slight changes were caused by mixing, whereas forming caused a comparable increase to the second grinding (factor 1.70, **Figure V-4B**). This might be attributed to a stronger LDH release from the muscle fibers due to the applied pressure. Considering the statics, the statistically significant increases in LDH were caused by the first and second grinding steps and forming. This result is partly comparable to the histologically determined ANIC (**Figure V-2A**). Moreover, this shows that LDH might serve as a chemical indicator for muscle fiber disintegration when the raw material is known and analyzed as a reference. An LDH increase was expected, as a disruption of muscle fibers by grinding and a subsequent release of muscle fiber content leading to an increase in LDH activity was reported earlier [12,15]. Similarly, LDH activity determination is a common measure to distinguish between chilled and frozen meat caused by increased enzyme release upon freezing [15].

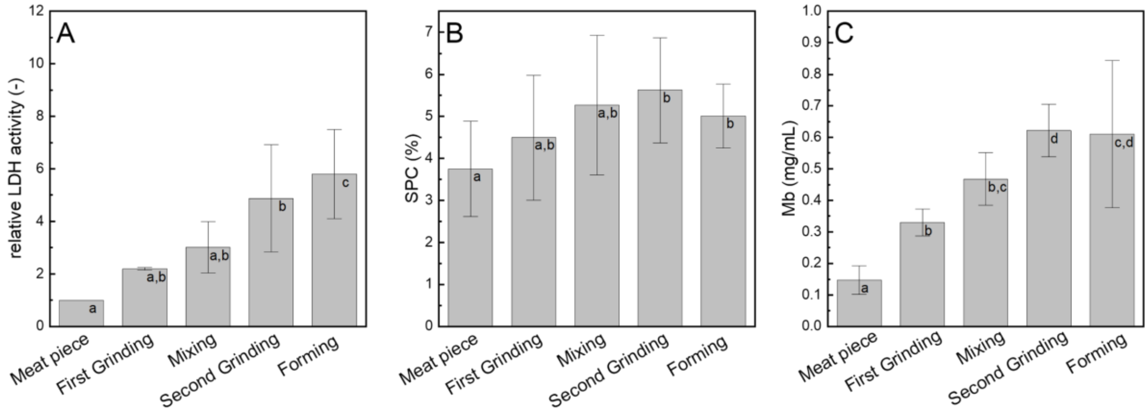


Figure V-4: Characterization of the chemical and quality properties: (**A**) relative lactate dehydrogenase activity (LDH), (**B**) myoglobin content (Mb), and (**C**) soluble protein content (SPC) of the extracted meat solutions at different processing stages. Data points with different letters are significantly different ($p < 0.05$), $n = 3$.

A slight increase in the amount of SPC during processing could be found (**Figure V-4B**). However, statistically significant differences were only analyzed between the second grind- ing step and forming and the meat piece. Moreover, SPC did not change in the same manner as the histologically determined ANIC. An increase in the soluble protein content was ex- pected, as Tyszkiewicz et al. [12] stated, that a mechanical rupture of the meat muscle fibers opens the internal structure of meat tissue, thus making the (myofibrillar) proteins available for extraction. They found a significantly higher proportion of extracted water-soluble (sarcoplasmic) protein in the ground sample compared with the sample treated with the tenderizer or activator. However, this trend was only slightly pronounced in the study. This might be traced back to the use of different analytical methods. Tyszkiewicz et al. [12] used the Helander procedure [29] which is used to detect the protein availability, whereas in this study the total amount of nitrogen compounds was determined. It is possible that other soluble nitrogen compounds, such as non-protein nitrogen compounds, dipeptides, or amino acids remain in the extract upon filtration and overlay the effect of the processing steps on the SPC.

During the first grinding step, Mb approximately doubled from 0.15 ± 0.05 mg/mL in raw material to 0.33 ± 0.04 mg/mL after the first grinding (**Figure V-4C**). The mixing and second grinding step further increased Mb (0.62 ± 0.08 mg/mL after second grinding). The subsequent forming step slightly reduced Mb. It can be concluded that the first and second grinding steps increase Mb the strongest. The Mb analysis is based on specific absorbance spectra of myoglobin derivates [30]. As myoglobin is present in cardiac and skeletal muscles and is released upon muscle fiber damage during acute myocardial infarctions, it serves as a biochemical indicator [31,32]. Meat grinding also disintegrates muscle structures, thus an increase of Mb with increasing ANIC is assumed. The strongest increase in the myoglobin content in the first and second grinding steps is in accordance with the findings of the histologically determined ANIC (**Figure V-2A**) and SPC (**Figure V-4B**). This proves the hypothesis that a higher ANIC leads to an enhanced release of intramuscular fibrillar compounds, such as myoglobin, and that the myoglobin content can serve as a chemical indicator for estimation of muscle fiber disintegration in this study.

The outcomes suggest that the grinding steps are key points for muscle fiber disinte- gration. When it comes to optimization toward a gentler beef hamburger manufacturing process, those should be taken under close investigation. Based on the present findings, it is assumed that adjustments in the grinding steps might lead to optimizations of the beef hamburger manufacturing process, thus optimizing the burger’s quality.

Mechanical Considerations

Figure V-5 illustrates changes in the meat structure as well as the mechanical stress acting on the meat during the different processing steps.

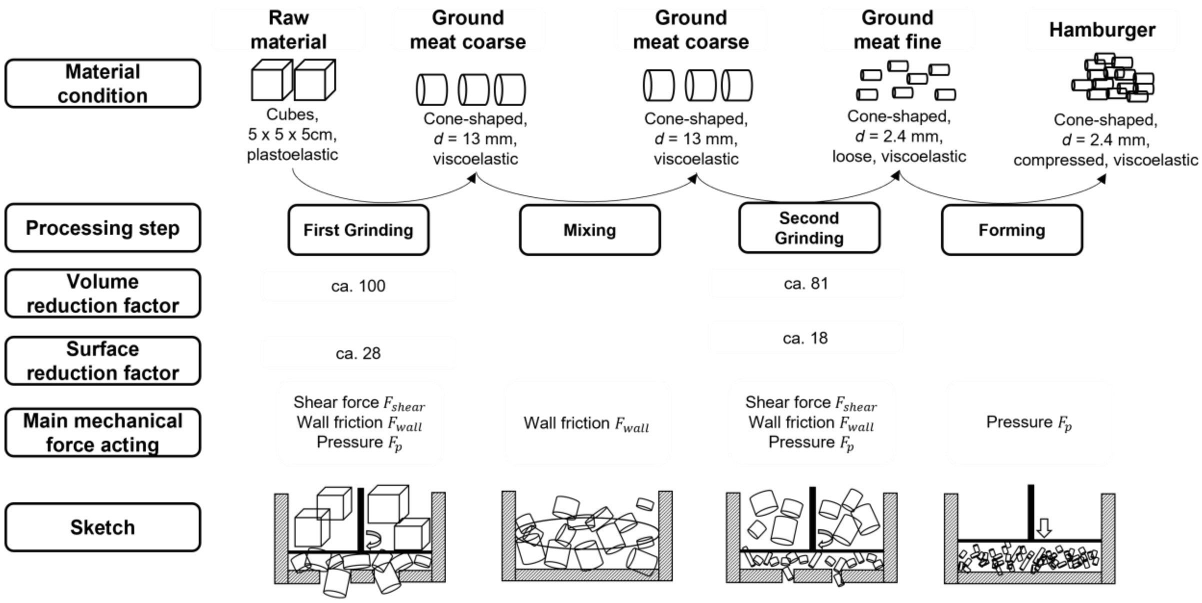


Figure V-5: Changes in material conditions and main mechanical forces in meat upon hamburger manufacturing.

As previously discussed, the first and second grinding steps account for the strongest structural, functional, and chemical changes. During grinding, particle sizes are reduced by cutting and shearing the meat through end-hole plates. Muscle fibers are fragmented into smaller pieces, muscle structures are disintegrated, and more open ends are created [2,10,13]. This can be underlined by the mechanical forces acting on the meat in comparison to the mixing and forming steps where mainly wall friction and pressure applied to the meat; shear force, wall friction, and pressure stresses the meat during grinding, therefore, applying a higher mechanical load. This leads to stronger disintegration of the material [10].

In the first grinding step, meat is cut from a cube into cylindrical-cone-shaped particles of 13 mm diameter, thereby reducing the surface area and volume per particle but increasing the total number of particles and the total surface area. This leads to an increased number of cut muscle fibers. During the second grinding, the particles are ground to a final particle size of 2.4 mm diameter, which further decreases the surface area (factor 18, **Figure V-5**) and volume per particle and the increasing total number of particles and total surface area. A stronger increase during grinding is in accordance with the findings of Beneke [2] who reported a cumulative increase in muscle fiber disintegration if several mechanical methods are combined. Another reason for the stronger increase in ANIC after the second grinding is the higher grinding screw pressure of 3.63 ± 0.68 bar in the second grinding step (**Figure V-6**) compared to a strongly variating and lower pressure in the first grinding step of 0.72 ± 0.29 bar (**Figure V-6**).

This might be attributed to the higher cutting resistance in already ground meat, which has more viscoelastic properties compared to the intact meat part in which elastoplastic properties dominate [10]. Higher pressure is, according to Wild et al. [33], associated with increased friction and destruction of the meat. Fluctuations in cutting set pressure might be caused by fluctuating raw material quality, as the processability of the meat strongly depends on the meat's properties and thus, determine the cutting set pressures.

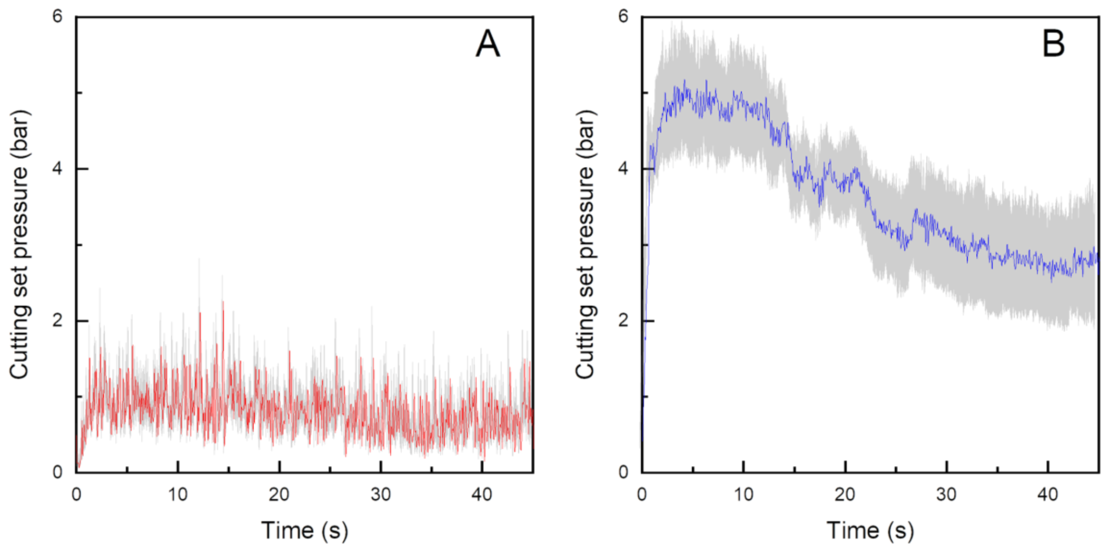


Figure V-6: Mean (red and blue lines) and standard errors (grey lines) of cutting set pressures during the first (red line) (**A**) and second (blue line) (**B**) grinding steps in beef patty manufacturing, n = 12.

Conclusion

It was shown in this study, that meat muscle fiber structure changes upon production of beef hamburgers, thus altering those attributes. Each process step contributes to a different extent to the material changes, as different forces act on the meat. The first and the second grinding were found to have the strongest impact as they caused a pronounced increase in the analyzed attributes, ANIC, LDH, Mb, and SPC. Those findings allow for an improvement of hamburger production by adjusting the grinding parameters, now known as the most influential processing step, and thus, to meet product quality and legal requirements.

Author Contributions

Conceptualization, L.M.B. and M.G.; data curation, L.M.B.; formal analysis, L.M.B. and M.G.; funding acquisition, F.W., N.T., J.W. and M.G.; investigation, L.M.B.; methodology, L.M.B.; project administration, L.M.B., F.W., N.T., J.W. and M.G.; resources, L.M.B.; software, L.M.B.; supervision, J.W. and M.G.; validation, L.M.B. and M.G.; visualization, L.M.B.; writing-original draft, L.M.B.; writing-review and editing, L.M.B., J.W. and M.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Most data are included in the article.

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Conflicts of Interest

The authors declare no conflict of interest.

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VI. Chapter:

Effect of Cutting Set Variations on Structural and Functional Properties of Hamburgers

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Abstract

Meat grinders are composed of a combination of individual functional elements (e.g., screw conveyor, perforated plates, knives). This setup, and in particular the chosen cutting set, influences the characteristics of ground meat and hamburgers produced. In this study, we took a closer look at the effect of cutting set variations and process parameters on structural, functional, and physicochemical properties of beef hamburgers produced. It was found that the specific mechanical energy input during grinding increased when cutting levels, i.e., a set of one hole plate and one knife, were increased, causing more cell disintegration ($r = 0.387$, $p = 0.02$). Surprisingly though, an influence on the functional and quality parameters of the hamburgers could not be found for most parameters tested. The findings indicate that variations in the cutting set affect the process parameters and the stress applied to the meat, but residence times in this zone are too small to cause noticeable effects on the analytical and qualitative properties of hamburgers. As such, there are options for energy and cost optimization of industrial grinding processes without sacrificing quality.

Keywords: Hamburger, process-structure-function relationship, perforated discs, knives, ground meat characteristics

Introduction

Sausages, ground meat, and other ground meat products are very important product classes in the German meat industry [1], using grinding as a key processing technique for size reduction. As the meat industry has shifted from small-scale, handcrafted to high-capacity, industrial-scale production [2] maintaining high product quality requires optimal process design based on in-depth knowledge of processes and mechanisms. The choice of a suitable cutting set, i.e., the specific combination of a series of hole plates and knives, is crucial for the size reduction in the grinder [3, 4], affecting the morphological structure and thus the physicochemical, sensory, and functional properties of ground products such as hamburgers [5-13].

Meat is an anisotropic, plasto-elastic material with a fibrous structure, able to store and release forces to a certain degree [4, 13-17]. When meat is conveyed through grinders, it is subjected to shear forces and wall friction between the rotating grinding screw, the meat, and the housing. In the cutting set zone, the material is being comminuted by the superimposition of irreversible deformations [4, 12, 17, 18]. During conveying, the grinding screw compresses the meat and creates a pressure gradient with the highest pressure in front of the cutting set [12, 17-19]. The comminution of meat already begins during the conveying and that associated compression, due to the applied pressure and shear forces [12, 15, 20, 21]. The applied forces disintegrate the inner structures of the meat due to mechanical overloading, while the rotating knives of the cutting set then cut the extruded meat strands by applying shear forces. This results in reduced particle sizes of the raw material [3, 12, 17, 22, 23].

A cutting set contains several static hole plates and rotating knives with different geometries, layouts, and amounts of combined plates and knives depending on the raw material, the process, and the desired product properties. The number of combined plates and knives determines the number of cutting levels [3]. The meat-cutting processes on the hole plates are mainly influenced by the knife sets, the number of blades, and the speed of rotation [2, 3]. The knife geometry affects the continuous flow of the raw material, following the principle of drag flow [3, 12, 18]. Increasing the number of blades per knife can result in a more uniform and smaller particle size of the ground meat, and significantly increases throughput [3, 17]. On the other hand, excessive knife speed can negatively affect the cutting process due to increased abrasion of the cutting tools and material [23]. The sharpness of the knife sets is crucial for the quality of the produced ground product. Sharp knives protect the material by cutting it rather than squeezing it, thus reducing frictional forces and juice leakage [2, 24]. A non-optimal adjusted arrangement and speed of the knives can negatively affect the process, e.g., by causing a blockage of the cutting set, increasing energy consumption, and abrasion [17, 23], and leakage of liquid, ultimately reducing product quality [25].

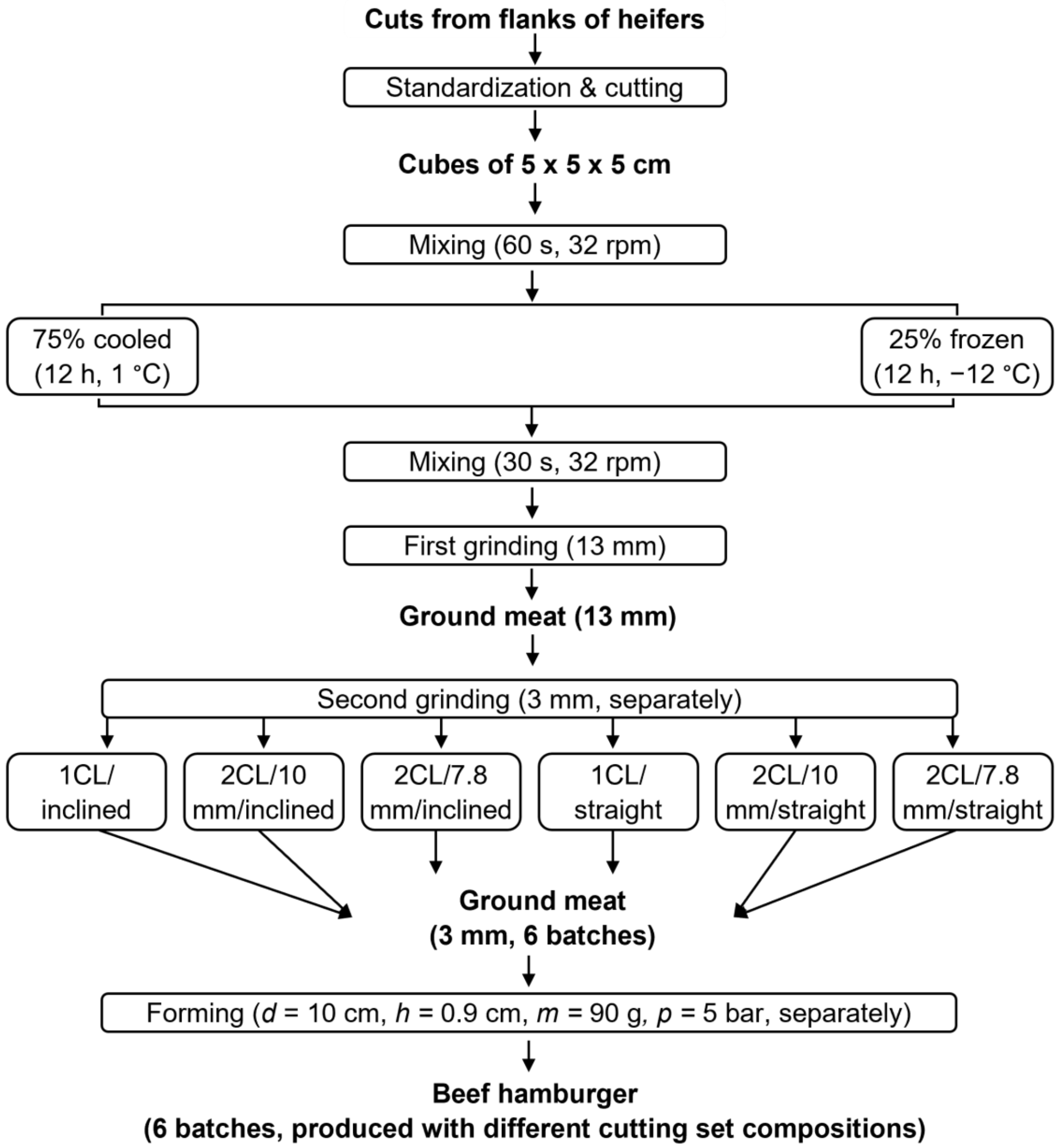
Intense processes might also cause stronger oxygen incorporation into the meat mass during manufacturing which accelerates the oxidation processed [26]. Besides the oxidation of myoglobin to metmyoglobin, fats and other components are oxidized due to higher oxygen exposure. As it is reported that the oxidation of lipids and myoglobin follow the same mechanisms based on oxy-free radical generation, myoglobin and metmyoglobin might be used as quality degradation indicators [27, 28].

The hole plates are essential for the size reduction performance and quality of the grinding process. Hole plates narrow the outlet area of the grinder and facilitate pressure build-up [12, 18], allowing for fixation of the raw material during rotational cutting and, ensuring the comminution of the raw material by partial ruptures through sharp-edged holes [29]. The size of the drill holes determines the particle sizes [29]. It was reported that applied forces are reduced the larger the drill holes of the plate [17]. A higher number of drilled holes increases the throughput of a grinder, but higher pressures on the bridges between the holes can increase abrasion [30]. Zhao and Sebranek [31] reported that an improved adjustment of the hole sizes of the plates improves the sensory quality of the products. Mechanical over-processing can cause cell structure disintegration and meat juice leakage [2, 29]. As a consequence, stepwise comminution in the cutting set results in the best comminution performance and involves combining knives and hole plates with varying diameters [2, 15]. This method pre-cuts meat at larger diameter hole plates and disintegrates the collagenous structures before the final size reduction. Knowledge about the influence of the cutting sets on structural and quality parameters of ground meat is primarily based on practical experience gained over several decades [19, 23, 32-34]. Based on this knowledge it was hypothesized, that cutting sets with inclined drill holes and with fewer cutting levels cause less cell disintegration, thus causing fewer changes in the structural, physicochemical, and functional properties of the hamburgers. The aim of the present study is therefore to investigate correlations between cutting set composition and the morphological, physicochemical, and functional properties of beef hamburgers.

Materials and Methods

Materials & sample preparation

The hamburgers were produced according to the production flowchart in **Figure VI-1**. Beef from the flanks of heifers (*M. obliquus externus abdominis, M. transversus abdominis, M. obliquus internus abdominis*; Prändl, Fischer [35]) was purchased from MEGA (MEGA – Das Fachzentrum für die Metzgerei und Gastronomie eG, Stuttgart, Germany), manually standardized to 20 % fat, roughly freed from tendons and cut into cubes of ca. 5 x 5 x 5 cm size. The standardized meat material was homogeneously mixed at 32 rpm for 30 s in left-hand rotation and for 30 s in right-hand rotation in a horizontal paddle mixer (Paddle mixer type RC-40, Equipamientos Cárnicos, S.L., Mainca, Barcelona, Spain). Until further manufacturing, 75 % of the meat was stored at T = 1 °C, and 25 % was frozen to a core temperature of T = - 12 °C overnight. The temperatures were chosen to ensure that frictional heat generated during grinding is sufficiently counteracted to prevent protein denaturation. The cooled and frozen meat cubes were mixed in the horizontal paddle mixer for 30 s at 32 rpm in left-hand rotation to ensure homogeneous distribution and first ground to a particle size of 13 mm using a meat grinder (Forschungsautomatenwolf Typ AE 130, Maschinenfabrik Seydelmann KG, Stuttgart, Germany) equipped with a 3-part cutting set (pre-cutter, 4-blade knife, 13 mm inclined end hole plate). Throughout the entire experiment the rotational speed of the meat grinder was set to 20 rpm at the feeding screw and 187 rpm at the grinding screw. The pre-ground 13 mm meat was ground to a final particle size of 3 mm in the second grinding step. Batches of ca. 10 kg were separately ground using different cutting set compositions (**Figure VI-1**). Detail of all cutting set parts are listed in **Table V-1** the cutting set variations are displayed in **Figure VI-1**. One cutting level (CL) is thereby defined as the combination of one hole plate and one knife. In straight hole-plates, the drill holes are drilled perpendicular to the surface of the perforated disc, whereas the drill holes in the inclined hole-plates are arranged in a spiraling pattern with a certain angle inclined to the surface [33, 34]. The ratio between the total drill hole area and total plate area is 0.42 for the straight 3 mm end hole plate and 0.44 for the inclined 3 mm end hole plate.

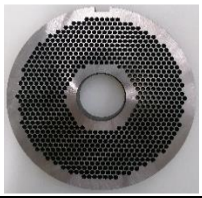


Sample code	Description of cutting set variation
1CL/inclined	Spacer - Knife 1 - 3 mm EHP inclined
2CL/10 mm/inclined	Spacer - Knife 1 - 10 mm MHP inclined - Knife 2 - 3 mm EHP inclined
2CL/7.8 mm/inclined	Spacer - Knife 1 - 7.8 mm MHP inclined - Knife 2 - 3 mm EHP inclined
1CL/straight	Spacer - Knife 1 - 3 mm EHP straight
2CL/10 mm/straight	Spacer - Knife 1 - 10 mm MHP straight - Knife 2 - 3 mm EHP straight
2CL/7.8 mm/straight	Spacer - Knife 1 - 7.8 mm MHP straight - Knife 2 - 3 mm EHP straight

Figure VI-1: Flow chart of the hamburger manufacturing with different cutting set compositions during second grinding and details on cutting set variations (CL = cutting level, MHP = middle hole plate, EHP = end hole plate).

Table VI-1: Details of all cutting set parts used in the first and second grinding.

Description	Product-Number	Producer	Photo
Plastic spacer for meat grinder	-	Maschinenfabrik Seydelmann KG, Stuttgart, Germany	-
Pre-Cutter	2031809 T	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
4-bladed knife 1	tc E130	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
4-bladed knife 2	tc E130	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
***Inclined* 13 mm** hole plate	T2 E13	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
***Inclined* 10 mm** hole plate	T2 E10	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
Straight 10 mm hole plate	E/10	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
***Inclined* 7.8 mm hole plate**	T2 E 7,8	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
Straight 7.8 mm hole plate	E/7,8	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	

Inclined **3 mm** hole plate	T2 E3	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	
Straight 3 mm hole plate	E/3	turbocut Joop GmbH, Bad Neustadt an der Sale, Germany	

The ground meat was formed using a modified hamburger press (MH-100 (modified), Equipamientos Cárnicos, S.L. (Mainca), Barcelona, Spain) by applying $p = 5$ bar forming pressure. The hamburgers weighed $m = 90$ g, had a height of $h = 0.9$ cm, and a diameter of $d = 10$ cm. The experiment was performed in duplicate.

Methods

Chemical composition of hamburgers

Before the water, fat, and protein determination, one hamburger per batch was homogenized for 30 s in a blender (Blixer® 2, Robot-Coupe, Montceau-en-Bourgogne, France). The water content was determined from the meat mass by the sea-sand method (§64 LFGB L 06.00-3 [36]), the fat content by Soxhlet extraction (Büchi 810, Büchi Laboratoriums-Technik AG, Flawil, Switzerland) from the residues of the water determination (§64 LFGB L 06.00-6 [36] and the protein content by the Dumas' combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) (§64 LFGB method L 06.00-20 [36] using a nitrogen-to-protein conversion factor of 6.25 [37]. The Dumas method as a rapid method was chosen due to the large sample sizes analyzed. The pH was determined using a puncture electrode (Microprocessor pH Meter 537 equipped with electrode WTW SenTix Sp, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany).

Histochemical analyses

The amount of non-intact cells (ANIC) in the hamburgers was histochemically assessed using a previously described method by Berger, Gibis [6] and Berger, Witte [7] following the official guidelines of § 64 LFGB (L 06.00-13) [36]. The ANIC was calculated based on the results of the point-count method, where the amount of non-intact cells in thin cryo-cut cross sections of the hamburgers was counted. Per sample at least $n = 6$ cross-sections were investigated, and two cross-sections each were averaged. The mean and the standard deviation of the three repetitions was calculated.

Determination of Drip Loss

The centrifugation method was used to determine the drip loss of the hamburgers. According to Berger, Gibis [6] and Berger, Witte [7], a 10 g sample was centrifuged for 20 min at 5 °C and 24,040 g (Centrifuge Z32HK, Hermle Labortechnik GmbH, Wehingen, Germany). The drip loss was obtained by differential weighing.

Determination of Firmness by Forward Extrusion

The forward extrusion method described by Berger, Gibis [6] was used to determine the firmness of the ground meat. For this, a texture measurement device (Instron, Model 3365, Instron Engineering Corporation Ltd, Massachusetts, USA) recorded the required force to press ground meat through a cylinder (d = 50 mm) with a 7.5 mm hole opening at a crosshead speed of 0.33 mm/s.

Grilling Procedure of Hamburgers

Frozen hamburgers were grilled on an electric contact grill (Nevada, Neumärker, Hemer, Germany) for 150 s at 200 ± 5 °C until a core temperature of 72 °C had been reached. Hamburgers were covered by a sheet of aluminum foil on both sides, to prevent sticking and disintegration on the surface of the grill. The hamburgers were allowed to cool to ambient temperature for at least 30 min and stored airtight in plastic bags until further analyses.

Determination of Hardness by Warner-Bratzler shear cell

Hamburgers were grilled as described in section “Grilling Procedure of Hamburgers” and allowed to cool to ambient temperature for at least 30 min. Then strips (width: 1.5 cm) were manually cut from the center to ensure sample homogeneity. The hardness of the grilled hamburger was analyzed by a texture analyzer (Model 3365, Instron Engineering Corporation Ltd., Massachusetts, USA) equipped with a V-shaped Warner-Bratzler guillotine (63° opening angle and 60 mm opening height). A crosshead speed of 250 mm/min was applied to cut through the sample completely. The maximal shear force (N) was recorded (n = 24 per sample).

Preparation of Extracted Meat Solution

Extracted meat solutions from all samples were produced to be used in further analyses. Following the instructions of Berger, Witte [7], hamburgers were diluted 1:10 with 10 mM potassium phosphate buffer (pH 7, T = 2 °C), filtered, and stored cooled in brown glass bottles until further analysis.

Determination of Soluble Protein Content (SPC)

The amount of soluble protein in the extracted meat solution was quantified in triplicate through rapid nitrogen analysis according to the Dumas combustion method (Dumatherm N Pro, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) [36]. The rapid Dumas method was used due to large sample sizes. A nitrogen-to-protein conversion factor of 6.25 according to Mariotti, Tomé [37] was used.

Determination of Myoglobin (Mb) and Metmyoglobin (MetMb) content

Oxidative changes of meat pigments were assessed by photometric determination of the myoglobin (*Mb*) and metmyoglobin (*MetMb*) content of the respective extracted meat solution, as already described by Berger, Gibis [6] (*MetMb* determination) and Berger, Witte [7] (*Mb* determination). For this, 100 µL of each meat extract was transferred into a 96-well transparent plate (NunclonTM Delta Surface, Thermo Fisher Scientific, Roskilde, Denmark) and absorption spectra were recorded at 25 °C using a microplate reader (Biotek Synergy HT, Biotek Instruments, Inc., Winooski, USA). The *MetMb* and *Mb* content were then calculated according to Trout [38].

Sensory and optical evaluation

Sensory and optical properties of grilled hamburgers (see section “Grilling Procedure of Hamburgers”) were evaluated using a 10-point rating scale. A sensory score of 5 points represents the compared reference standard. The reference was defined as standard in pretests by sensory experts. For the sensory evaluation, grilled and cooled (T = 7 °C) hamburgers were cut into quarters using a standardized cutting template. For reheating before the sensory testing, one hamburger was placed on a melamine plate (white melamine plate 190 x 145 mm, WACA-Kunststoffwarenfabrik, Halver, Germany), covered with another plate of the same type and reheated in a microwave (model HF15M541, Siemens Aktiengesellschaft, Munich, Germany) under standardized conditions (P = 800 W; t = 45 s) to a core temperature of T = 70 ± 2 °C. The parameters hardness, juiciness, texture, and overall acceptability of the hamburgers were assessed. For optical evaluation, cooled hamburgers were cut cross-sectionally with a slicer (Bizerba VS8A, Wilhelm Kraut GmbH & Co. KG, Balingen, Germany), and the inner structure was evaluated in terms of the coarseness of the particles, amount of batter-like substance and overall acceptability. The sensory evaluation was performed using a 10-point scale (0 = harder/

dryer/ compacter/ worse/ finer/ more batter-like substance and 10 = softer/ juicer/ looser/ better/ coarser/ less batter-like substance). Each sample was evaluated by at least n = 18 panelists. The panel consisted of persons of different genders, ages, and backgrounds making the sensory test diverse and enabling to reduction of errors. Informed consent was obtained from each panelist prior to their participation in the study.

Process control parameters

The grinding screw torque was recorded during the second grinding by the operating software of the meat grinder (S7-To-Excel Tool - Expert - for Windows, Träger Industry Components, Weiden, Germany) for all batches produced with different cutting set compositions. The idle torque of the grinding screw was previously determined to be M = 6.9 ± 1.1 Nm (data not shown). The mass flow was determined by weighing the output of ground meat over the course of 15 s.

Determination of Specific Mechanic Energy input (SME)

The mass-specific mechanical energy input *SME* (J/kg) as the degree of shear strain during grinding was calculated according to the following equation VI-1 and VI-2 [18, 39-41].

$$SME = \frac{P}{\dot{m}} \quad \text{VI-1}$$

$$P = (M - M_{empty}) \cdot n_{rad} \quad \text{VI-2}$$

With P = average power during grinding process (W), M = average grinding screw torque during grinding (Nm), M_{empty} = idling torque of grinding screw (Nm), $\dot{m} = \frac{m}{t}$ being the mass flow (kg/s), $n_{rad} = \frac{2\pi \cdot n_{grinding\ screw}}{60}$ being the radial rotational speed of the grinding screw (s^{-1}), $n_{grinding\ screw}$ = rotational speed of the grinding screw (s^{-1}). The average torque during grinding is determined as the torque during the plateau phase of grinding.

Statistical analyses

Two independent experiments (biological replicates) with at least three analytical replicates (technical replicates) were performed per cutting set variations. All results are presented as mean ± standard deviation or mean ± standard error (torque profiles), both calculated by MS Excel (Microsoft, Redmond, WA, USA), plotted with OriginPro 2020 (OriginLab Corporation, North Hampton, MA, USA), and statistically analyzed using SPSS (IBM SPSS Statistics 26, IBM Deutschland GmbH, Ehningen, Germany).

Shapiro-Wilk and Levene tests were used to check the normal distribution and variance homogeneity of the data. An analysis of variance (ANOVA) was performed by applying the Tukey post hoc test for data sets tested for variance homogeneity (confidence interval of 95 %, $p = 0.05$). Additionally, a two-factorial analysis of variance (two-way ANOVA) was performed for the sensory and optical attributes using the Tukey post hoc test (confidence interval of 95 %, $p = 0.05$). For data sets without homogeneous variances, a robust Welch-ANOVA with a Games-Howell post hoc test was performed (confidence interval of 95 %, $p = 0.05$). To assess the linear correlation between two normally distributed variables a Pearson correlation analysis was carried out. A statistically significant difference between means was considered if $p < 0.05$ and is indicated by small letters attached to the mean value of the measurement.

Results and Discussion

Chemical composition

The hamburgers in this study were produced according to German guidelines for meat and meat products and contained beef only, i.e. no salt or spices were added [42]. They were composed of 60.9 ± 0.8 % water, 19.8 ± 0.7 % fat, and 18.75 ± 0.07 % protein and had a pH of 5.75 ± 0.03.

Structural changes

As a parameter for structural changes in hamburgers upon manufacturing with different cutting set compositions, the amount of non-intact cells (ANIC) was determined. **Figure VI-2** shows the ANIC of the hamburgers at different cutting set compositions. The ANIC of the hamburgers ranged from 10.82 ± 0.84 Vol.% in the system equipped with one inclined cutting level (1CL/inclined) to 14.43 ± 0.18 Vol.% in the system with two straight cutting levels and a 7.8 mm middle hole plate (MHP) (2CL/7.8 mm/straight). The statistical analysis revealed that there was a significant difference between the 1CL/inclined and 2CL/straight with both sizes of MHP. All other combinations showed no significant differences (**Figure VI-2**). As initially hypothesized, the cutting set with the inclined drill holes resulted in lower or comparable ANIC as the straight drill holes. This is in agreement with the results of Büchs [34] and Haack [43] who reported that the use of inclined hole perforated discs in the grinding process improved the quality of the resulting ground meat. This might be caused by the entrance angle of the meat in the cutting set, which is lower if the drill holes are inclined, thus frictional forces are reduced, and fewer cells get disintegrated [4, 33, 34, 43]. Further, the assumption that fewer cutting levels cause less cell disintegration could be confirmed. This result contrasts with the finding of Barbut [2] and Haack, Schnäckel [3], who stated that the size reduction is most gentle if the size is reduced gradually. Considering the working principle and size reductions mechanism in a meat grinder, which is based on pressure build-up and shearing, the difference can be explained by less frictional force and less pressure acting on the meat if fewer cutting levels are used [3, 17, 44, 45]. The effect of reduced forces probably outweighs the large difference in the particle size before and after grinding.

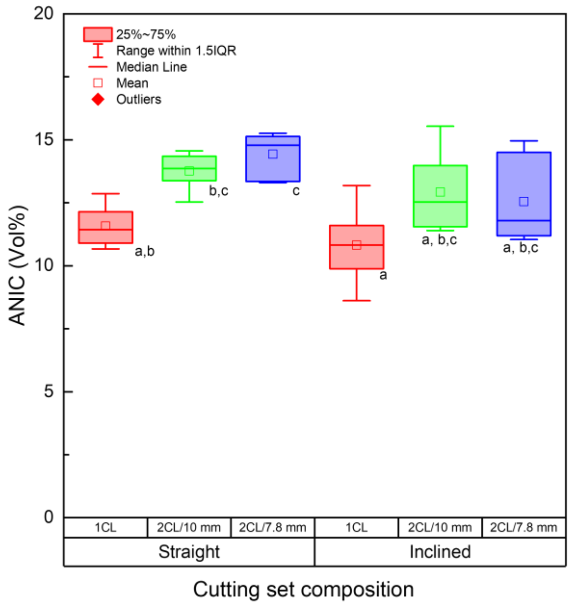


Figure VI-2: Data distribution of amount of non-intact cells (ANIC) as a boxplot for samples ground with different cutting set compositions (CL = cutting level). Data points with different letters are significantly different ($p < 0.05$).

However, the difference between the lowest and the highest ANIC amounts to about 4 Vol.% thus structural changes due to cutting set variations were relatively small. Furthermore, none of the examined cutting set variations exceeded the legal limit of 20 Vol.% of destructed cells [42].

Changes in mechanical process control parameters

The specific mechanical energy input was used as a parameter to rate the mechanical load of the material during the second grinding [18, 40, 41]. The SME during the second grinding with different cutting set compositions is illustrated in **Figure VI-3**

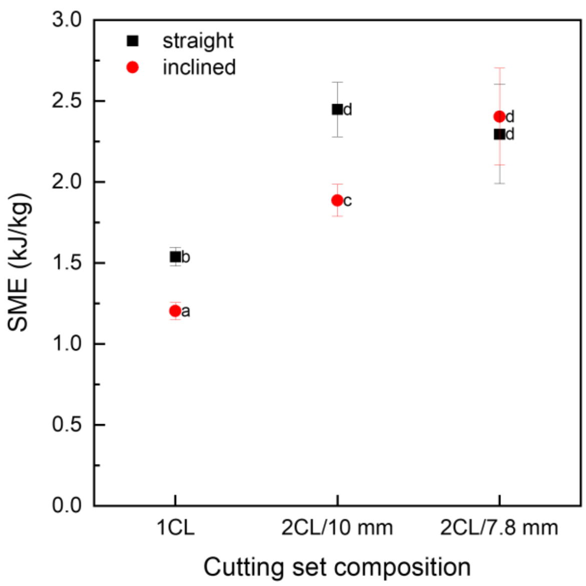


Figure VI-3: Specific mechanical energy input (SME) during the second grinding of beef equipped with different cutting set compositions (CL = cutting level). Data points with different letters are significantly different ($p < 0.05$).

Figure VI-3 shows that the SME was significantly reduced at fewer cutting levels (1CL/inclined: 1.2 ± 0.05 kJ/kg; 2CL/7.8 mm/inclined: 2.4 ± 0.3 kJ/kg) and when hole plates with inclined drill holes were used (1CL/inclined: 1.2 ± 0.05 kJ/kg; 2CL/straight: 1.54 ± 0.06 kJ/kg). This might also be attributed to the entrance angle of the meat to the drill holes, as previously described [17, 23, 33]. However, the use of different sizes of MHP did not significantly affect the energy input during grinding. Since Haack, Schnäckel [3] and Schnäckel, Krickmeier [46] reported a gentler grinding if particle sizes were reduced gradually, it was assumed that the 7.8 mm MHP might result in lower energy use, as the size reduction was more uniform. However, this could not be proven in this study.

Figure VI-4 shows the grinding screw torque during the second grinding with different cutting set compositions. Similar to the SME, the torque was lower when using fewer cutting levels (1CL/inclined: ca. 25 Nm; 2CL/straight: ca. 40 Nm). Torque is defined as a measure of the energy the motor needs to provide into the process [18]. Following, higher torque values go along with a stronger load in the processed meat which confirms the higher ANIC when using more cutting levels (**Figure VI-2**). Furthermore, it must be emphasized that pressure differences in the cutting set compositions occurred (**Figure VI-4**). Although the pressure in the cutting set using one cutting level was higher than that using two cutting levels, the ANIC (**Figure VI-2**) and SME (**Figure VI-3**) was lower. A pressure difference between the same composition at different geometry, e.g., 1CL/straight vs. 1CL/inclined, was not detected, thus the small difference in total drill hole area did not influence the pressure build-up. One can thus conclude that the additional shear forces due to the additional cutting plate outweigh the influence of the higher pressures.

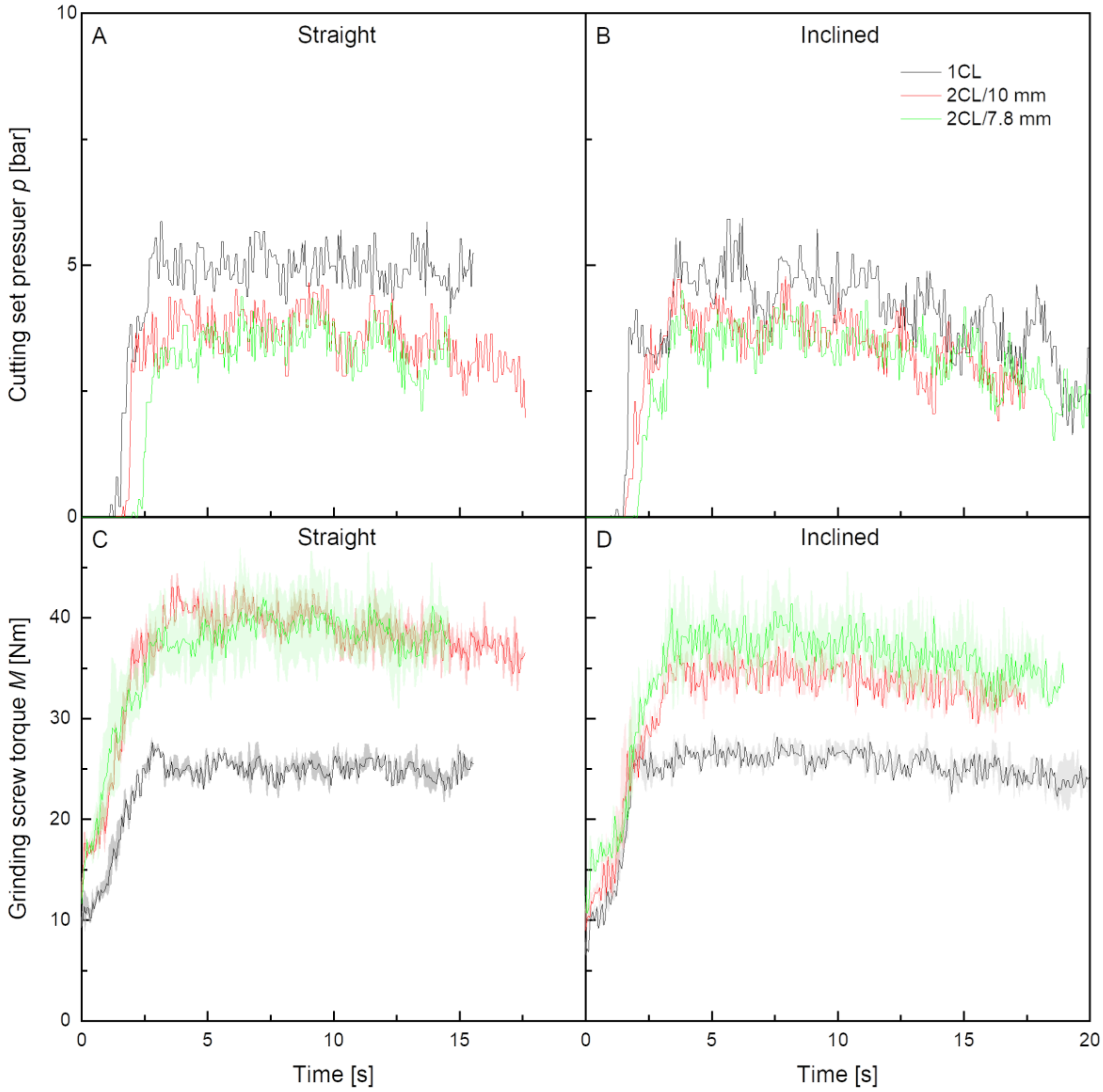


Figure VI-4: (A, B) Cutting set pressure in front of the final end hole plate and (C, D) grinding screw torque M during the second grinding of beef equipped with different cutting set compositions (CL = cutting level) and different drill hole geometries (straight (A, C), inclined (B, D)).

A Pearson correlation analysis between the process-related parameter SME and the structural parameter ANIC showed a significant, positive correlation ($r = 0.387$, $p = 0.020$). This underlines the hypothesis that increased mechanical stress caused more cell disintegration and is in agreement with previous studies [6, 47, 48]. It further indicates that both the composition of the cutting set as well as the choice of the hole plates and the size of the drill holes influence the induced frictional forces occurring during the grinding process and thus the quality of the end product. This conclusion was also reached by Haack, Schnäckel [3].

Influence on physicochemical and functional properties of hamburgers

The effect of cutting set composition on the physicochemical and functional properties of the hamburgers was investigated in terms of the raw hamburgers drip loss (DL) and firmness, the extracted meat solutions soluble protein content (SPC), the myoglobin (Mb) and metmyoglobin (MetMb) content and the fried hamburgers hardness. An optical and sensory evaluation was performed to assess the quality of the hamburgers. Further, possible correlations of the parameters with the ANIC and SME were checked and summarized in **Table VI-2**.

Table VI-2: Pearson correlation coefficients *r* of specific mechanical energy input (SME), amount of non-intact cells (ANIC), drip loss (DL), soluble protein content (SPC), metmyoglobin content (MetMb), myoglobin content (Mb), firmness (via forward extrusion) and hardness (via Warner-Bratzler shear cell (WBS)) of hamburgers and meat extracts produced with different cutting set compositions.

Parameter	SME (kJ/kg)	ANIC (%)	DL (%)	SPC (%)	MetMb (mg/mL)	Mb (mg/mL)	Firmness (extrusion) (N)	Hardness (WBS) (N)
SME (kJ/kg)	1	0.387*	-0.167	-0.409*	-0.364*	-0.542**	-0.129	-0.030
ANIC (%)	0.387*	1	0.082	-0.224	-0.300	-0.366*	-0.187	-0.337*
DL (%)	-0.167	0.082	1	0.554**	0.194	0.499**	-0.689**	0.127
SPC (%)	-0.409*	-0.224	0.554**	1	0.481**	0.767**	-0.546**	0.122
MetMb (mg/mL)	-0.364*	-0.300	0.194	0.481**	1	0.788**	-0.322	0.083
Mb (mg/mL)	-0.542**	-0.366*	0.499**	0.767**	0.788**	1	-0.554**	0.219
Firmness (extrusion) (N)	-0.129	-0.187	-0.689**	-0.546**	-0.322	-0.554**	1	-0.323**
Hardness (WBS) (N)	-0.030	-0.337*	0.127	0.122	0.083	0.219	-0.323**	1

*The correlation is significant at a level of 0.05

**The correlation is significant at a level of 0.01

The drip loss of the raw hamburgers ranged from 3.79 ± 0.99 % for the sample produced with one cutting level and inclined drill hole to 4.87 ± 1.07 % for the sample produced with two cutting levels, the 10 mm EHP and straight drill holes, as shown **Figure VI-5**. However, no statistical differences could be found between the samples. Since literature reports an increased leakage of intracellular contents from samples with an increased number of destructed cells [3, 25], it was initially postulated that the DL of the samples increases with increasing ANIC and SME. However, Pearson correlation analyses revealed no statistically significant correlation of the DL with the ANIC (r = 0.082, p = 0.634) or with the SME (r = -0.167, p = 0.329).

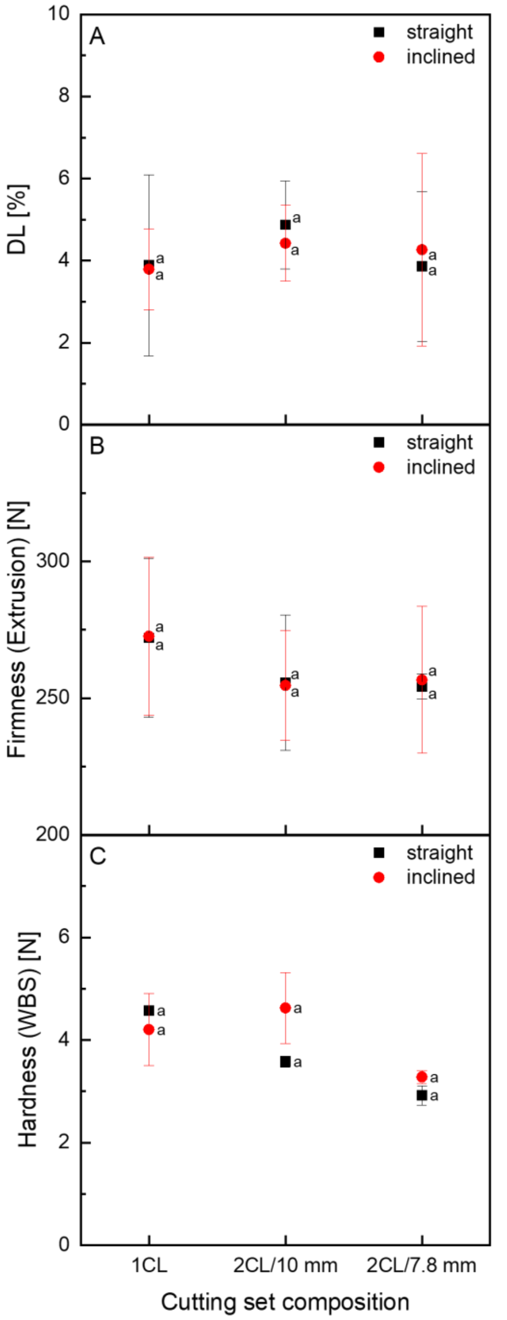


Figure VI-5: Characterization of the functional properties (A) drip loss (DL), (B) firmness, (C) hardness (via Warner-Bratzler shear cell (WBS)) of beef hamburger ground with different cutting set compositions (CL = cutting level). Data points with different letters are significantly different ($p < 0.05$).

The firmness of the raw ground meat was found to range from 254.27 ± 4.69 N (2CL/10mm/straight) to 272.67 ± 28.77 N (1CL/inclined). There too, no statistically significant differences were detected between the samples (**Figure VI-5**). In their research, Acton [25] and Chesney, Mandigo [49] reported that with decreasing particle sizes the cohesiveness and binding strength of burger patties increased due to increased cell disruption and release of intracellular contents. Based on those findings, it was assumed that samples having a higher

ANIC and thus consisting of a higher number of smaller cells would have a firmer structure. A Pearson correlation analysis however did not show a correlation between the firmness and the ANIC in the present study (*r* = -0.187, *p* = 0.288).

The hardness of the grilled hamburgers was analyzed using the Warner-Bratzler shear cell and varied between 2.92 ± 0.18 N (2CL/7.8 mm/straight) and 4.62 ± 0.69 N (2CL/10mm/inclined) (**Figure VI-5**). **Figure VI-5** shows that there is no statistically significant difference between samples ground with different cutting set compositions. As the Pearson correlation analysis revealed a significant negative correlation between the hardness and the ANIC (*r* = -0.337, *p* = 0.045, **Table VI-2**), the results are in line with the findings of Chesney, Mandigo [49] who reported that the maximum shear force in flaked pork meat decreased with decreasing particle size.

The SPC in the samples produced with different cutting sets ranged from 4.05 ± 0.96 % (2CL/7.8mm/straight) to 5.02 ± 5.07 % (1CL/ inclined) with no statistically significant differences between the different treatments (**Table VI-3**). Based on previous findings that intracellular compounds such as proteins or myoglobin may leak out when cell gets disintegrated, an increased SPC was assumed [3, 25, 49]. The Pearson correlation analysis revealed again no significant correlation between the SPC and the ANIC (*r* = -0.224, *p* = 0.188, **Table VI-2**), but a significant, negative correlation between SPC and the SME (*r* = -0.409, *p* = 0.013, **Table VI-2**). This indicates a reduced SPC with increasing SME and might result from the incorporation of soluble proteins in network formation upon increasing energy input. The results of this study are in line with the findings of a previous work, where the authors also found a decrease of SPC with increasing mechanical treatment of the samples [6]. However, this is in contrast with the report of Acton [25] and Haack, Schnäckel [3], as the present correlation would mean a reduced SPC with increasing SME.

Table VI-3: Characterization of the physicochemical properties soluble protein content (SPC), myoglobin content (Mb), and metmyoglobin content (MetMb) of extracts meat solutions from hamburgers produced with different cutting set compositions. Data points with different letters are significantly different ($p < 0.05$).

	Cutting set composition	SPC (%)	Mb (mg/mL)	MetMb (mg/mL)
Inclined	1CL	5.02 ± 0.57 [a]	0.06 ± 0.02 [a]	334.21 ± 256.18 [a]
	2CL/10 mm	4.58 ± 0.73 [a]	0.06 ± 0.02 [a]	280.37 ± 149.87 [a]
	2CL/7.8 mm	4.60 ± 0.61 [a]	0.05 ± 0.01 [a]	198.08 ± 51.50 [a]
Straight	1CL	4.19 ± 0.69 [a]	0.05 ± 0.01 [a]	147.41 ± 52.08 [a]
	2CL/10 mm	4.62 ± 0.64 [a]	0.05 ± 0.01 [a]	183.09 ± 34.22 [a]
	2CL/7.8 mm	4.05 ± 0.96 [a]	0.04 ± 0.01 [a]	138.98 ± 13.42 [a]

The Mb content of the extracted meat solutions was determined as a measure for chemical changes, such as oxidation. Mb is an intracellular compound and is released during intense processing. Similar to the SPC, it was therefore assumed that the Mb content increases in samples with higher ANIC [38, 50]. Contrary to that hypothesis, no differences in the Mb content in the samples' meat extract could be detected in the present study (**Table VI-3**). The Mb content of the samples produced with different cutting set compositions was around 0.05 mg/mL for all samples. A Pearson correlation analysis (**Table VI-2**) based on this, however, was able to find a significant negative correlation between Mb and ANIC ($r = -0.336$, $p = 0.028$), as well as a highly significant negative correlation between Mb and SME ($r = -0.542$, $p = 0.001$). This shows that the correlation is generally valid but does not apply to the range of the examined parameter variations, since the differences are too marginal.

The sensory and optical perception of the hamburgers were tested by a sensory panel. The quality of the hamburgers was rated in terms of hardness, juiciness, texture, and overall acceptability in the sensory analysis as well as the coarseness, the amount of batter-like substance, and the overall acceptability in the optical analysis. **Table VI-4** shows the results of the sensory and optical evaluation by the panel. All parameters in the sensory evaluation ranged around a sensory score of 5, indicating that all tested samples were comparable with each other and the pre-defined standard (= sensory score of 5). The statistical differences in the sensory attributes of the hamburgers produced with different cutting set compositions were either non-existent (juiciness, overall acceptance, **Table VI-4**) or only marginally expressed (hardness, texture, **Table VI-4**). A Pearson correlation analysis between the firmness of the hamburger in the forward extrusion testing and the sensory parameters juiciness and acceptability, respectively, revealed a significant negative correlation (juiciness: $r = -0.246$, $p = 0.040$, acceptability: r = -0.303, p = 0.011). This indicates that samples were rated juicer and better than the standard if the ground meat was less firm, i.e., less force was necessary to extrude the sample in the forward extrusion. An explanation for this might be that samples with lower firmness might be able to better keep the moisture which improves the sensory perception of juiciness. Comparable results were also obtained in the sensory evaluation of the samples as the sensorially determined hardness positively correlated to the juiciness of the samples (r = 0.595, p = 0.001), Thus, this trend was underlined.

Table VI-4: Sensory evaluation of hamburgers in terms of hardness, juiciness, texture, and overall acceptability produced with different cutting set compositions and optical assessment of hamburgers halves in terms of coarseness, amount of batter-like substance, and overall acceptability. Prior to each evaluation, a reference was offered for examiner training (sensory score = 5). Sensory score 0 = harder/ dryer/ compacter/ worse/ finer/ more batter-like substance and 10 = softer/ juicer/ looser/ better/ coarser/ less batter-like substance. CL = cutting level. Data points with different letters are significantly different ($p < 0.05$).

		Sensory				Optical		
Cutting set composition		Hardness	Juiciness	Texture	Overall acceptability	Coarseness	Amount of batter-like substance	Overall acceptability
Inclined	1CL	5.00 ± 1.35[a,b]	4.73 ± 1.55[a]	4.61 ± 1.47[a,b]	4.73 ± 1.44[a]	5.11 ± 0.99[c]	5.06 ± 1.14[c]	4.93 ± 1.10[a]
	2CL/10 mm	5.38 ± 1.20[a,b]	4.65 ± 1.32[a]	5.02 ± 1.21[a,b]	4.88 ± 1.34[a]	4.84 ± 0.97[b,c]	4.66 ± 1.19[a,b]	4.83 ± 0.72[a]
	2CL/7.8 mm	5.48 ± 1.38[b]	5.27 ± 1.38[a]	5.16 ± 1.50[b]	5.33 ± 1.61[a]	4.28 ± 1.24[a,b]	4.24 ± 1.23[a]	4.53 ± 1.21[a]
Straight	1CL	4.99 ± 1.22[a,b]	4.55 ± 1.24[a]	4.84 ± 1.31[a,b]	4.82 ± 1.29[a]	4.78 ± 0.97[b,c]	4.44 ± 1.03[a,b]	4.70 ± 0.85[a]
	2CL/10 mm	4.54 ± 1.30[a]	4.31 ± 1.62[a]	4.19 ± 1.30[a]	4.71 ± 1.17[a]	4.26 ± 1.19[a,b]	4.32 ± 1.23[a,b]	4.59 ± 0.94[a]
	2CL/7.8 mm	5.53 ± 1.41[b]	5.06 ± 1.68[a]	5.34 ± 1.56[b]	5.14 ± 1.49[a]	3.98 ± 1.30[a]	3.87 ± 1.52[a]	4.38 ± 1.13[a]

The overall acceptability of all hamburgers in the optical evaluation ranged around the standard with a sensory score of 5 and was therefore comparable amongst all samples (**Table VI-4**). However, some differences were detected in the optical evaluation in terms of particle size. The hamburger produced with the inclined cutting set using one cutting level (1CL/inclined) was rated as the coarsest sample, whereas the hamburger produced with the straight cutting set having a 7.8 mm MHP was rated the finest. Comparable results were obtained for the ANIC, which is reasonable since they are interrelated. A Pearson correlation analysis indicated a significant positive correlation between the firmness of the ground meat in forward extrusion and the optical assessed particle size (coarseness: $r = 0.359$, $p = 0.003$, amount of batter-like substance: $r = 0.323$, $p = 0.006$). These results indicate that finer meat masses with smaller particle sizes were softer and less cohesive. Those observations are in accordance with the findings of Berger, Witte [7], who found a lower firmness in samples with finer particle sizes. The highly significant negative correlation between the optically assessed particle size and the SME ($r = -0.217$, $p = 0.001$) underlines the previous statement. Samples with lower SME input during grinding were rated as coarser whereas samples with higher SME were described to have a finer structure.

A two-way ANOVA ($p \leq 0.05$) was performed, which compared the influence of cutting set composition and the geometry of the drill holes on sensory parameters. It was found that the differences are small and if differences occur, the cutting set combination had a stronger influence than the geometry of the drill holes. In most cases, the combination 2CL/7.8 mm differed from the other samples. However, even though small differences in the individual sensory parameters could be identified, the overall acceptability in the sensory and optical evaluation was the same for all samples.

As the differences in the sensory and optical analyses are quite small, all samples are ranging around the standard reference (sensory score of 5). This indicates that the sensory quality of the samples is neither increased nor decreased by the cutting set composition. These results go along with the analytical physicochemical (**Table VI-3**) and functional parameters (**Figure VI-5**) which also did not point out any major differences between the samples produced with different cutting sets. Taking all results together, it can be concluded that the variation of the cutting set did not, or only to a small extent influence the functional and quality parameters of the hamburgers.

Conclusion

When grinding with different cutting set compositions, differences in SME and smaller differences in ANIC were observed, i.e., the variation in the cutting set influences the stress and the structure of the meat to some extent. However, these relationships cannot be determined analytically or by sensory analysis in the present study. This shows, that although the SME of the grinding with variating cutting sets differed significantly, the structure of the meat (ANIC) was only altered to a small extent. The study confirms previous assumptions that the grinding process due to the short residence time of meat in the grinding zone is already designed to be relatively gentle and that fluctuations in ANIC are more likely to be caused by raw material variations, e.g., different breeds, age, species of cattle or processing temperature. Under the conditions tested, the composition of the cutting set was found to be of minor importance to product quality and functionality. Moreover, results suggest that even though structural parameters vary as a function of process parameters (SME), this may not noticeably influence the function and quality of the product, as long as the cutting set operates optimally (i.e., holes plates are not abraded, and knives remain sharp). The results of this study are of practical relevance, as they broaden the possibilities of production processes design and setup without having an impact on the product functionality and quality.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

All relevant data is included in the article, further details are available upon request.

Authorship

Lisa M. Berger: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Project administration, Resources, Writing - original draft, Writing - review & editing, Methodology, Software, Validation. Felix Adam: Data curation, Investigation. Monika Gibis: Funding acquisition, Project administration, Supervision, Validation, Writing - review & editing. Franziska Witte: Funding acquisition, Project administration, Writing - review & editing. Nino Terjung: Funding acquisition, Project administration. Jochen Weiss: Funding acquisition, Project administration, Supervision, Writing - review & editing.

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Concluding Remarks and Impact of the Thesis

The study showed that there is a general relationship between structure and functionality in ground meat. The basic mechanism of the interaction of the different components and their effects on the interactions were examined and could be clarified. Thus, it has been shown that the system "ground meat" can be described as a "mixed sample", in which the product characteristics are defined by mixing effects of less and highly comminuted components of the different phases. The decisive element for changes in the physicochemical and functional properties is the interaction of larger and smaller cell fragments as well as dissolved compounds. The properties of the product are predominantly defined by the properties of the dominant phase (**Chapter II**). A correlation between the amount of non-intact cells (ANIC) and functionality (e.g., drip loss $r = -0.749$, soluble protein content $r = -0.670$, firmness $r = -0.389$; $p < 0.05$) could therefore be confirmed but the importance of this observation was limited in the sense that effects only occurred at very high ANIC (ANIC > 35 Vol.% at $x_{meat\ batter} > 25$ %). These high ANIC values up to 35 Vol.% cannot be expected to be reached with standard processing methods and are therefore not of practical relevance.

Since a correlation between structure and functionality could be established in certain ranges (**Chapter II**), a correlation of process to functionality was also assumed based on literature research. Accordingly, various investigations were carried out.

It could be shown that the characteristics of the raw materials, in particular, their temperature (-6 °C, -12 °C) and the proportion (0, 15, 30, 45 %) of frozen material, influence the load on the raw material during processing and thus lead to a change in the ANIC (**Chapter III**). These higher loads can be measured by increased torques as machine parameters. A higher proportion of frozen meat results in colder processing temperatures. The meat thus has higher specific cutting forces, which increases the applied forces during grinding and thus the ANIC. However, since the temperature of the frozen meat had little effect, it was concluded that the frozen temperature only affects the specific cutting force to a lesser extent and thus has minimal effect on the overall system. These results indicate a range in which raw material preparation can be customized to the manufacturing conditions. An adjustment of the frozen content and temperature can however yield ecological and economic advantages. Since an influence on analytical parameters such as firmness or hardness could be determined, the variation of temperature and the proportion of frozen meat represents a possibility to specifically influence product parameters in a certain range.

Based on the previous findings in **Chapter III**, it was assumed that re-feeding frozen, partly crushed hamburgers would cause similar effects. Contrary to expectations, it was shown that re-feeding frozen hamburgers up to a proportion of 20 % does not affect the structure and functionality of hamburgers (**Chapter IV**). This can be explained by the formation of smaller fragments and exhibition of mixed mass bulk properties, already small pre-grinding particle size of re-fed material, and temperature equalization between the frozen and cooled material causing softening of the frozen particles. The results show that up to 20 % of frozen, already processed hamburgers can be added to the manufacturing process without affecting the properties of the final product. This provides manufacturers of ground meat and ground meat products with the opportunity to recycle visually deviating hamburgers without incurring quality deterioration, which has both economic and ecological advantages.

Investigation of a standard hamburger production process revealed that the greatest changes in structure and functionality were induced by the process step of grinding. Mixing and forming the hamburgers had little effect (**Chapter V**). This implies that the grinding process should be optimized if the processing of ground meat and ground meat products should be designed more gently. Since the main comminution performance takes place in the cutting set, the effects of changing the cutting set variation on structure and functionality were investigated (**Chapter VI**). Different cutting set variations have been shown to affect the stress during grinding (SME) and the structure (ANIC), but only to a small extent. Moreover, these process variations could not be confirmed analytically or by sensory tests. Therefore, the cutting set composition is not decisive in making the process gentler.

As a quintessence, this work has shown that through gentle processes of mincing on a pilot plant scale, an amount of non-intact cells of around 25 - 30 Vol.% is usual for craft style and industrial processes of meat. To facilitate a gentle process, a reduction of frozen meat content (max. frozen meat content = 30 %) and an optimal setting of process parameters, e.g., the maintenance and composition of the cutting set, are of importance.

It must be emphasized that cell disintegration, to a certain extent, characterizes the intended unit operation of particle size reduction and is therefore unavoidable during the grinding process. It has been shown that there is a fundamental relationship between process load, the formation of ANIC, and structural change affecting hamburger functionality (**Chapter II**). Histochemical analyses are, for now, the only method to detect cell disintegration in meat products and enable a correlation of structure (ANIC) and process (SME) parameters as done in this thesis.

However, it was found that raw material variations have a strong effect on the analytical parameters. It is known that factors such as sex, age, or breed of the animals impart key product characteristics such as juiciness, water-holding capacity, or hardness. Despite the careful selection of the raw materials, raw material-related fluctuations were also observed in the present investigations, which were noticeable in the large standard deviations of the measured values.

Thus, raw material selection and raw material quality control must be emphasized once again as important tools for quality assurance when generating meat preparations such as hamburgers or meat products such as sausages. In general, it was observed that even if effects on ANIC could be achieved, i.e., changes in structure were apparent, changes in analytical or sensory properties were rarely detected. This means that the histologically determined ANIC does not automatically allow conclusions to be drawn about the quality of the hamburger and customer perception.

This has two main implications:

i) Reliability of the official method

In Germany, the histological method according to § 64 LFGB (L 06.00-13) is mainly used to monitor and validate the quality of ground meat and ground meat products. Here, the ANIC is determined visually and manually by the point-count method. A defined limit value of 20 Vol.% should not be exceeded to be allowed to label the product accordingly. In our investigations, it was found that the ANIC depends not only on the raw material but also on the operator and the embedding method used. Thus, the method is very subjective and hardly comparable, which makes the validity of the method as a means of official monitoring questionable. In addition, the set limit of 20 Vol.% must be put into question, since values above the limit value were already found during the most gentle processing of the meat in the standard process on a pilot plant scale. Most importantly, this did not have any negative effects on the functionality and quality of the samples. In addition, the fluctuations resulting from sample preparation and the operator outweigh the informative value of the method at this limit. To optimize the existing histological method, automation, and machine learning techniques, e.g., AI-assisted evaluation, might be explored. For this, the software would need to be trained with a large and versatile database. This would not only eliminate the subjectivity of the evaluation but also enable the rapid examination of a larger sample size thereby improving reproducibility and accuracy of the method. In addition, standardization of sample preparation (e.g., cryo or paraffin embedding) would facilitate a better comparison of data from different sources. As previously mentioned, the histological method is susceptible to raw material variations. To be able to consider these in the sample evaluation, an examination and characterization of the raw material would be advantageous. This would allow the values to be correlated and normalized. However, the practical implementation of this in large-scale production is difficult.

ii) Redefinition of the quality terms for hamburgers

The redefinition of the quality standards of hamburgers could lead to a redefinition and/or expansion of the analytical parameters of the official control procedure of ground meat and ground meat products.

In the investigations, it was found that there are no or only minor correlations between ANIC, physicochemical and functional properties of the hamburgers. Consequently, although ANIC describes the structure of the meat, it does not provide any direct information about the quality of the product. Thus, the concept of quality for ground meat and ground meat products should be redefined and not based solely on the parameter of a histologically determined ANIC. Direct quality parameters (e.g., color, loss on frying, sensory evaluation) must be determined to assess the quality of the hamburgers. In addition, a large-scale consumer study reflecting general consumer expectations is still lacking and might be worth carrying out then in future studies. An indirect derivation via physical parameters is not sufficient but can serve as a reference.

Outlook

The studies in this thesis have led to new insights into the bulk properties of ground meat and ground meat products with a special emphasis on hamburgers (**Chapter II**) and the effect of raw material (**Chapters III & IV**) and process parameter variations (**Chapters V & VI**) on the characteristics of hamburgers. In the following, some research areas that could be of interest to further studies are outlined:

Investigation of further raw material variations

This work focused on the use of beef as raw material for ground meat and hamburger production. However, the use of pork meat is also of substantial importance in the meat industry, with the main application of ground meat for home use or further use in meat product manufacturing. In this thesis, it was demonstrated that the function principle mainly depends on the interaction of the raw material and the grinder. Thus, raw material properties strongly affect the efficiency and quality of grinding. It is known that basic characteristics, e.g., cutting resistance, differs among different species and chemical composition, e.g., fat content. Following, variations in raw material origin and composition might change the interaction of meat and grinder and thus the processability. Further research should thus focus on the effects of raw material characteristics on its processability and the impact on the product characteristics. To achieve optimal product quality an adapted process for each raw material, e.g., pork, beef, chicken, might be necessary and could be set up with the gained knowledge of this further research.

Investigation of further grinding parameters

In addition, several other grinding parameters are known to influence grinding efficiency and quality. Future studies might therefore focus on the effect of the grinding screw design, the raw material feeding system, the design of knives, the sharpness of knives and plates (i.e., maintenance control cycle), and their combination with the rotational speed of the screw on structure, functionality, and quality of ground meat. It is hypothesized, that changes in the screw design strongly influence the conveying behavior and the pressure buildup in the grinder, that a continuous raw material mass flow and an optimized rotational speed enable an optimal mass flow, and that the sharpness of knives and plates positively impact the structure by facilitating clear cuts. Furthermore, the use of process automation based on integrated temperature, pressure, and torque monitoring could contribute to process optimization. This coordinated measurement control cycle would be able to optimize the grinding process.

It is further assumed, that knife geometry, number of blades, and angle of blades further impact the cutting behavior and the volume flow and might therefore also be investigated to design a customized production process. This might be combined with the knowledge of the previously mentioned aspect of raw material properties. These findings might not only be used for quality optimization and to deliver targeted solutions for specific use cases but also process optimization regarding economic and ecological aspects.

Industrial application scale-up

To achieve an ecological and economic impact, the gained knowledge must be transferred into application-oriented solutions, to facilitate gentler craft style and industrial-scale production of ground meat and hamburgers. Since ground meat and ground meat products are popular and frequently consumed product categories, small changes and optimization in production may have a large impact. Future studies and projects should therefore target feasible and viable solutions for scale-up and could also include process automatization and inline measurement approaches.

Defining consumer expectation

It was shown that the actual procedure to assess the quality of beef hamburgers in official food control lacks the assessment of quality attributes. Thus, the quality perception of ground meat and ground meat products must be defined. Therefore, a large-scale consumer study reflecting general consumer expectations might be carried out in future studies and could help to define basic quality parameters for ground meat and ground meat products.

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Eidesstattliche Versicherung

Eidesstattliche Versicherung über die eigenständig erbrachte Leistung

gemäß § 18 Absatz 3 Satz 5 der Promotionsordnung der Universität Hohenheim für die Fakultäten Agrar-, Natur- sowie Wirtschafts- und Sozialwissenschaften

- Bei der eingereichten Dissertation zum Thema *Process, Structure and Function Relationship in Ground Meat* handelt es sich um meine eigenständig erbrachte Leistung.

- Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

- Ich habe nicht die Hilfe einer kommerziellen Promotionsvermittlung oder -beratung in Anspruch genommen.

- Die Bedeutung der eidesstattlichen Versicherung und der strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt.

Die Richtigkeit der vorstehenden Erklärung bestätige ich: Ich versichere an Eides Statt, dass ich nach bestem Wissen die reine Wahrheit erklärt und nichts verschwiegen habe.

Hohenheim, 11.07.2023

Ort und Datum

Unterschrift

Curriculum Vitae

Name	Lisa Marie Berger
Address	Eckbergstr. 14
	72135 Dettenhausen, Germany
Date of birth	27.09.1993 in Tübingen
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Education

Oct. 2016 – Oct. 2019 **Master of Science "Food Science and Engineering"**
Universität Hohenheim, Stuttgart
Master Thesis: "Influence of Water Content in Egg White Powder on their Functional Properties and Microbial Safety"
Final Mark: 1.5

Oct. 2012 – Apr. 2016 **Bachelor of Science "Lebensmittelwissenschaft und Biotechnologie"** Universität Hohenheim, Stuttgart
Bachelor Thesis: "Physicochemical and Technofunctional Properties of Selected Plant Proteins"
Final Mark: 1.8

Sept. 2004 – July 2012 **Abitur** (Mark 1.4)
Geschwister-Scholl-Schule Tübingen

Professional Experience

Since April 2022 **Co-founder and Food Technologists at Better Food Consulting**
Main Topics: Alternative Proteins, Novel Ingredients, Process and Product Development and Optimization, Startup Consultancy, Upcycling, Sustainability

Since Sept. 2020 **Co-founder, Managing Associate, CMO & CTO at ZBS Food UG (haftungsbeschränkt)**
Main Topics: Product Development, Sustainability, Circular Food System, Food Waste Reduction, Quality Management, Website and Online Shop Support

Since Dec. 2018	**PhD candidate at University of Hohenheim, Dept. of Food Material Science** Topic: "Process, Structure and Function Relationship in Ground Meat."
June - July 2018	**Student assistant at University of Hohenheim** Supervise practical course of the department of "Technology and Analyses of Plant Food Stuff"
Aug. - Sept. 2017	**Holiday job at Daimler Gastro GmbH, Sindelfingen** Work in the canteen of Daimler
Mar. - Aug. 2016	**Internship at Unilever Deutschland Holding GmbH, Heilbronn** In the Research & Development department with specification on dry products for major consumers (Team Food Solutions Dry Products). Major tasks: data analysis; optimization of dry products regarding scientific methods; setups and analysis of „Design of Experiments"; Research in the field of powder flowability and using the knowledge for optimization and improvement of the line efficiency.
Mar. - Aug. 2015	**Internship at Schwartauer Werke GmbH & Co. KGaA, Bad Schwartau** In the department „Innovation and Quality" with focus on product development for „Corny". Major Tasks: developing new recipes and technologies; manufacturing of samples; product optimization; preparation, performance and analysis of different sensory tests.
Sept. - Oct. 2014	**Internship at Schönbuch Braumanufaktur, Böblingen** in product monitoring and quality management
Aug. - Sept. 2013	**Holiday job at Daimler, Sindelfingen** Department: Factory Development
Aug. - Sept. 2012	**Holiday job at Daimler, Sindelfingen** Department: Electrical Engineering
Aug/ Sept. 2009	**Social Internship at GWW, Holzgerlingen** Facility for disabled people
April 2008	**BOGY – Internship for vocational orientation at nutrition counselling at the children's hospital in Tübingen**

Languages and IT-Skills

Native Language	German
Further Languages	English (UniCert II) Latin (Latin proficiency certificate "Großes Latinum")
Microsoft Office	very good skills
WordPress	good skills
SPSS & Origin	very good skills

Different recipe and administration software

Additional Skills

Dez. 2020	**DEKRA-Certificate „IBQsys Corporate Planning"** Training in International Business Qualification and Corporate Planning
June 2019	**Participation at SEA:Start Workshop** Training in Social Entrepreneurship
Sept. 2018	**Six Sigma Yellow Belt** Training in Quality Management
Jan.- Dec 2018	**Participation at EIT Food project "Foodio-Food Solution Master Class"** Development of an innovative, sustainable, and dairy based product. The project covers all essential steps of the product development process e.g., idea generation, market research, development of business case and finical planning, production trails and packaging development. It takes place in an international team with the collaboration of different international universities and partners from the food industry.
Oct. 2015	**Participation in sensory workshop at Universität Hohenheim** Learning basics in sensory and sensory methods
Sept. 2015	**UniCert II-Certificate** (Mark: 1.9) with scientific specialisation
May 2012	**Classified as "Trainer C Breitensport/ DSV Grundstufe Ski Alpin**"
Jan. 2011	**Honorary work as a „Skimentor" at Geschwister-Scholl-Schule Tübingen**
Jan. – May 2011	**Participation at "Deutschen Gründerpreis für Schüler"** 1st place in Baden-Württemberg, 10th place nationwide

Honorary Work

2019 - 2021	**Honorary Ski School Directory at sports club VfL Dettenhausens**
Since 2019	**Honorary Board Member at „s'Dettahäuser Fleckatheater"**
Since 2013	**Member of the Education Team of the Skiing Club of the VfL Dettenhausen**
Since 2011	**Honorary Board Member of the Skiing Club of the VfL Dettenhausen**
Since 2008	**Honorary work at the sports club VfL Dettenhausen**

Prizes and Awards

April 2023	**Travel Award "Universitätsbund Hohenheim" to present actual research at the IFT First Exhibition in Chicago, USA**
July 2012	**e-fellows scholarship**

Hohenheim, 11.07.2023

Place and Date

Signature