# PLANT PROTEIN GELS AS BINDERS IN MEAT PRODUCT ANALOGUES

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Submitted by
Eva Maria Herz

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| | |
|---|---|
| Dean: | Prof. Dr. rer. nat. Uwe Beifuss<br>Fachgebiet Bioorganische Chemie<br>Institut für Chemie<br>Universität Hohenheim |
| 1st Reviewer: | Prof. Dr. Jochen Weiss<br>Fachgebiet für Lebensmittelmaterialwissenschaft<br>Institut für Lebensmittelwissenschaft und Biotechnologie<br>Universität Hohenheim |
| 2nd Reviewer: | Prof. Dr.-Ing. Mario Jekle<br>Fachgebiet für Pflanzliche Lebensmittel<br>Institut für Lebensmittelwissenschaft und Biotechnologie<br>Universität Hohenheim |
| 3rd Examiner: | Prof. Dr. Anja Wagemans<br>Fachgebiet Food Colloids<br>Institut für Lebensmitteltechnologie und Lebensmittelchemie<br>Technische Universität Berlin |
| Oral examination on: | 17.02.2023 |

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- **Chapter I:** Shared first authorship of Eva Herz and Pascal Moll, who conceptualized and wrote the original draft. Christophe Schmitt proof-read the manuscript and was provided funding. Prof. Dr. Jochen Weiss also conceptualized, wrote, and proof-read the manuscript.
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- **Chapter IV:** Eva Herz designed and supervised the study, interpreted results, and wrote the manuscript. Till Kinne conducted most of the experiments as part of her master thesis. Dr. Nino Terjung was involved in project administration and funding acquisition.
- **Chapter V:** Eva Herz designed and supervised the study, interpreted results, and wrote the manuscript. Till Kinne conducted most of the experiments as part of her master thesis. Dr. Nino Terjung was involved in project administration and funding acquisition.

# List of Publications

## Publications in peer-reviewed journals

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Herz, E., Schäfer, S., Terjung, N., Gibis, M., Weiss, J. (2021) 'Influence of transglutaminase on glucono-δ-lactone-induced soy protein gels' ACS Food Science and Technology, 1, 8, pp. 1412–1417

Herz, E., Kinne, T., Terjung, N., Gibis, M., Weiss, J. (2023) 'Influence of extrudate to SPI-gel-binder ratios and transglutaminase crosslinking on texture of a plant-based salami analogue' Future Foods, 7, 100235

Herz, E., Moll, P., Schmitt, C., Weiss, J. (2023)'Binders in foods: definition, functionality, and characteristics' Food Hydrocolloids, 145, 109077

## Forthcoming publications in peer-reviewed journals

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## Oral presentations

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## Other publications

Ralla, T., Herz, E., Salminen, H., Edelmann, M., Dawid, C., Hofmann, T., Weiss, J. (2017) ‘Emulsifying properties of natural extracts from *Panax ginseng L*’ Food Biophysics, 12 (4), pp. 479–490

Zeeb, B., Herz, E., McClements, D.J., Weiss, J. (2014) ‘Impact of alcohols on the formation and stability of protein-stabilized nanoemulsions’ Journal of Colloid and Interface Science, 433, pp.196–203

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# Symbols and Abbreviations

| Symbol | Definition | Unit |
|---|---|---|
| $a_w$ | Water activity | - |
| $\gamma$ | Strain | % |
| $\dot{\gamma}$ | Shear rate | s-1 |
| $\gamma_L$ | limit of linear viscoelastic region | % |
| G’ | Storage modulus | Pa |
| G’’ | Loss modulus | Pa |
| h | Hours | (h) |
| $\omega$ | Angular frequency | rad/s |
| pH | Potential of hydrogen | - |
| pI | Isoelectric point | - |
| rpm | Revolutions per minute | 1/min |
| t | Time | s |
| T | Temperature | °C |
| $T_g$ | Glass Transition Temperature | °C |
| w/w | Weight per weight | - |
| $\zeta$-potential | Zeta-potential | mV |

**Abbreviation**

| | |
|---|---|
| AiF | Arbeitsgemeinschaft industrieller Forschungsvereinigungen |
| ANOVA | Analysis of variance |
| CLSM | Confocal laser scanning microscopy |
| DM | Dry matter |
| EC-FCN | emulsified and crosslinked fat crystal network |
| e.g. | for example (Latin: exempli gratia) |
| FEI | Forschungskreis der Ernährungsindustrie e.V. |
| GDL | Glucono-δ-lactone |
| GHG | Greenhouse gases |
| i.e. | that is to say (Latin: id est) |
| IEP | Isoelectric point |
| LVE | Linear viscoelastic |
| p-value / P | Probability value |
| RH | Relative humidity |
| SPI | Soy protein isolate |
| TG | Transglutaminase |
| TPA | Texture profile analysis |
| WG | Wheat gluten |

# Summary

Concerns about the environmental, ethical and health impact associated with especially large quantities of meat consumption have fostered the development of plant-based analogues of meat products, which has become a major trend in the food industry during the last years. Although many meat analogue products are nowadays available in supermarket shelves, scientific knowledge, especially of composite meat analogues, ingredient interaction, and texture formation are still scarce. Processed meat products, such as sausages, usually consist of comminuted fibrous meat particles, comminuted adipose tissue, resulting in fat particles and solubilized myofibrillar meat proteins that are solubilized from the muscle meat by comminution and the addition of salt. This salt-solubilized meat proteins have later the function as binder. These three major components should therefore be mimicked to obtain meat analogue products with similar texture and nutritional properties compared the original meat products. Anisotropic structures that resemble the fibrous texture of meat can be formed with globular plant proteins, such as soy protein, with processing techniques, such as extrusion or the shear cell technology. To mimic the texture of animal fat tissue, which has in contrast to plain plant fat elastic properties, an approach has recently been presented, where plant oil was emulsified with plant proteins. The emulsion was subsequently cross-linked covalently with the enzyme transglutaminase, leading to an emulsion-gel with similar texture properties as animal fat tissue. To combine these two materials and hold them together in a matrix for plant-based meat analogues, a binder is necessary. Binder materials for meat analogues have so far not been studied systematically and knowledge on their properties and design is lacking. In commercial products, methylcellulose or other hydrocolloids are often used as binder, however, consumer acceptance of such ingredients is declining. Thus, the aim of this thesis was to investigate the properties of plant protein gels as binders for meat analogues. The approach was based on the hypothesis that plant protein suspensions will be mixed with comminuted extrudates and fat mimetic particles and subsequently gelled to form a coherent matrix with similar texture properties as meat products. Different gelling mechanisms, such as heat-, acid- and enzyme-induction as well as drying were investigated.

In the first part of this thesis (**Chapter II**), the ability of a soy protein gel to act as binder between a layer of high-moisture extrudate and a fat mimetic was investigated. First, heat-induced gelling properties of concentrated soy protein isolate (SPI) suspensions (10-16% SPI) with various coagulants ($CaSO_4$, $CaCl_2$, $MgCl_2$), acid-forming glucono-δ-lactone (GDL) and

the enzyme transglutaminase (TG), inducing covalent cross-links, were studied. The results showed that with TG, the gel hardness could be increased significantly. Thus, in the following a heat-induced (85 °C) and a TG-induced SPI gel (14% w/w) were applied in different quantities as binder between a layer of extrudate and fat mimetic. With a tensile test, the maximum force and work of adhesion were analyzed. The results showed that the harder, TG-induced SPI-gel resulted in distinctly higher binding strength. We therefore concluded that covalent, isopeptide bonds probably result in improved binding strength that is likely to be strong enough for further processing of meat product analogues, such as slicing.

While a TG-induced SPI-gel was shown to be a promising binder for meat product analogues with neutral pH, the enzyme's pH-optimum of 7 causes a challenge for the application in acidic food products, such as fermented sausage analogues. Therefore, in (**Chapter III**), a different approach was used in which TG and slowly acidifying GDL were added at the same time, to see if cross-linking of the proteins could be facilitated before the pH became too low. To that purpose, TG and GDL were added separately or simultaneously to 10-15% SPI-suspensions. Texture analysis showed that SPI gels, induced by a combination of GDL and TG, at pH 5−6, were harder than gels induced only by the addition of GDL and as hard as gels induced by TG at the optimum pH of 7. Decreased tan δ values and confocal laser scanning microscopy images indicated that protein cross-linking had taken place in combined gels. Taken together, the results imply an initial covalent network formation induced by TG, while the slow acidification by GDL leads to agglomeration of non-crosslinked proteins to the network, due to weakened electrostatic repulsion. In summary, the combined addition of TG and of slowly acidifying GDL leads to acidic gels with enhanced textural properties and therefore constituting a promising option for the use as binder is acidic meat analogue products, such as fermented sausage analogues.

In **Chapter IV**, the previously studied SPI-gels were then applied as binder to hold together fat mimetic particles and extruded proteins with a fibrous structure to resemble the structure of classical dry-fermented sausages. GDL-induced and GDL+TG-induced SPI-gels were compared and applied in different binder-to-extrudate ratios (30-70%). The sausage analogues were processed similarly to traditional sausages by chopping, smoking, and drying and analyzed in terms of composition, instrumental texture, and sensory analysis. The texture analysis showed that with increasing binder content, the cohesiveness increased, while the hardness decreased. This was confirmed in a sensory analysis. Beyond that, the addition of TG to the

binder resulted in slightly increased hardness and cohesiveness but drying of the sausages had a distinctly higher influence on increasing the hardness. From the results of the study, we concluded that with a SPI-gel as binder, the structure is dominated by the extrudates at low binder contents, resulting in high hardness but a lack of cohesiveness, while at higher binder contents, the binder dominates the structure, leading to sufficient cohesiveness but decreased hardness. A sufficient cohesiveness and hardness could not be achieved with a single formulation using SPI-gels as binder, indicating that in addition to hardening by network formation, adhesiveness might be another key requirement for a binder.

In **Chapter V**, hydrated gluten (gluten:water 2:1) was applied as a binder due to its known adhesive and viscoelastic properties at different ratios of binder to extrudate (7.5 – 37.5% gluten binder) in a similar sausage composition as in Chapter IV. Texture and sensory properties were again analyzed. With gluten as binder, cohesiveness and springiness increased with increasing binder content. The hardness was not influenced by the binder content but increased with drying. The results of the sensory analysis correlated well except for hardness, where an increase was perceived with increasing binder content. Furthermore, a decrease in dryness was perceived with increasing binder content. The results of the study confirmed the hypothesis that a high gel strength is necessary for binder and showed furthermore, that also adhesive properties resulting in strong interaction between the binder and the to-be-bound particles are necessary.

Taken together, the results of this thesis have shown that plant protein suspensions are principally suitable as binders for meat analogue products if they meet the key requirements of *(i)* sufficient hardening from network formation, which might be induced e.g. by heating, acidification, the addition of enzymes or a combination of those mechanisms and might furthermore be enhanced by drying and *(ii)* adhesive properties to enable sufficient cohesiveness between the binder and embedded particles, such as fibrous plant proteins and fat mimetics. This thesis provides furthermore insights on formulation- and process-based approaches to modulate the texture of meat analogue products.

# Zusammenfassung

Diskussionen über die ökologischen, ethischen und gesundheitlichen Auswirkungen, die mit dem Verzehr besonders großer Mengen an Fleisch verbunden sind, hat die Entwicklung von Fleischersatzprodukten gefördert, die in den letzten Jahren zu einem bedeutsamen Trend in der Lebensmittelindustrie geworden sind. Obwohl inzwischen viele Produkte in den Supermarktregalen erhältlich sind, liegen noch immer wenige wissenschaftliche Erkenntnisse zu deren materialwissenschaftlichen Designkriterien vor, insbesondere hinsichtlich der Zusammensetzung von Fleischersatzprodukten, Wechselwirkungen zwischen den Inhaltsstoffen und deren Auswirkung auf Textur und Sensorik. Verarbeitete Fleischerzeugnisse, wie z. B. Wurstwaren, bestehen in der Regel aus zerkleinertem Fleisch, zerkleinertem Fettgewebe und gelösten Fleischproteinen, die durch Zerkleinerung und die Zugabe von Salz aus dem Muskelfleisch herausgelöst werden und später als Binder dienen. Diese drei Hauptbestandteile müssen daher unter Verwendung alternativer, nicht-tierischer Stoffe strukturell und technofunktionell analog konzipiert werden, um Fleischersatzprodukte mit entsprechender Textur und den ursprünglichen Fleischprodukte ähnliche ernährungsphysiologischen Eigenschaften zu erhalten. Anisotrope Strukturen, die der faserigen Textur von Fleisch ähneln, können mit globulären Pflanzenproteinen, wie z. B. Sojaproteinen, verfahrenstechnisch durch Extrusion oder Scherzelltechnologie erzeugt werden. Um die Textur von tierischem Fettgewebe zu imitieren, das im Gegensatz zu reinem Pflanzenfett elastische Eigenschaften hat, wurde in einer früheren Dissertation ein Ansatz entwickelt, bei dem eine Emulsion aus pflanzlichem Öl und Pflanzenproteinen hergestellt wurde, die anschließend mit dem Enzym Transglutaminase kovalent vernetzt wurden, was zu einem strukturierten Lipidsystemen mit ähnlichen Textureigenschaften wie tierischem Fettgewebe führte. Um diese beiden Materialien zu verbinden und in einer Matrix zusammenzuhalten, ist jedoch ein Binder erforderlich. Binder für Fleischersatzprodukte wurden bislang noch nicht systematisch untersucht und es gibt wenige Erkenntnisse über deren Konzeption und Funktionalität. In derzeit kommerziell erhältlichen Produkten werden häufig Methylcellulose oder andere Hydrokolloide als Bindemittel verwendet. Deren Akzeptanz ist allerdings bei den Verbrauchern stark rückläufig. Aber auch die sensorischen Eigenschaften bedingen, Alternativen zu finden, da diese Stoffe oft leicht adstringierend sind und Trockenheit beim Kauprozess im Gaumen erzeugen können. Ziel dieser Dissertation war es daher, die Möglichkeit der Nutzung von Pflanzenproteingelen als alternative Binder für Fleischersatzprodukte zu untersuchen. Dem lag die Hypothese zugrunde, dass Pflanzenproteinsuspensionen mit zerkleinerten Extrudaten und

strukturierten Lipidsystemen vermischt und anschließend geliert werden können, um eine kohärente Matrix mit ähnlichen Textureigenschaften wie Fleischprodukte zu bilden. Dazu wurden verschiedene Gelbildungsmechanismen, wie Hitze-, Säure- und Enzyminduktion sowie der Einfluss der Trocknung, untersucht.

Im ersten Teil dieser Arbeit (**Kapitel II**) wurden technofunktionelle Eigenschaften eines Sojaproteingels als Binder zwischen einer Schicht aus pflanzlichen Protein-Nassextrudaten und einem pflanzlichen Analog eines tierischen Fettgewebes untersucht. Zunächst wurden die hitzeinduzierten Gelbildungseigenschaften von konzentrierten Sojaproteinisolat-(SPI)-Suspensionen (10-16% SPI) mit verschiedenen Salzen ($CaSO_4$, $CaCl_2$, $MgCl_2$), säurebildendem Glucono-δ-Lacton (GDL) und dem Enzym Transglutaminase (TG), das kovalente Bindungen induziert, betrachtet. Die Gelfestigkeit konnte dabei nur mit TG deutlich erhöht werden. Daher wurden im Folgenden ein hitzeinduziertes (bei 85 °C) und ein TG-induziertes SPI-Gel (beide 14 % w/w) in unterschiedlichen Mengen als Binder zwischen der Schicht aus Extrudaten und dem Fettsystem untersucht. In einem Zugversuch wurden die maximale Kraft sowie „Work of Adhesion“ bestimmt. Die Ergebnisse zeigten, dass das härtere, TG-induzierte SPI-Gel zu einem deutlich besseren Zusammenhalt der zwei Schichten führte. Daraus konnte der Schluss gezogen werden, dass die Bildung kovalenter Isopeptidbindungen zu einer verbesserten Bindung führt, die weitere Verarbeitungsschritte von Fleischersatzprodukten ermöglicht, wie z. B. ein Schneiden oder Schnetzeln.

Während sich das TG-induzierte SPI-Gel als vielversprechender Binder für Fleischersatzprodukte mit neutralem pH-Wert erwies, stellte das pH-Optimum des Enzyms von 7 jedoch eine Herausforderung für die Anwendung in sauren Lebensmitteln, wie z.B. fermentierten Wurstanalogen, dar. Daher wurde in **Kapitel III** der Ansatz untersucht, TG und das langsam säuernde GDL gleichzeitig zur SPI-Suspension hinzuzufügen, um zu untersuchen, ob eine enzymatische Vernetzung der Proteine stattfinden kann, bevor der pH-Wert die Aktivität des Enzyms inhibiert. Dazu wurden TG und GDL getrennt oder gleichzeitig zu 10-15%igen SPI-Suspensionen hinzugegeben. Die Texturanalyse zeigte, dass SPI-Gele, die durch eine Kombination von GDL und TG bei einem pH-Wert von 5-6 hergestellt wurden, härter waren als Gele, die nur durch die Zugabe von GDL verfestigt wurden, und genauso hart waren wie Gele, die durch TG bei einem optimalen pH-Wert von 7 hergestellt wurden. Verminderte tan δ-Werte und konfokale Laser-Scanning-Mikroskopie-Bilder belegten, dass in den kombinierten Gelen eine Proteinvernetzung stattgefunden hatte.

In Summe deuten die Ergebnisse auf eine anfängliche kovalente Netzwerkbildung durch TG hin, während die langsame Ansäuerung durch GDL aufgrund der abgeschwächten elektrostatischen Abstoßung zur Agglomeration nicht-vernetzter Proteine an das Netzwerk führt. Eine kombinierte Zugabe von TG und langsam säuerndem GDL führte zu Gelen mit niedrigem pH sowie verbesserten Textur-Eigenschaften und stellt somit eine vielversprechende Option für die Verwendung als Binder in Fleischersatzprodukten mit niedrigem pH, wie z.B. Rohwurst-Ersatzprodukten, dar.

In **Kapitel IV** wurden dann die zuvor untersuchten SPI-Gele als Binder eingesetzt, um zerkleinerte strukturierte Lipidsysteme und Extrudate in eine kohärente Matrix zu überführen, die in ihrer Struktur der klassischer Rohwürste ähnelt. GDL-induzierte und GDL+TG-induzierte SPI-Gele wurden verglichen und in unterschiedlichen Verhältnissen von Binder zu Extrudat (30-70 %) eingesetzt. Die Wurstanaloga wurden ähnlich wie herkömmliche Würste durch Zerkleinern, Räuchern und Trocknen hergestellt und hinsichtlich ihrer Zusammensetzung, Textur und sensorischen Eigenschaften analysiert. Sowohl die Textur- als auch die sensorische Analyse zeigten, dass mit steigendem Binderanteil die Kohäsivität zunahm, während die Festigkeit abnahm. Darüber hinaus führte die Anwendung von TG im Binder zu einer leicht erhöhten Festigkeit und Kohäsivität. Ein Trocknen der Würste erhöhte die Festigkeit signifikant. Aus den Ergebnissen der Studie lässt sich schließen, dass bei einem SPI-Gel als Binder die Struktur bei niedrigem Binderanteil durch die Extrudatpartikel dominiert wird, was zu einer hohen Festigkeit, aber zu geringer Kohäsion führt. Hingegen dominiert bei höherem Anteil der Binder die Struktur, was zu einer verbesserten Kohäsion, aber geringerer Festigkeit führt. Mit dem SPI-Gel als Binder konnte in keiner Formulierung sowohl eine ausreichende Kohäsion als auch Festigkeit erzielt werden. Dies deutet darauf hin, dass neben der Verfestigung von Bindern durch Netzwerkbildung die Adhäsion eine weitere wichtige Eigenschaft eines Binders ist.

In **Kapitel V** wurde hydratisiertes Gluten (Gluten:Wasser 2:1) aufgrund seiner bekannten adhäsiven und viskoelastischen Eigenschaften als Binder in unterschiedlichen Verhältnissen zu Extrudat (7,5 - 37,5% Glutenbinder) in einer ähnlichen Rezeptur wie in Kapitel IV verwendet und Textur und sensorische Eigenschaften analysiert. Mit Gluten als Binder nahmen Kohäsion und Springiness mit steigendem Binderanteil zu. Die Härte wurde durch den Bindemittelgehalt nicht beeinflusst, nahm aber durch die Trocknung zu. Die Ergebnisse der sensorischen Analyse bestätigten die Ergebnisse aus der Texturanalyse, mit Ausnahme der Festigkeit, die mit

zunehmendem Binderanteil anstieg. Darüber hinaus wurden die Produkte mit abnehmendem Binderanteil als trockener wahrgenommen. Die Ergebnisse der Studie bestätigten die Hypothese, dass eine hohe Gelstärke für den Binder erforderlich ist, und zeigten darüber hinaus, dass auch adhäsive Eigenschaften, die zu einer starken Wechselwirkung zwischen dem Binder und den zu bindenden Partikeln führen, notwendig sind.

Insgesamt belegen die Ergebnisse dieser Arbeit, dass Pflanzenproteinsuspensionen aus hydratisierten Pflanzenproteinen prinzipiell als Bindemittel für Fleischersatzprodukte geeignet sind, wenn sie folgende Schlüsselanforderungen erfüllen: *(i)* eine ausreichende Verfestigung durch Netzwerkbildung, die z.B. durch Erhitzen, Säuern, Enzyme oder eine Kombination dieser Mechanismen induziert und durch Trocknung verstärkt werden kann, und *(ii)* adhäsive Eigenschaften, die zu einem ausreichenden Zusammenhalt zwischen dem Binder und den eingebetteten Partikeln, wie faserigen Pflanzenproteinextrudaten und strukturierten Lipidsystemen, führen. Insgesamt leistet die Dissertation damit einen Beitrag zur Entwicklung formulierungs- und prozessbasierter Ansätze zur Verbesserung der Textur von Fleischersatzprodukten.

# I. Chapter: Introduction

## Review: Binders in Foods: Definition, Functionality, and Characterization

**Eva Herz[a1], Pascal Moll[a1], Christophe Schmitt[2], J. Weiss[1♣]**

[1]University of Hohenheim, Department of Food Material Science, Institute of Food Science and Biotechnology, Garbenstrasse 21/25, 70599 Stuttgart, Germany

[2]Nestlé Research, Nestlé Institute of Material Sciences, Department of Chemistry, Vers-chez-les-Blancs, CH-1000, Lausanne 26, Switzerland

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*This review will also be published as part of the PhD thesis of Pascal Moll.*

[a] Both first authors.

♣ To whom correspondence should be addressed: Phone +49 711 459 24415, Email: j.weiss@uni-hohenheim.de

# Abstract

*Background*

Binding agents have emerged as an important and required class of ingredients to manufacture a wide range of new food products, especially semi-solid or solid vegan meat and dairy analogues.

*Scope and Approach*

In this review, we give a definition for this class of ingredients, look at what constitutes required functionalities for binders, and highlight what molecular mechanisms are involved to give rise to these functionalities. Furthermore, an overview on food binders, such as animal-derived / plant-based proteins (myofibrillar proteins, egg white protein, gluten etc.) and carbohydrate-based (methylcellulose, carrageenans etc.) is given and their functions are explained.

*Key Findings and Conclusion*

Three functions have been identified as critically important: stickiness, hardening and water binding. Analytical methods to determine these characteristics are presented and it is recommended to study those simultaneously in model systems, where methylcellulose or gluten might serve as benchmark.


**Keywords**: binder, binding agent, stickiness, hardening, water holding, functions, mechanics, analytical methods

# Introduction

With emerging plant-based alternatives to replace traditional animal-based foods such as meat and meat products (e.g. burger, bacon, sausages) or dairy and dairy products (e.g. yogurt, cheeses), the need for a "binder" to create new vegan matrices with desirable organoleptic and technofunctional characteristics has been clearly identified. The role of the binder compound is to glue structural elements such as protein extrudates and fat particles or oil droplets together to form a coherent matrix. If one investigates historical records, binders have been identified and used by humans for quite some time – not only in the food science area, but in many other technological applications as well. However, their discovery and use have been based mostly based on trial and error approaches (Grossmann, 2003).

One of the oldest use of binders involved for example the manufacture of paints. There, pigments had to be bound together to form a coherent, viscous mass that could be applied to solid surfaces to create paintings. Materials used as binders included egg, wax, honey, lime, whey, linseed oil or bitumen. Of these, egg used to be one of the most used binders for paints until it was replaced in the early 16th century by oil. It is interesting to note that the unique ability of egg to act as a binder and compounding material has also been long recognized in the culinary arts, where it has been used for the preparation of various dishes and foods where binding is needed, such as for example breaded coatings or steak tartare. It is therefore not surprising that many of the first generation of vegetarian meat product analogues were manufactured using egg as a binder material (Mühle, 2020). But what exactly is a binder? What are its functions? How do these compounds do what they do, and what are their molecular characteristics?

In this review paper, the current state of knowledge on binders was therefore reviewed to provide some answers to the above raised questions. The literature research showed that there is only limited knowledge available in the food science area, and therefore various adjacent fields have also been screened for technologies and formulations that required similar functionalities including the emerging science behind 3D printing, pharmaceutical compounding, and industrial adhesives. Based on this and the authors own work in this area, a model for their fundamental functionality in vegan product matrices has been proposed. From this, options for the development and/or discovery of new binders have been suggested.

# Functions

There is currently no accepted universal definition of a binder in the context of food applications, but Wikipedia loosely classifies such materials as "….any material or substance that holds or draws other materials together to form a cohesive whole mechanically, chemically, by adhesion or cohesion" (Wikipedia, 2020). It specifies further that "…binders are liquid or dough-like substances that harden by a chemical or physical process and bind fibers, filler powder and other particles added into it". Such compounds include glue, adhesives and thickening agents, and organic compounds that may be used for such purposes include bitumen, animal and plant glues, polymers, as well as inorganic compounds such as lime, cement, gypsum, or liquid glass. With respect to food applications, a binder may thus be defined as **a compound that is able to "bind" particulates together (e.g. fat, meat or dairy analogue particulates), i.e. facilitate an interaction between otherwise inert, non-interacting particles to form a heterogeneous but coherent food material matrix.** Thereby, the matrix becomes a system with a percolating structure, and consequently, mechanical properties are altered such that the mass can for example be formed maintaining its shape for some time, which is an essential prerequisite for a burger or a sausage mix, but also dairy product analogues. Moreover, it is important that the binder can also immobilize a solvent during processing to create for example in vegan meat analogue products a certain juiciness and moistness, preventing also excessive water losses during food preparations such as frying, grilling, or boiling.

The term binder has also been used more widely for ingredients that not only provide gluiness but also ingredients that thickening a matrix, or in general compounds that improve the overall texture of a product (Siegel et al., 1979; Kyriakopoulou et al., 2021). In this section, we shortly review those functions and explain why they are required.

## Stickiness

For a long time, stickiness was regarded as an undesirable property in the food industry as sticking of material to surfaces leads to increased cleaning efforts, product loss, production inefficiency, equipment wear and contamination (Michalski et al., 1997; Adhikari et al., 2001; Frabetti et al., 2021). However, stickiness is an essential prerequisite for binders to hold heterogeneous components together (Wall & Huebner, 1981) that would otherwise be inert to each other and non-interacting. It is required in several food products, *e.g.* for sticking batter to

meat sticks (Cunningham & Tiede, 1981), fixing ingredients on bakery products (Ghorbel & Launay, 2014), agglomerating food powders (Boonyai et al., 2004), gluing ingredients in cereal bars (Burke & Hartel, 2021; Melati et al., 2021), and binding different layers in 3D printing applications (Varvara et al., 2021). A food category of particular interest is the growing field of meat analogues where the ability of different ingredients to bind to each other is of great importance for desirable organoleptic and technofunctional product characteristics since the frequently used texture vegetable proteins possess only a limited binding ability on their own (Bakhsh et al., 2021; Kyriakopoulou et al., 2021; Sakai et al., 2021). To showcase the different functions of binders, we present a well-working and established system that is also the benchmark for the binding in meat analogues, namely meat proteins (**Figure I.1**). First, meat and fat are cut into smaller non-sticky pieces and ground using a meat grinder into smaller particles, that are bound together by a small number of soluble proteins that stem from disintegrated cells. Further mechanical forces such as kneading, mixing, or compression leads to more protein being pushed out of the cell into the serum phase thereby increasing stickiness. While this is only partly wanted for burger patties with a high amount of intact cells (**Figure I.1A**), the meat mass is deliberately made stickier in heated sausage production through salt addition and mechanical forces (**Figure I.1B**) (G.H. Lu & T.C. Chen, 1999; Desmond, 2006). Severe mechanical forces cause a higher degree of meat cell disruption and salt facilitates solubilization of the myofibrillar proteins that benefit stickiness and hold the now even smaller meat and fat particles together. Next, the meat batter is filled in casings and heated yielding a coherent three-dimensional crosslinked meat protein network with embedded meat and fat particles that lost its stickiness (Knipe, 2014). This hardening function is explained next.

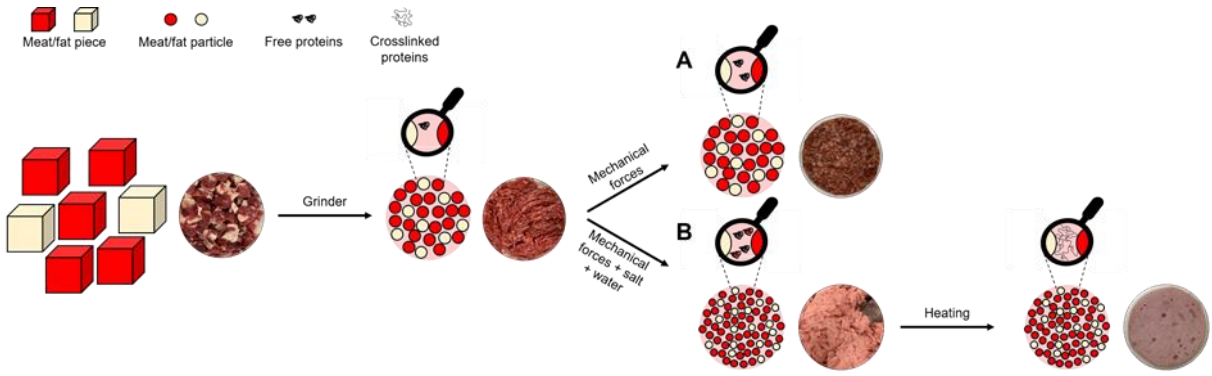


**Figure I.1:** Schematic drawing exemplifying the stickiness and hardening function of meat proteins. From left to right: Non-sticky meat and fat pieces are ground into sticky meat and fat particles before being processed into burger patties (A) and heated sausages through further mechanical forces, salt addition and heating (B). Note that the sizes of meat/fat pieces and particles is not true to scale.

## Hardening

Binders used in semi-solid and solid food products need to be hardened to some degree, as the texture of the binder will also have an influence on the overall texture of the composite product and/or embed the different structural elements of composite products. While in a "first phase/sticky state" the binder and particles are brought and hold together by the stickiness, in a second state, hardening is needed to fixate the structure. Moreover, stickiness is not a desired property for consumption of a food and thus the hardening will also have a strong impact on ability to orally process the food material. Ultimately, consumer acceptance relies strongly on this second step as it determines the final product texture. During processing, a liquid state of the binder is desired to facilitate an even distribution in the product as well as facilitate a certain processing of the material such as pumping or pouring the mass in molds or casings. Furthermore, after hardening, the binder ideally loses its stickiness due to increased molecular interactions and decreased molecular mobility (Anvari et al., 2015; Beniwal et al., 2021). A common way to induce hardening in biopolymers such as proteins or polysaccharides is cross-linking, which may be done either physically or chemically. Considering the example of meat processing (**Figure I.1**), where salt-extracted, partially solubilized myofibrillar proteins act as binders, hardening is induced by heating, leading to denaturation and aggregation of those proteins (Fukazawa et al., 1961; Samejima et al., 1969; Gordon et al., 1992). This yields the desired texture properties, as otherwise the product would not be sliceable, but spreadable, which is the case for sausages where no cross-links are formed (Hilbig et al., 2020).

Another example where hardening of the binder is essential to achieve the desired texture properties of the final product way to induce is the class of confectionary products, where sugar-based binders are employed (Hartel et al., 2011) or in chocolate, where texture properties are strongly influenced by presence of fat crystals (Glicerina et al., 2016). Finally, products made with a hardened, no longer sticky, binder allow for an easy and complete removal from the products' packaging and ensure that the product also keeps it's formed shape.

## Water holding

Especially in meat science, the term "binding" is often associated with water and fat binding capacity, rather than with stickiness or the ability to form a coherent matrix (Fukazawa et al., 1961; Liu et al., 2016). Water holding capacity describes the ability of a (biopolymer) matrix to absorb and hold water against gravity, including bound water, hydrodynamic water, capillary

water and physically entrapped water, which benefits juiciness and tenderness (Damodaran, 2017). It is directly related to the capacity to hold water during processing, such as slicing or cooking (Warner, 2017). The water holding capacity is also of great importance for the previously mentioned binder-example of emulsion-type sausages (**Figure I.1**), as here water is added during processing in the form of ice to prevent overheating during chopping thus keeping the protein-fat mixture at moderate temperatures preventing fat melting and protein denaturation. There, water must be bound in the matrix and kept enclosed in the voids of the protein network after heating to achieve the desired texture and juiciness in the final product. As stated above, also fat binding is important for the desired texture and mouthfeel. As such, an inclusion of water holding capacity in the list of essential "binder properties" is reasonable for many products, such as e.g. whole-cut meat analogues, where stickiness of the binder is unnecessary as no particles have to adhere to each other but the products benefits from water and fat retention for improvements in mouthfeel and juiciness (Kyriakopoulou et al., 2021).

### Other considerations

From the described phenomena above it becomes clear that binders are complex ingredients displaying a wide range of properties. These functionalities must be modulated and adjusted to serve the needs arising from the manufacturing and consumer side. For example, during a certain production step e.g., shaping, pumping, slicing, different subsets of functions of a binder are needed. Moreover, processing may induce changes in binder functionality and transitions in material properties (Weiss et al., 2019). Therefore, it is of outermost importance to understand the mechanism behind the different functionalities, so that properties can be tuned.

## Mechanistic considerations

The different functions of binders give rise to the question how they can be altered and controlled by users that are in need to create working food formulations and manufacturing processes. A thorough understanding of the underlying molecular reasons can in turn help to work with existing binders as well as implement healthier and novel ones (Laricheva & Mikhailova, 2020; Burke & Hartel, 2021).

## Stickiness

Stickiness of amorphous and semi crystalline foods (< 10% moisture) has been well reviewed by Boonyai et al. (2004) and Chen and Özkan (2007), and is mostly related to their glass transition. The glass transition temperature is a mean temperature that describes the temperature windows across which a food material will transition from a liquid to a glassy or amorphous state. In the latter state, there is little chance for adhesion to take place whereas above the glass transition temperature the material is in the rubbery state - able to be deformed and to stick (Adhikari et al., 2001; Kasapis, 2004). This is of great importance, especially for sugar-based binder systems (Jannin et al., 2008; Tita et al., 2012; Wang & Hartel, 2021). In general, an increase in water content leads to a decrease in glass transition temperature because of the plasticizing effect of water.

In this review, we focus mostly on the stickiness of semi-solid binders with higher water content that serve in the aforementioned defined way. They can be described as so-called pressure sensitive adhesives (PSA). PSAs stick to a variety of materials by applying a slight pressure due to them having well balanced liquid-like and solid-like characteristics. Their stickiness depends on both the adhesive strength between the binder and the adherend as well as the cohesive strength of the binder itself (Heddleson et al., 1994; Duncan et al., 1999; Baron et al., 2009; Baït et al., 2020; Wang & Hartel, 2020). If a binder fails to possess both characteristic, it is not sticky in the sense of a binder. For instance, oil clings easily to surfaces and therefore has a high adhesion but it fails to stick things together because of a lack of cohesive strength. On the other hand, a rubber with great cohesive strength cannot adhere to surfaces and is consequently not sticky. The question is, how adhesion and cohesion can be tuned for improving binder stickiness? Unfortunately there is no general agreement on which factors and forces are actually involved in stickiness (Adhikari et al., 2001) since earlier food science studies aimed for stickiness reduction to solve a particular problem in a single system instead of understanding the fundamental mechanism behind binder stickiness. Ultimately, a governing structure-function relationship needs to be established (Michalski et al., 1997). The following aims to give an overview on what factors influence adhesion and cohesion.

## Adhesion

Adhesion is the ability of a binder to instantly form a bond with the adherend, which is the substance that is to be bound (Duncan et al., 1999). Since binder and adherend are dissimilar,

adhesion is an interfacial property (Adhikari et al., 2001). There are several theories as to why adhesion occurs. These were excellently reviewed by Wang and Hartel (2021) and it should be noted that no single approach explains all observed adhesion mechanisms (Baldan, 2012; Nussinovitch, 2016) especially for foods because of their various compositions and structures (Michalski et al., 1997).

*Thermodynamic adsorption and intramolecular forces:* The thermodynamic adsorption theory being the most popular one states that adhesion between two phases is thermodynamically favorable when the surface energy of the binder is greater than that of the adherend. Furthermore, the involved intramolecular forces have to match which makes the chemical composition of the binder and the adherent of key importance (Dobraszczyk, 1997; Adhikari et al., 2001; Van Der Leeden & Frens, 2002; Ghorbel et al., 2003; Laukemper et al., 2021). For instance, it was found that during probe tack test (see section *Stickiness*) materials with high surfaces energies such as metals produce higher stickiness values because of stronger intermolecular forces between the materials, *i.e.*, stronger adhesion (Kilcast & Roberts, 1998; Laukemper et al., 2021; Averina et al., 2022). Consequently, the adhesion of a binder depends on the material to be glued (Dong et al., 2021). Specifically, Brandner et al. (2021) showed that adhesion of gluten to particles was improved when the particles had amino-functionalized surfaces, thereby being more similar to the chemical composition of the gluten. Chemical, thermal, and enzymatic modifications are applied to primarily proteinaceous binder for exposing reactive site groups for better interactions to take place with the adherend. For instance, alkaline treatments of plant proteins are done to improve accessibility of functional groups through unfolding that benefits external binding and adhesion. So far, such approaches have rather been used in wood applications instead of food science (Wall & Huebner, 1981; Vnučec et al., 2016; Averina et al., 2021). Some studies relate adhesion to the amount of soluble ingredients, yet the exact mechanism is under debate (Chen & Hoseney, 1995; Lee & Yoo, 2020). However, these studies suggest that processing operations such as homogenization that increase solubility of e.g. proteins (Moll et al., 2021) or using a soluble protein fraction (Moll et al., 2022b) could benefit adhesion.

*Surface constitution:* Furthermore, mechanical interlocking because of surface irregularities can provide additional adhesive strength (Michalski et al., 1997). In general, surface roughness increases adhesion as long as the binder manages to penetrate into those cavities (Noren et al., 2019). This condition is increased for softer binders (Sun et al., 2013) or when the irregularities

are not too distinct thereby preventing the binder from interactions with the actual adherend surface underneath as it was observed by Laukemper et al. (2021). A binder has to physically be able to spread on the adherend (= wetting) and be able to deform to allow for intermolecular interactions to occur (Wang & Hartel, 2021). For instance, Burke and Hartel (2021) reported that sugar syrups failed to adhere to the probe when the surface of the sugar was partly covered with particles. Naturally, a higher contact time and pressure leads to a better distribution of the binder over the adherend surface and thus an increase in the effective area of interaction (Adhikari et al., 2001; Ghorbel & Launay, 2014; Laukemper et al., 2021). Furthermore, studies reported an increase in adhesion when water content was increased (Chen & Hoseney, 1995). This is due to the plasticizing effect of water (Adhikari et al., 2001) and a similar transition can be observed when temperature increases that benefits molecular mobility (Heddleson et al., 1994). Contrarily, a decrease in water content and temperature decreases wettability and increases cohesion leading to little or no adhesion, which was observed for e.g. sugar syrups (Burke & Hartel, 2021) and doughs (Ghorbel & Launay, 2014)

## Cohesion

Cohesion is the ability of the binder to resist rupture. Since cohesion is an internal property of the binder, it is predominately a function of the strength of intermolecular forces of similar molecules (Duncan et al., 1999; Fiszman & Damásio, 2000; Noren et al., 2019; Burke & Hartel, 2021; Rosenthal & Thompson, 2021). When it comes to cohesion, the pioneering work of Rumpf (1962) must not remain unmentioned, since he outlined a relationship between particle diameter and relative contribution of interparticle attraction for powder agglomeration (**Figure I.2**). This may also play a role for hydrated systems. Interestingly, the displayed intermolecular forces at short distances (intermolecular and electrostatic forces) are rather ascribed to the phenomena of adhesion, while solid bridges fall into the realm of cohesion. In fact, the contribution of adhesion to stickiness decreases with distance to the adherend and cohesion becomes more important (Michalski et al., 1997). It should be mentioned though that in hydrated systems, solid bridges hardly occur and phenomena such as entanglement and crosslinking that are more common in hydrated systems increase in importance. In general, cohesive strength is increased through a decrease in temperature and water content as well as an increase in molecular weight of the involved hydrocolloids because of more entanglement and stronger molecular interactions with less polymer slippage (Wall & Huebner, 1981;

Sengsuk et al., 2021). Furthermore, crosslinking of polymers through covalent and non-covalent bonds increases cohesion (Azeredo & Waldron, 2016; Silvestre et al., 2021).

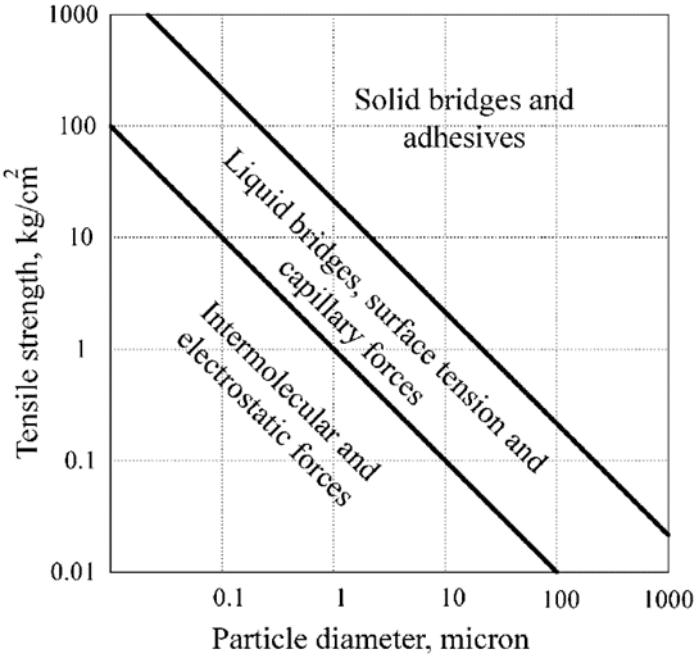


**Figure I.2:** Strength of bridges to hold particles together (adapted from Rumpf (1962)).

Taken together, the conditions benefiting adhesion and cohesion and ultimately stickiness are partly contradictory, which is why a combination of both is of importance. Viscoelasticity – which also involves a balance of these - was thus reported to be key (Dobraszczyk, 1997; Baron et al., 2009). For example, during bonding (typically at frequencies of < 1 Hz), binders must be able to deform thereby improving wetting and bond formation (= adhesion) while during debonding (characteristic frequency > 100 Hz) they must be rather elastic (= cohesion) to resist separation (Heddleson et al., 1994; Wang & Hartel, 2021). As such, industrial PSA consist of cohesion-providing polymers in combination with small-molecular weight tackifiers that enhance adhesion (Schneberger, 1983; Foley & Chu, 1986; Feldstein et al., 2015). Similar approaches transposed and implemented with edible ingredients provide an interesting way to increase stickiness in food binders. Lastly, it should be noted that it is barely possible to alter a single textural characteristics such as adhesion through *e.g.* temperature increase without affecting another such as cohesion as well (Kazemeini et al., 2021). This makes optimizations challenging. However, this can also be advantageous in processing. For instance, chocolate or other sugary coatings containing value-adding ingredients such as nuts, adhere to ice cream because of their viscous properties at temperatures above their melting point. Once the

temperature is decreased to below the crystallization point, hardening of the coating that acts as binder for the nuts occurs, and the product becomes fully solid.

## Hardening

As stated above, hardening can be induced by chemical and physical crosslinking of polymers. Several reviews and books have explained in detail involved gelation mechanisms, especially for proteins (Totosaus et al., 2002; Phillips & Williams, 2009; Damodaran, 2017). A detailed review on cross-linking of food-grade films has recently been published by Azeredo and Waldron (2016). Biopolymer gels can be formed via non-covalent cross-links (hydrophobic interactions, hydrogen bonds, electrostatic interactions), which can be induced by heating, cooling, change of pH or ionic strength or covalent cross-links (e.g., disulfide bonds, interpeptidic bond). The latter can be induced by heat, pressure, cross-linking agents, or enzymes. The type of cross-links and hence the gel strength/hardness depends on inherent factors (hydrophobicity, electrostatic interactions, disulfide bonds, molecular weight, chemical composition and reactive groups) and adaptable factors (biopolymer concentration, pH, temperature, ionic strength and type of ion, pressure) (Totosaus et al., 2002). For example, gel hardness correlates often with molecular weight of proteins or hydrocolloids, but for proteins also with cysteine and cystin residues as sulfhydryl-disulfide interchange reactions occur during heating, which increases the molecular weight (Ferry, 1948; Phillips & Williams, 2009). In general, covalent interactions are stronger than non-covalent interactions and thus facilitate increased hardening. The polymer concentration is furthermore important as there is usually a critical gelling concentration and with increasing concentration, and the gel hardness increases (Zayas, 1997). Protein gels are typically formed by covalent (disulfide, gamma-glutamyl) and/or noncovalent (hydrogen bonds, hydrophobic interactions, electrostatic interactions) bonds, often a combination of both (Damodaran, 2017). Hydrocolloid gels are mostly formed by physical interaction of junction zones, for example, by hydrogen bonding, hydrophobic association or cation-mediated crosslinking (Phillips & Williams, 2009). Moreover, the hardness of biopolymer gels or hydrated biopolymers can be influenced by phase transition, which has for example been studied for gluten (Toufeili et al., 2002). Often, the involved biopolymers govern the possible type of crosslinking, which in turn determines subsequent processing approaches. For instance, sugar-based binders can be transitioned from a sticky rubbery (low viscosity) state to a glassy state (high viscosity) by lowering the temperature to below their glass transition temperature (**Figure I.3A**) (Wang & Hartel, 2021). This transition

can be described as hardening. On the other hand, methylcellulose solutions display thermo-reversible transitions with a certain gelling temperature, below which they are in a sticky sol state (low viscosity), whereas above they possess a low stickiness accompanied by a high viscosity due to hydrophobic-induced hardening (**Figure I.3B**) (Li et al., 2001). It should be noted that other temperature-dependent transitions may be non-reversible. Furthermore, most food hydrocolloids such as gelatin possess cold-setting gelation properties and therefore do not fall into the categories illustrated in Fig. 3. This demonstrates the complexity of food binders and how their properties are affected differently depending on the environmental circumstances such as changes in temperature.

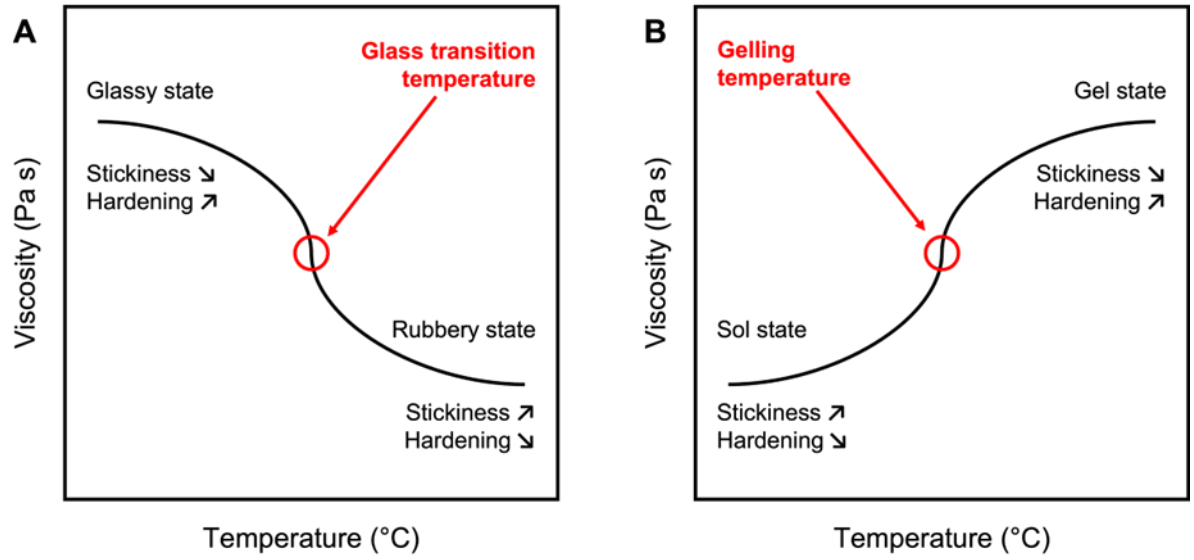


**Figure I.3:** Viscosity as influenced by temperature for (A) sugar-based binders with glass transition temperature and (B) methylcellulose binder with gelling temperature. Inspired by Wang and Hartel (2021) and Li et al. (2001).

Especially enzymes possess the ability to induce cross-links in binders and therefore to induce or increase hardening. For binder applications in food, mostly the enzyme transglutaminase (TG), that induces protein cross-linking by catalyzing an acyl-transfer reaction between the amino group of lysine and the carboxamide group of glutamine residues (Nonaka et al., 1989) and the enzyme laccase, that can induce cross-links in pectin through oxidative coupling of feruloyl groups (Micard & Thibault, 1999) have been used so far (see section ***Fehler! Verweisquelle konnte nicht gefunden werden.***). In addition, laccase can potentially cross-link proteins through tyrosine oxidation (Sakai et al., 2021).

The formation of a gel or a network from a previously liquid dispersion via a glass transition is an important hardening approach for many food binders (Bhandari & Roos, 2016). Most of

these transitions are into the amorphous state only as stated above, but some binding agents may also undergo a partial crystallization as may for example be the case for sugar depending on applied cooling conditions (Mahato et al., 2019). Such mixed transitions lead to very different textures in the final food product and should therefore be recognized when they happen. They are observable by looking at changes in for example the specific volume of samples (Rahman et al., 2007). A low molecular weight substance prone to crystallization changes its specific volume abruptly and transitions occur at a single temperature with slopes being indicative of the thermal expansion coefficient of the liquid and the crystalline solid, respectively. In contrast, higher molecular weight compounds display a change in slope over a temperature range, with the $T_g$ being an indicator of the onset of the transition. As the glassy state is a non-equilibrium state, the cooling speed is a key determinant of the number of crystals and amorphous glass that may in the end be present in such a mixed matrix.

Finally, since many foods made with a binder exists in the end as so-called particle-filled gels, the influence of particle-matrix interactions should also be shortly addressed. As shown by Gravelle et al. (2019), particles that have strong interfacial adhesion with the surrounding matrix, so-called active fillers, support the matrix and increase gel strength, while particles that do not adhere, so-called passive fillers, yield weaker matrices. Therefore, the hardness of a particle-filled gel does not only depend on the hardness of the binder or particles itself but also on the interactions between the components.

## Water holding

Within the context of meat products, the water holding capacity is usually influenced by salt and pH (Warner, 2017). When salt is added, $Cl^-$ ions bind to positively charged protein side groups, screens positive charges, and breaks salt bridges, resulting in separation of filaments and increased hydration (Hamm, 1986). For NaCl concentrations of 0.3 – 1 M, a salting-in effect has been reported, resulting in "swelling" of the myofibrillar filaments and increased water holding, while > 1 M, a salting-out phenomena causes reduced water holding (Offer & Trinick, 1983; Chen et al., 2017). Furthermore, phosphates and polyphosphates are often used in comminuted meat products to increase the water holding capacity (Warner, 2017). When looking at non-meat proteins, it has been shown that generally, the addition of salt increases the gel strength of globular protein gels up to a certain ionic strength (Foegeding et al., 1995), except for soy protein, where water holding capacity decreased with increasing ionic strength (Lakemond et al., 2003). Beyond that, a study showed that with the use of HPP, the cooking

loss of comminuted meat patties could be reduced, especially with reduced salt content, which could be of interest for salt-reduced, healthier food (Macfarlane et al., 1984). Furthermore, the water holding capacity and even more the water release of a food, is also influenced by the microstructure of the product. For example, elongated cavities in fibrous meat analogues have an influence on the water release (Cornet et al., 2020), while in (soy protein) gels, a high protein concentration as well as a porous microstructure have been associated with increased water release (Sun & Breene, 1991; Herz et al., 2021a). As many polysaccharides/fibers, such as carrageenan, pectin or cereal fibers have a high water binding capacity themselves, they have been added to comminuted meat products to increase the water holding capacity and juiciness of the product (Talukder, 2015). It should be kept in mind that water binding and hardness are at some point often competing factors, for example when an increased polymer concentration leads to increased hardness but decreased water holding capacity.

As the term "binder" can also be connected to fat binding, this topic should also be shortly addressed: the stabilization of fat in comminuted meat batter has been described as either emulsion-type, where proteins act as emulsifier and form a film around fat globules or as physical entrapment, where the fat is hold in place by the protein matrix (Gordon et al., 1992).

# Examples

Having looked at functions and mechanistic origins of binder properties, we provide an overview of food binders described in scientific literature (Tab. 1) and furthermore a more detailed description of the most commonly used binders in foods.

## Proteins

### Meat proteins

Meat proteins usually function as binder in various meat products, such as sausages (heated as well as raw sausages), formed ham, minced meat, but also restructured meat.

Batter-type meat products, including most finely comminuted and heated sausages, are produced by comminution of lean meat with the addition of salt to extract the myofibrillar proteins. While the formation of a weak gel has already been observed in the cold state of the meat batter, denaturation and aggregation by heating strengthen the gel, leading to immobilization of fat, water and other components as well as the desired texture of the products

(Gordon et al., 1992). In raw- or dry-fermented sausages, similarly proteins are extracted by salt during grinding or mincing at the beginning of the production process. The solubilized proteins form a sticky protein film around the comminuted meat particles. Later, acidification by lactic acid bacteria or acidifiers such as glucono-δ-lactone (GDL) decreases the pH close to the isoelectric point of the meat proteins, leading to coagulation and gel formation of the myofibrillar proteins. Drying or heating will furthermore strengthen the gel later in the manufacturing process (Toldrá & Hui, 2014).

Restructured meat refers to a group of products, where meat is comminuted into smaller size particles and then reformed into a shape that resembles that of a product of an intact muscle. For this, a binder is usually added or, as mentioned before, myofibrillar proteins are extracted by salt addition from the meat (Seideman & Durland, 1983). Furthermore, a study by Macfarlane et al. (1977) showed that sarcoplasmic proteins enhanced the binding strength at low ionic strength, while with increasing salt concentrations, the binding strength decreased, which was attributed to salt induced denaturation or precipitation of sarcoplasmic proteins.

In addition, several studies have investigated the use of animal blood proteins as binders for meat products: For example, plasma powder was used in combination with NaCl as binder for restructured dry ham (Romero de Ávila et al., 2014). Besides that, several studies have been published on the application of a binder system called “Fibrimex”, containing blood plasma derived thrombin and fibrinogen (Boles & Shand, 1998, 1999; Lennon et al., 2010). The binding mechanism is based on the blood clotting mechanism: when mixed and applied to meat pieces or particles, the thrombin enzyme converts fibrinogen into fibrin, which is then cross-linked by the enzyme transglutaminase, which is also present in the extracted fibrinogen (Wijngaards & Paardekooper, 1988).

**Egg white**

The outstanding binding ability of egg white to act as a binding agent has been known for quite some time. Egg white is highly appreciated in the culinary arts, where it has been used for the preparation of various dishes and foods where binding is needed, such as for example breaded coatings or steak tartare (O'Dea & Hewson, 2015). As previously mentioned, many of the first generation of vegetarian meat product analogues contained egg as a binder material. In fact, for a long time egg white was found to be the only suitable binder for meat analogues (Mullen et al., 1971). Egg white in raw and powdered form was used by G.H. Lu and T.C. Chen (1999)to glue muscle chunks together. It must be noted that powdered egg white was left to rehydrate on

the muscle chunks and rehydration time as well as available water on the adherend, i.e., the meat chunk, were found to be of great importance for binding. Interestingly, the binding ability was better for muscle to muscle instead of when fat was involved most likely due to differences in the surface characteristics (roughness, surface activity, etc.) as mentioned before. A heating step in a microwave was applied, which is why one may assume that hardening took place to some degree. During heating egg white proteins denature and unfold at specific temperatures revealing hidden reaction sites. Those reaction sites allow for the aggregation of single molecules through protein-protein interactions into larger entities and the development of a continuous network that entraps water (Mine, 2014). Therefore, high gel hardness, water-binding as well as possible interactions with filler particles can be considered as binding properties of egg white protein.

**Milk proteins**

Milk-derived binding agents mostly come in powdered form and can be categorized into whole milk, skim milk, whey, and casein powder (Fox et al., 1998). Therefore, their binding performance highly depends on the characteristics of the powders (e.g. water content dictates glass transition temperature and thus stickiness) and the application procedure such as rehydration time, concentration, and possible heat treatments. Andiç et al. (2010) reported that addition of whey powder and skim milk powder (2%) increased cooking yield and moisture retention of beef patties indicating that those binders improved water holding. However, skim milk powder decreased the cohesiveness of patties suggesting that cohesion of meat particles was weakened (Andiç et al., 2010). The authors do not provide an explanation for this observation, but it may be that the intrinsically present binding mechanism provided by solubilized meat proteins was disturbed. The addition of whey powder (2-4%) to beef meatballs increased cooking yield, but no effect on juiciness was observed (Serdaroğlu, 2006). A paper glue based on whey protein was found to have similar bonding strength to a commercial, synthetic polymer-based glue (Wang et al., 2013). Interestingly, the authors used a thermal denaturation step to induce whey protein unfolding and polymerization for desired viscosity. Then, the polymerized whey proteins were mixed with different quantities of polyvinyl pyrrolidone acting as co-binder until bonding strength was optimized (Wang et al., 2013). Taken together, concentration adjustments and biopolymer mixing were used to tune for desired adhesion and cohesion properties.

**Gluten**

Gluten is not a single protein, but a group of wheat storage proteins composed of 55% glutenins and 45% gliadins, composed of various subgroups (α-/β-gliadins (soluble in dilute alcohols), γ-gliadins (cysteine-rich intrachain disulfide bridge spiked proteins), and ω-gliadins (soluble in acidic acetonitrile); high-molecular-mass (HMW) and low-molecular-mass (LMW) glutenin aggregates). Gluten is known for providing unique viscoelastic properties to baked products, such as bread, which is based on the interaction of glutenins and gliadins by hydration and application of mechanical forces (Morel et al., 2020). Furthermore, gluten proteins are unique amongst other plant proteins with respect to their properties, as they have been described to form filamentous polymer-like gels rather than particulate networks like most others (Schreuders et al., 2021). The peculiar mixture of high molecular weight glutenin and low molecular weight gliadins in gluten has been compared to elastomer-tackifier systems as they occur in industrial pressure-sensitive adhesives, where a large polymer provides an elastic component and a lower molecular tackifier imparts viscous properties (Schneberger, 1983; Levine & Slade, 1990; Heddleson et al., 1994; Feldstein et al., 2015). Therefore, it is not surprising that gluten with its unique properties have also been used as binder. For example, gluten was found to be the best binder for meat pieces in the presence of 8% salt and 2% sodium tripolyphosphate after heating to 75 °C as determined by the force to separate them and this was attributed to its ability to interact with the available myosin of the meat pieces (Siegel et al., 1979).

**Soy Protein**

Mullen et al. (1971) patented a soy protein modification that improved coagulation of the soy protein acting as a binder by raising the pH value to about 9, followed by lowering the pH again to 5.5 – 8.0. The authors reported that alkaline treatment led to degradation of the soy proteins into smaller subunits and in combination with increased repulsive negative charges at alkaline pH resulted in increased solubilization of the proteins (Mullen et al., 1971). Furthermore, a study by Sun et al. (2023) showed unfolding and increased surface hydrophobicity of soy proteins after alkaline treatment. Probably, increased hydrophobicity and recombination of subunits at lower pH-values led to increased gel hardness and after heating. Similarly, Hager (1980) improved soy protein solubility through a treatment with a cation exchange resin that gelled more easily and as such being a suitable binder in meat analogues. We speculate that the

solubility increase did not only improve hardening but also prior to this sticking of the soy proteins to the adherend.

**Enzymes**

The enzyme transglutaminase (TG) is known to induce protein cross-linking by catalyzing an acyl-transfer reaction between the amino group of lysine and the carboxamide group of glutamine residues (Nonaka et al., 1989). TG has been studied as binding agent for restructured meat in presence of NaCl as well as without NaCl but in combination with other food proteins such as sodium caseinate, whey protein and gelatin. Good binding quality was found for TG and NaCl as well as TG and sodium caseinate, which showed that salt-solubilized myofibrillar meat proteins as well as sodium caseinate are good substrates that can be cross-linked by TG (Kuraishi et al., 1997). Furthermore, transglutaminase was used for crosslinking soy proteins as a binder in restructured meats. Interestingly, the addition of sodium bisulfite increased the binding strength as it reduced disulfide linkages to free thiol groups that were then available for interaction between soy protein (binder) and muscle proteins (adherend) (Tsao et al., 2002). Increased binding strength by TG-induced cross-links compared to only heat-induced network formation have also been shown as superior for the assembly of extrudate and soy protein binder layers for a complex meat analogue (Herz et al., 2021b). TG has also been studied as binding agent in combination with fibrin/thrombin combination to induce cross-links between fibrin molecules but between fibrin and collagen. The addition of this binding agent resulted in improved textural properties of cooked ground meat with reduced salt level, probably due to induced cross-links, but also in reduced cooking loss, compared to only TG as binder, indicating high water binding capacity of fibrin/thrombin (Esra Atilgan & Birol Kilic, 2017). In a plant-based burger patty, TG as addition to a soy protein binder increased hardness, springiness and cohesiveness which can be attributed to protein cross-links (Lee & Hong, 2020).

Recently, laccase was used to crosslink sugar beet pectin acting as binder in patties based on soy textured vegetable proteins with methylcellulose serving as benchmark binder (Sakai et al., 2021). Laccase is an oxidative enzyme that can oxidize feruloyl groups present in sugar beet pectin resulting in covalent bond formation between ferulic acid residues and therefore induce gel formation of sugar beet pectin (Micard & Thibault, 1999). It was found that cross-linking of sugar beet pectin by laccase resulted in increased hardness of patties compared to patties with (Nonaka et al., 1989) methylcellulose as binder (Sakai et al., 2021).

## Carbohydrates

### Cellulose derivatives

The ubiquitous utilization of cellulose derivatives such as methyl cellulose and carboxymethyl cellulose as binder in meat analogues is owed not only to its low cost but mainly to its unique thermo-reversibility (Wittek et al., 2020; Dong et al., 2021). At room temperature cellulose derivatives can form adhesives layers on the adherend (Amboon et al., 2012) prior to gelling upon heating at 50 to 55 °C due to an increase of hydrophobic interactions that leads to physical crosslinking (Sarkar, 1995; Desbrières et al., 2000). Although the exact gelation mechanism between proteins and e.g. methyl cellulose is not yet fully understood, the compound has been widely appreciated for its binding and moisture retention ability as well as texture modifier (e.g. firmness) (Bakhsh et al., 2021). Interestingly, there are no studies regarding the stickiness or cohesion of methyl cellulose as influenced by etherification, molecular weight, or concentration despite its long history of use.

### Alginate

Means and Schmidt (1986) exploited the gelling properties of alginate with calcium to act as binder in structured beef steak. The main advantage was that this mechanism can be used for raw and uncooked material. Yet, it should be noted that here the cohesion of meat pieces was evaluated and adhesion probably played minor role (Means & Schmidt, 1986). Similarly, Mitchell et al. (1976) filed a patent for using pectin with a low degree of esterification as a binder in combination with calcium for obtaining a coherent product with proteinaceous materials.

### Carrageenan

In a study by Arora et al. (2017) carrageenans were found to be the best binder in mushroom based sausage analogues as compared to soy protein concentrate, casein and xanthan gum. The sausages were evaluated among emulsion stability, cook loss and textural properties and therefore the exceeding binder properties of carrageenan can be explained by its higher water holding capacity and gel hardness compared to the proteins tested (Arora et al., 2017).

## Mixtures

Furthermore, mixtures of biopolymers bear potential as sticky binder. For instance, proteins from soy/pea mixed with pectin were shown to have distinct viscoelastic properties making them a promising food-grade PSA (B. L. Dekkers et al., 2018; Moll et al., 2022c). More particularly, altering the mixing ratio of pea protein isolate and pectin was a suitable method to tune adhesion and cohesion thereby providing different degrees of stickiness (Moll et al., 2022a). As such this mixture fulfilled one key function of a binder, i.e. stickiness. When the pectin was derived from sugar beet, treatment with laccase facilitated crosslinking between the two biopolymers and triggered the transition from a viscoelastic and sticky mixture to a solid-like mass (Moll et al., 2023c). This hardening reaction within the binder was later shown to provide cohesiveness in a burger type meat analogue (Moll et al., 2023b) as well as binding performance between different layers in a bacon type meat analogue (Moll et al., 2023a). These studies illustrated that the binding system must possess a certain liquid-like character to be applied easily on the surfaces of the adherent such as TVP particles. Furthermore, a certain cohesion should be provided that the TVP mixed with the binder can be formed into e.g., a burger patty. Finally, crosslinking facilitated the required hardening to set the TVP in a solid-like structure that is then not sticky anymore.

Interestingly, in the cement of sandcastle worms, a mixture of proteins is secreted as complex coacervate that adheres to inorganic matter and later on solidifies due to a change in pH (Brubaker & Messersmith, 2012). Understanding such phenomena in nature might be key to find edible ingredients with superior binding ability. This approach has for example been used by Kaur et al. (2011) to produce a synthetic glue, mimicking the adhesive of the sandcastle worm, for medical applications such gluing of bone fractures or implants (Winslow et al., 2010).

Taken together, the list of regularly used and available binders remains relatively short, illustrating the need to find new compounds or mixtures thereof that can serve as binders in food products. To do this, analysis of binder properties is a prerequisite. Consequently, available analytical methods to characterize binders and their functionality are listed next.

**Table I.1:** Overview of food binders, their function and product applications described in scientific literature.

| Binder | Product applications | Function | References |
|---|---|---|---|
| **Myofibrillar protein** | Processed meat (minced meat, burgers, sausages etc.) restructured meat products | Heat- or acid-induced gelation, water- and fat-binding, network formation | *Gordon et al. (1992), Toldrá and Hui (2014)* |
| **Eggwhite protein** | Meat analogue products, meat pieces | Heat-induced gelation, network formation | *G H Lu and T C Chen (1999), Mine (2014)* |
| **Skim milk powder, Whey** | Beef patties, meatballs | Water-holding | *Andiç et al. (2010)* |
| **Gluten** | Meat pieces | Adhesiveness, network formation | *Siegel et al. (1979)* |
| **Soy protein** | Meat analogue products | Coagulation, Gelation | *Mullen et al. (1971), Hager (1980), Herz et al. (2023)* |
| **Transglutaminase** | Meat products, restructured meat, meat analogue products | Protein cross-linking | *Kuraishi et al. (1997), Herz et al. (2021b),E. Atilgan and B. Kilic (2017), Lee and Hong (2020)* |
| **Laccase** | Meat analogue products | Cross-linking of sugar beet pectin | *Sakai et al. (2021)* |
| **Methylcellulose** | Meat analogue products | Adhesion, (reversible heat-induced) gelation, water-binding | *Amboon et al. (2012), Bakhsh et al. (2021)* |
| **Pea starch and fiber** | Beef burgers | Water-holding, gelation | *Pietrasik et al. (2020)* |
| **Alginate** | Restructured meat | Calcium-induced gelation | *Means and Schmidt (1986)* |
| **Carrageenan** | Meat analogue product | Gelation, water-binding | *Arora et al. (2017)* |
| **Pea protein and pectin** | Meat analogue products | Gelation, cohesion | *Moll et al. (2023b), Moll et al. (2023a)* |

# Analytical methods

## Stickiness

For a long time, there were no standardized testing procedures for stickiness in the food industry (Dobraszczyk, 1997), which is why adjacent fields such as the adhesive industry were screened for suitable methods and gradually adopted. Now, the probe tack test (**Figure I.4**) is the most common stickiness test in the food industry (Noren et al., 2019). Here, a probe of a certain size or shape is brought into contact with the adhesive food material for a defined time and contact pressure before withdrawal at a certain speed takes place while measuring the tensile strength (Adhikari et al., 2001). The recorded force-distance curve is used for the assessment of stickiness, sometimes combined with weighing the residual sample (Michalski et al., 1997). While the maximum force is an indicator for instant bond formation (adhesive strength), the distance the adhesive sample is extended to gives a measure of cohesion (Duncan et al., 1999; Grausgruber et al., 2003). Nevertheless, a complete differentiation of adhesion and cohesion is not possible with a single mechanical test (although it would be of greatest importance) (Dobraszczyk, 1997; Hoseney & Smewing, 1999; Frabetti et al., 2021) and the most suitable parameter for stickiness is therefore the work of adhesion being the area under the curve (Dhaliwal et al., 1990; Ghorbel et al., 2003) (**Figure I.4**). In addition, probe tack tests can be combined with video monitoring to better understand the debonding between probe and adhesive (Sun et al., 2013). Cohesive failure happens when the adhesive strength of the sample exceeds its cohesive strength and the sample fractures leaving residual sample on the probe. Adhesive failure occurs when no residual sample remains on the probe combined with a rapid return to zero force. (Kilcast & Roberts, 1998; Frabetti et al., 2021). The results depend on the used equipment and the applied testing conditions especially contact force, contact time, withdrawal rate (Kilcast & Roberts, 1998; Ghorbel & Launay, 2014; Burke & Hartel, 2021) as well as the surface energy of the probe (Heddleson et al., 1994; Laukemper et al., 2021). It should be mentioned that that the conditions during probe tack test should resemble the conditions occurring in the food matrix as closely as possible to conceptually test a binder (Brenner & Nishinari, 2014), e.g. choosing a probe with similar surface energy as the food particles that shall be bound.

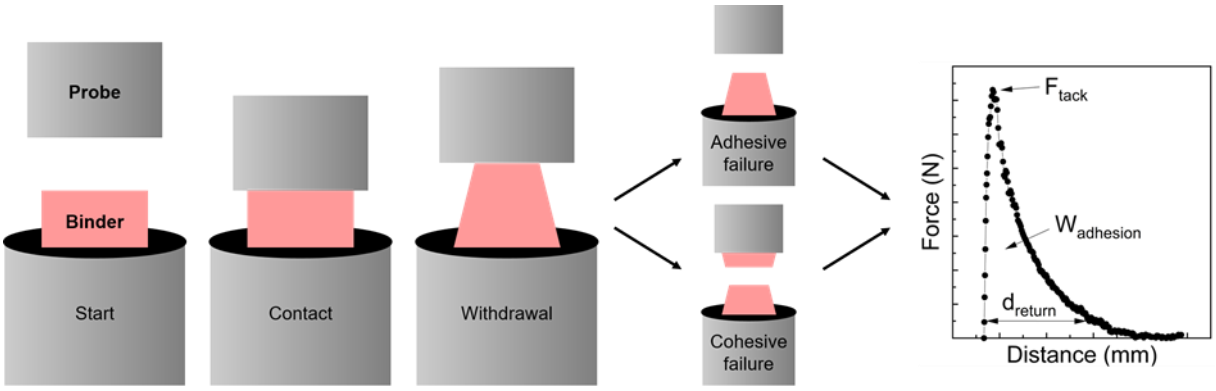


**Figure I.4:** Exemplary illustration of a probe tack test undergoing adhesive or cohesive failure and an associated force-distance curve as adopted from Moll et al. (2022d).

The widely adopted texture profile analysis produces a term called "adhesiveness", which should be taken with some caution though since the stickiness of pressure-sensitive adhesive such as foods is highly sensitive to the applied pressure. The harder the sample, the higher the force the plunger penetrates the sample thereby increasing adhesion between sample and plunger artificially. Furthermore adhesiveness changes when fracturing during the first compression occurs (Brenner & Nishinari, 2014). As a consequence, the texture profile analysis is not very suitable for adhesion tests (Wang & Hartel, 2021).

Other methods include peel tests where a strip of material being attached to the testing machine is placed on the adhesive sample and pulled off while recording the required force. When the adhesive is placed in a 180° fashion to the testing machine, it is prone to rupture during bending. An angle of 90° requires a complex system for maintaining this angle throughout the test (Kilcast & Roberts, 1998; Adhikari et al., 2001). This limits the wider use in food applications, and it is therefore mostly applied in testing food stickiness to packaging material (Noren et al., 2019; Frabetti et al., 2021).

Besides the afore-mentioned direct methods, there are indirect measures that bear the potential to further understanding the phenomena of stickiness. Microscopic approaches can be used to visualize adhesion and wetting on a molecular level, e.g. by detecting cracks of the adhesive after curing or determining the roughness of the material that shall be bound (Averina et al., 2021; Laukemper et al., 2021). Furthermore, contact angle measurements provide information on the wetting of the adherend by the adhesive (Michalski et al., 1997; Wagoner & Foegeding, 2018). In general, stickiness quantification is very product-specific, this is why food powders are usually not measured with a probe tack test (Noren et al., 2019), but with other techniques such as the shear cell method or the sticky point temperature method for which the reader is

referred to other reviews (Adhikari et al., 2001; Boonyai et al., 2004). Last, it should be noted that rheological measurements are powerful to understand how binders respond to applied deformations under conditions that can be tuned to resemble the conditions in the food matrix (Tabilo-Munizaga & Barbosa-Cánovas, 2005). As a matter of fact, it is well-known that viscoelasticity is one of the most important characteristics of pressure-sensitive adhesive to fulfill stickiness (Sun et al., 2013; Ghorbel & Launay, 2014; Wagoner & Foegeding, 2018; Wang & Hartel, 2020).

## Hardening

Rheological properties of gels and specific methods have been described in various books and reviews (Peleg, 1976; Mitchell, 1980; Mezger, 2006). The most important characteristics and tests to determine and describe gelation properties of biopolymers are storage modulus (G') and loss modulus (G'') and the linear-viscoelastic region (LVE), often determined in an amplitude sweep. Here, the G' values within the LVE indicate the hardness or stiffness of the sample, while the limit of the LVE-region ($\gamma_L$) and the development of G'' give more information on polymer interaction or cross-linking as well as elasticity or stiffness of the gel (Renkema, 2004; Mezger, 2006). The frequency dependency of the sample as measured during frequency sweep indicate the type of gel (entangled, physical, chemical) (Stading & Hermansson, 1990; Tunick, 2011). Rheology is a very sensitive measurement and testing methods can be precisely controlled, repeated, and compared. However, gel properties can only be measured up to the point of fracture and therefore can't be directly correlated to the chew down phase (Foegeding, 2007). In addition, time- and temperature sweeps can be used to mimic process induced changes of the binder (Ahmed et al., 2016). Creep recovery tests were used to model viscoelasticity of caramel and correlate it to its adhesiveness (Wagoner & Foegeding, 2018).

The texture analysis of food has been extensively shaped and described by Bourne, who invented also the "Texture Profile Analysis". This method, which is still widely used today to characterize food texture, is performed by a double uniaxial compression of a sample with an instrument that records force versus distance. From the resulting curve, various texture parameters can be calculated, such as hardness, cohesiveness, or springiness. The maximum force of the first compression cycle is considered the "hardness" (Bourne, 1968). If only the parameter hardness is of interest, it can also be measured by a simple compression or penetration test (Bourne, 2002). Advantages of this test include the easy and fast performance as well as good correlation with sensory analysis (Szczesniak, 1987; Bourne, 2002). Especially

semi-solid foods can also be analyzed by extrusion testing, either forward extrusion (where the sample is pressed through a cylinder with an outlet at the bottom) or backward extrusion (where the probe is forced to flow through a small gap between plunger and container) (Liu et al., 2019). However, all texture analysis results are strongly influenced by the dimension of the sample and the measurement geometry as well as extend of compression or penetration. Therefore, results of different studies are often not directly comparable (Bourne, 2002).

## Water holding capacity

Various methods to measure the water holding capacity have been reviewed by Trout (1988). Classically, measurements like the compression and filter paper or centrifugation method have been used. With the first method, a sample of a defined size is compressed by a specific force on a filter paper. The released water from the sample is absorbed by the filter paper and can be measured by weighing or by the visible area on the filter paper. For the centrifugation method, specific centrifuge tubes are used, which allow for a separation of the sample and the exuded water released by the application of centrifugal force can be weighed (Kristensen & Purslow, 2001).

Water and fat binding or in this case rather water and fat release are related to the sensory property juiciness in meat products. It was suggested that the first impression of juiciness is generated by the rapid release of fluids, related to the water content, while a sustained impression of juiciness during chewing is related to the fat content. This interaction can only reliably be measured by a sensory panel (Winger & Hagyard, 1994).

## Further analytical approaches

The binding strength of beef burgers with different starch and fiber fractions acting as binder was tested by pulling apart the sample just beyond the point of rupture while recording the force (Pietrasik et al., 2020). A similar tensile strength test was done by Herz et al. (2021b) for a bacon analogue composed of extruded plant proteins and plant-based fat mimetics with soy protein isolate acting as a binding agent. Similarly, G.H. Lu and T.C. Chen (1999) tested the binding strength of egg white and plasma powder acting as a binder for muscle chunks with a modified Warner-Bratzler shear device. Furthermore, texture properties of composite foods can be determined with a sensory test. A first method for assessing texture systematically with a sensory panel was described by Brandt et al. (1963), where members of a trained panel

evaluated mechanical characteristics on a numerical scale with appropriate reference samples presented for each characteristic. Another method to obtain a specific texture profile by an untrained sensorial panel, the “consumer texture profile technique” was developed by Szczesniak et al. (1975). A detailed description of various established sensory tests was published by Meilgaard et al. (1999) and a review on novel sensory methods has been written by Varela and Ares (2012).

## Conclusions

Taken together, the survey of the existing literature shows that there is still a relatively inadequate understanding of structure-function relationships when it comes to binders for use in concentrated, hydrated systems such as plant-based meat or cheese analogues. It appears clear though that at a minimum three functions are critically important for such compounds, namely (a) stickiness, (b) hardening and (c) water binding. Individually, studies have been carried out to characterize one or the other of these properties. While the origin of stickiness and the ability of a compound to bind individual particles together has been studied in powders and attributed there to liquid and solid bridges, constituents of vegan food systems such as protein extrudates or plant fat particles are apparently mostly bound through polymer entanglement and/or attractive interaction forces balancing adhesion and cohesion. A more holistic approach to binder functionality is therefore recommended by looking simultaneously at stickiness, hardening and solvent binding for a range of binder candidates in well-defined model matrices such as burger mixes or vegan raw fermented sausage masses. Such studies could yield insights into what approaches are promising to tailor such components to optimize their performance with respect to the above-mentioned functions. To that purpose, one might start using methylcellulose gums as a benchmark but employing methylcellulose gums with different degrees of etherification and molecular weights, and then assess the degree to which they provide stickiness, hardness, and water binding. In gluten, one might try and modify the used ratios of glutenin to gliadin and modulate hydration temperatures and shearing so as to change the overall structure of the complex which could lead to an altered ability to entangle and to bind water. Some candidates for new binders have been listed previously and include but are not limited to rice bran, yellow pea, chickpea and lentil, psyllium, teff, quinoa buckwheat maize, chia, and acorn. There, investigations might focus on generating and characterizing different fractions such as fiber, protein, or mixtures thereof in order to better understand this ingredient class.

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# Aims of the study

The aim of this thesis was to investigate the potential of plant protein gels as binders in meat analogue products. Processed meat products, such as sausages, are usually produced by comminuting lean meat and fat tissue and the addition of salt. Comminution of the meat and especially the addition of salt lead to partial solubilization of myofibrillar protein. This partially solubilized myofibrillar protein serves as binder for those meat products by "gluing" the comminuted meat and fat particles together. During processing of sausages, the solubilized proteins are usually gelled by heating (e.g. emulsion-type sausages) or acidification (e.g. raw-fermented sausages) and leading to hardening and substantial contribution to the final texture and shape of the product. To produce corresponding analogues of existing meat products, we therefore hypothesized that three components are necessary: fibrous plant proteins, plant fat particles with elastic and melting properties as well as gel-forming (soluble) plant proteins that can act as binder. While a lot of research has been done on fiber formation of plant proteins by extrusion or shear cell technique over the last years and recently a novel approach to mimic animal fat tissue by emulsification of oil or oil-fat-mixtures with plant proteins followed by enzymatic cross-linking, systematic studies on binders in meat analogues are scarce. Nowadays, various hydrocolloids and predominantly methylcellulose are used in meat product analogues on the market, but consumer acceptance of these ingredients is declining.

We hypothesized that a binder should be in sol-state in the beginning, which enables processing, such pumping and filling, and a gelation should be induced at a later state to embed fibrous protein and fat particles in a continuous matrix and to result in sufficient hardness to contribute to the expected texture of the final meat analogue product. While a wide range of burger and finely comminuted sausage analogues has become established on the market, the range of products in lacking in acidic salami-style sausages as well as non-comminuted products, such as bacon. Therefore, this thesis focused on the challenges of application in these products.

To test the hypothesis, different mechanistic approaches such as heat-, salt-, and acid-induced gelation and enzymatic crosslinking or combined approaches for network formation were investigated. In addition, the binding strength of those gel particles, when applied as binder system between a layer of high-moisture extrudate and a layer of fat mimetic was characterized (Chapter II). The gelation properties and the microstructure of soy protein isolate gels, induced by simultaneous addition of slowly acidifying glucono-δ-lactone and the enzyme

transglutaminase (TG) was investigated, as this is of particular interest for meat product analogues with low pH-values, which is the case for traditionally fermented foods (Chapter III). In this context, the binding capacity at different ratios of the characterized GDL-induced soy protein gel to high-moisture extrudate was investigated, to study the influence of the quantity ratio and the addition of transglutaminase on the texture of salami-style sausage analogues (Chapter IV). In addition, hydrated gluten in various ratios to a high-moisture extrudate was applied as an alternative binder system in sausage analogues similar to the previously studied, to show the influence of this binder on the texture of a plant-based sausage analogue (Chapter V).

# II. Chapter

# Influencing Factors on the Ability to Assemble a Complex Meat Analogue Using a Soy-Protein-Binder

**Eva Herz[1], Laura Herz[1,2], Johannes Dreher[1], Monika Gibis[1], Joydeep Ray[2], Patrick Pibarot[2], Christophe Schmitt[2], and Jochen Weiss[1*]**

[1]Department of Food Material Science, University of Hohenheim, Garbenstrasse 25, 70599 Stuttgart, Germany

[2]Nestlé Research, Nestlé Institute of Material Sciences, Department of Chemistry, Vers-chez-les-Blanc, Lausanne 26, CH-1000, Switzerland

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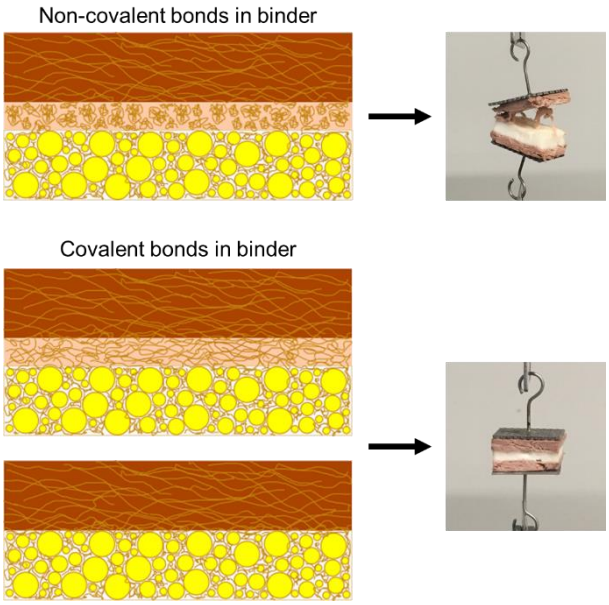
*Corresponding author: Tel: +49 711 459 24415; E-mail address: j.weiss@uni-hohenheim.de

# Abstract

Existing complex meat analogues such as bacon often do not resemble meat-based ones in appearance, texture, and techno-functionality. This has been due to them being only composed of a differently colored homogeneous protein matrix, but lack in presence of a fat phase which contributes to frying performance and taste perception. We hypothesized that extruded plant proteins and plant-based fat mimetics can be assembled into a performance-enhanced bacon analogue by using a suitable binder system based on soy protein isolate (SPI), where, furthermore, adhesion can be improved by increasing gel strength. The results showed that extrudates and fat mimetics could not be adhered sufficiently by a heat-induced 14% (w/w) SPI-gel as binder, independent of the applied quantity. Successful adhesion was only achieved when transglutaminase was added to the binding suspension, and only the hardest, transglutaminase cross-linked gels resulted in acceptable cohesion. Overall, our results showed, that gels needed to be formed via covalent, isopeptide bonds to act as functional binders in a bacon analogue whereas gels formed via physical bonds were insufficiently adhesive to function as a binder.


**Keywords**: complex meat analogue, binder, gelation, texture, soy protein isolate, transglutaminase

**Graphical Abstract**



# Introduction

The design of consumer-acceptable analogues of meat and meat products is gaining attraction due to the desire of consumers to reduce the amount of meat in their diets, a fact that is related to environmental, ethical, and health concerns associated with their consumption. The consumption of processed red meat has been associated with health risks, especially cardiovascular diseases, coronary heart diseases and cancer with potential mechanisms linked to the content of saturated fat, cholesterol, iron, phosphatidylcholine, and carnitine (Anand et al., 2015). On the other hand, vegetarian diets, and diets high in plant foods and plant protein sources have been associated with a lower prevalence for coronary heart diseases and obesity (Anand et al., 2015; Appleby & Key, 2016). Additionally, ethical concerns about animal welfare and slaughtering lead consumers to decrease or exclude meat from their diet and increase consumption of meat analogue products instead (Piazza et al., 2015; Apostolidis & McLeay, 2016). Agricultural food production is very resource intensive as more than 70% of the world's water use is currently in the area of agriculture (FAO, 2020). Moreover, the food supply chain has been estimated to produce about 26% of all anthropogenic greenhouse gas emissions (GHG) (Poore & Nemecek, 2018). Studies have indicated that excluding animal products from the diet could reduce the food's land use by 76%, GHG emissions by 49% and freshwater withdrawals by 19% (Poore & Nemecek, 2018). While beef, pork and poultry have footprints of 45-640, 20-55 and 10-30 GHG kg $CO_2$-eq $kg^{-1}$ protein, vegetarian meat substitutes, containing egg- or milk protein, have a footprint of 17-34 GHG kg $CO_2$-eq $kg^{-1}$ protein. In comparison, completely plant-based meat substitutes range lower with 6-17 GHG kg $CO_2$-eq $kg^{-1}$ protein (Nijdam et al., 2012). Therefore, plant-based meat substitutes bear the potential to supply protein-rich meals at a lower environmental impact, thereby providing opportunities to make the global food supply system more sustainable.

Protein structuring approaches including extrusion and shear cell technologies have sufficiently advanced by now to provide a good combination of scalability and resource efficiency. They can be used to create anisotropic fibrous structures from biopolymer blends of plant proteins thereby providing the basis for the creation of meat analogues (Birgit L. Dekkers et al., 2018). For example, fibrous structures were manufactured at high temperatures using a conical shear cell from soy-protein-gluten-mixtures and pea-protein-gluten-mixtures with tensile strengths being in the same range or even harder than those of chicken meat (Schreuders et al., 2019).

Yet, there hasn't been much research on the more complex assembly of analogues using these and other components such as structured fat systems (aka fat mimetics).

Bacon is a very popular cured meat product, especially in the US but also in Europe. It is produced from pork belly by trimming, curing, smoking, pressing and optionally slicing. In distinction to other meat products, fibrous muscle tissue and fat tissue are visually distinctly layered (Devine & Jensen, 2004). There are a few vegan bacon analogues on the market, but to date those are mostly composed of a (structured) biopolymer phase, that has been colored differently, but lacks a structured fat phase which is essential for the performance during frying where the fat is rendered providing for crispiness and a characteristic taste. In this study, we hypothesized that assembly of a fat mimetic system with extrudates may yield a product that more closely resembles animal-based bacon. In this context, the functionality of a suitable binder providing the required adhesion between extrudate, and the fat mimetic was seen as critical. The term "binding" has been used by meat scientists (among several other meanings) in the context of having meat pieces adhere to each other (Siegel et al., 1979; Terrell et al., 1982; G.H. Lu & T.C. Chen, 1999). Following this definition, the term is similarly used to describe the functionality of components that provide adhesion between plant-based components such as extrudates and structured fats (Arora et al., 2017; Lee & Hong, 2020).

In meat processing, the extraction of salt-soluble myofibrillar protein is typically a first step in order to obtain several types of meat products, although targeted functional properties vary. In finely comminuted emulsion-type-sausages, formation of a weak gel is already observed prior to heating and the gel strengthens upon heating as proteins denature and aggregate. But the term "binding" can also be associated with the incorporation of water and fat into the meat batter (Fukazawa et al., 1961; Gordon et al., 1992; Liu et al., 2016). In restructured meat-products, the focus has been on the ability to adhere separate meat pieces together to form a composite solid, such as a restructured ham (Siegel & Schmidt, 1979; Chen & Trout, 1991). In all these cases, solubilized myofibrillar protein plays a crucial role as a binder compound to generate coherent, solid meat products upon heating (Fukazawa et al., 1961; Samejima et al., 1969; Nakayama & Sato, 1971). Our base hypothesis was therefore that plant protein suspensions may similarly provide the required binder functionality as long as they are capable of undergoing a heat induced gelation. It should be noted that when discussing binder functionality of solubilized meat as well as soy protein, water holding capacity (WHC) is also of importance. It is crucial to product performance in meat products and there, an ionic strength of up to

0.8 - 1.0 M NaCl has shown to increase water holding capacity, whereas higher salt concentrations decrease the water holding capacity (Lawrie, 2006). In contrary, increasing ionic strength has shown to decrease WHC in soy protein gels (Lakemond et al., 2003) but generally increase gel strength of globular protein gels up to certain ionic strength values (Foegeding et al., 1995). It was further postulated that gel strength and the ability to form a sufficient number of intramolecular bonds as well as bonds with the used composites of protein extrudates and structured fat phases may be important factors influencing quality attributes of the assembled products (Furukawa et al., 1980). To test this hypothesis, different influencing factors (SPI-concentration, temperature, coagulants, transglutaminase) on the gel strength of SPI-gels were analyzed to obtain first insights into the suitability of these systems as binders. Subsequently, selected gels were applied as a binder between slices of protein extrudates and structured fat analogues (Dreher et al., 2020a, 2020b) and their ability to provide cohesion in composite samples was investigated using a tensile test.

# Materials and Methods

## Materials

Soy protein isolate (Supro Ex 37 HG IP) was purchased from Solae Europe S.A. (Geneva, Switzerland). Transglutaminase (Transglutaminase Activa WM) was bought from Ajinomoto Foods Europe SAS, Hamburg, Germany. Glucono-δ-lactone (GDL) was obtained from Roquette GmbH, Frankfurt, Germany. Calcium sulphate ($CaSO_4$) was obtained from ThermoFisher (Kandel) GmbH, Karlsruhe, Germany and ferrous sulphate ($FeSO_4$) from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany. Calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) were purchased from Carl Roth GmbH + Co. KG, Karlsruhe, Germany. NaCl was bought from Südwestdeutsche Salzwerke AG, Heilbronn, Germany. High-moisture plant protein extrudate (pea/gluten-based, ~54% dry matter) and stearin shea butter fraction (from AKK, Hull, UK) were provided by Nestlé Research, Lausanne, Switzerland. Canola oil was bought from a local wholesaler (MEGA eG, Stuttgart, Germany).

## Methods

*Experimental procedure.* In a first step, the influence of gel strength and consequently, the hardness of SPI gels under varying conditions such as SPI-concentration, heating, addition of

coagulants, acidification by GDL and additional covalent crosslinking by transglutaminase was analyzed to select suitable gels to act as binders in subsequent assembly studies.

Firstly, the effect of SPI content (10, 12, 14 and 16%) and heating temperature (70, 80 and 90 ºC) on the hardness of the gels was analyzed. Secondly, we investigated how the addition of various coagulants ($CaSO_4$, $FeSO_4$, $CaCl_2$, $MgCl_2$) in concentrations of 0.3 – 5% (w/w) influenced the hardness of SPI gels. The applied concentration range of 0.3 – 5% for the divalent salts was chosen based on design and results of various other studies (DeMan et al., 1986; Kohyama et al., 1995; Tang et al., 2011) and adapted to the increased protein concentration of the studied samples. Also, the influence of acidification on gel performance was investigated. To that purpose, a concentration of 1.3% (w/w) GDL was added to the SPI suspension to induce a pH decrease to 4.5. This pH was chosen since it represents the isoelectric point of the used soy protein isolate where net charges are zero (data not shown). Divalent salts and GDL were chosen because they are typically used for soy protein gelation in tofu production (DeMan et al., 1986). Furthermore, the reduction of repelling charges by adjusting the pH close to the isoelectric point or salt addition can optimize the cohesion and adhesion of proteins (Wall & Huebner, 1981). The protons released by GDL and calcium ions neutralize the net charge of the proteins allowing hydrophobic interactions to become predominant, and promoting interaction of proteins (Kohyama et al., 1995). The functionality of coagulants was tested at a SPI concentration of 14 (w/w) % in the suspension since viscosity was sufficiently low to facilitate an even distribution in the suspension.

Furthermore, TG was added to induce covalent crosslinking in SPI suspension. There, the optimum concentration for achieving a maximum hardness had been tested previously (Herz et al., 2021a) indicating that 25 mg TG per g SPI were required.

Finally, the effect of the 16% SPI system, added in different quantities (4, 5 and 6 g), TG-gelled binder (6 g) and in-situ gelling of fat mimetic between a high-moisture extruded plant protein sheet and a fat mimetic composed of an emulsified and cross-linked fat crystal network (EC-FCN)) (Dreher et al., 2020a, 2020b)to form vegan bacon analogue, and their ability to maintain the mechanical integrity of the composite product was investigated.

*Gel preparation.* Soy protein isolate (SPI) (10-16% w/w) and, if applicable, coagulants were dispersed in water with a kitchen aid mini food processor (Whirlpool Germany GmbH, Stuttgart, Germany) for 30 s at level 1, followed by a further mixing for 60 s at level 2.

Afterward, the protein suspensions were filled to the brim into ten 30 mL Nalgene cups (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and heated for 30 min at 70, 80, 85 or 90 °C using a heated water bath (Compact Thermostat M20, Lauda Dr. R. Wobser GmbH & Co. KG, Germany). After cooling to room temperature, the samples were stored at 2 ºC prior to measurements.

*Hardness.* The hardness of gels was measured by a puncture test with an Instron Universal Texture Analyzer (Instron Model 3365, Instron Engineering Corporation, Norwood, Massachusetts, USA). Sample SPI gels made in Nalgene cups with sample volumes of 30 mL (see above) were penetrated at a speed of 1.5 mm s-1 to 75% length scale with a pin geometry (d=13 mm). Measurements were repeated using 10 different samples per manufactured gel.

*Fat mimetic preparation.* An emulsified and cross-linked fat crystal network (EC-FCN), that closely resembles animal fat tissue in terms of mechanical properties, was prepared following the procedure of Dreher et al. (2020a). Briefly, a 70% O/W-Emulsion with SPI as emulsifier was prepared and gelation induced by the addition of transglutaminase (TG). The oil phase consisted of 70% (w/w) canola oil and 30% (w/w) shea butter stearin fraction, which was melted into the liquid oil in order to mimic melting properties of animal fat. The water phase consisted of a 10% (w/w) SPI-suspension, resulting in a total SPI-concentration of 3% (w/w) in the emulsion. The SPI-suspension was prepared by dispersing SPI in water with a kitchen aid mini food processor, followed by a slow addition of the oil phase while blending. After emulsification, 1% (w/w) NaCl and 0.75% TG (w/w; 25 mg/g SPI) were added. The emulsion was filled into rectangular aluminum forms, covered with foil and placed in a heating chamber. Heat treatment was performed at 45 ºC chamber temperature until a core temperature of 40 ºC was reached. Temperature was kept constant for 1 h to enable protein-crosslinking by TG. Afterward, the sample was heated at 90 ºC to a core temperature of 85 ºC and then stored at 2 ºC prior to further use.

*Assembly of extrudate and fat mimetic.* SPI-suspensions were spread evenly between a layer of the extrudate and a layer of the fat mimetic and subsequently heat treated to adhesion. To that purpose, both the fat mimetic and the used extrudates were cut into pieces of 8.3x12.3x0.2 cm (=102.1 $cm^2$). Binder amounts of 29.6 $mg/cm^2$ (3 g per sample), 39.5 $mg/cm^2$ (4 g per sample) and 59.3 $mg/cm^2$ (6 g per sample) were tested. Samples were pressed with a defined weight of 8 $g/cm^2$ and incubated for 1 h at 40 ºC, followed by heating for 1 h at 85 ºC.

*In-situ gelation of fat mimetic.* In order to briefly test also the ability of the uncrosslinked fat mimetic system to act as binder, the emulsion was spread onto an extrudate layer and allowed to gel.

*Tensile strength.* The cohesion of products composed of adhered extrudate and fat mimetics was analyzed using a tensile test, where samples were pulled apart using a texture analyzer (Instron Model 3365, Instron Engineering Corporation, Norwood, Massachusetts, USA). There, the maximum force and work were recorded. The test was based on the adaptation of a method for bacon previously described by Teye et al. (2006). From each sample, 10 squares of 2 cm diameter were cut and glued with cyanoacrylate glue to stainless steel stabs of the same diameter. The stabs were hooked into the measurement geometry of the texture analyzer. The samples were pulled apart at speed of 1 mm $s^{-1}$ and force versus distance curves were recorded. The maximum force and area under the curve referring to the work of adhesion were calculated. Additionally, pictures of ruptured samples were taken.

*Statistics.* Statistical analysis of variance (one-way ANOVA) and Tukey's post hoc tests were carried out with OriginPro 2020 (OriginLab Corporation, Northampton, USA). All factors included in the statistical analysis are shown in the graphs; maximum force and work of adhesion (**Figure II.3**) were analyzed independently. Differences at $P< 0.05$ were considered to be significantly different. The assumption of normality and equal variance was tested ($p<0.05$). Experiments were carried out in duplicate and measurements were performed on at least five samples for puncture tests and ten samples for tensile tests. Graphs were also prepared with OriginPro (OriginLab Corporation, Northhampton, USA).

# Results and Discussion

## Gelation behavior of concentrated SPI suspensions

Initially, the effect of SPI concentration and heating temperature on the hardness of SPI-gels was investigated, as heat denaturation is known to alter the adhesive and cohesive properties of globular proteins, as well as being an essential mean to solidify many composite systems (Wall & Huebner, 1981). The hardness of heat-induced SPI gels (12-16% w/w) increased with increasing SPI concentration, but was not much affected by the heating temperature (**Figure II.1**). While hardness values of SPI gels with 10% and 12% (w/w) at all heating temperatures were in a range of ~0.1-0.9 N and did statistically mostly not differ significantly from each

other, the hardness increased significantly with increasing SPI concentration to ~ 1.9-2.3 N with 14% (w/w) SPI and to ~ 4.7-5.8 N with 16 (w/w) % SPI. The results are in good agreement with those obtained by Shimada and Cheftel (1988), who suggested that heat-induced gel formation is more dependent on hydrophobic interactions, hydrogen bonds, and S-S interchange than on the formation of additional S-S bonds. An increasing protein concentration leads to an increasing gel firmness as more intermolecular interactions between proteins are possible and the area occupied by the protein network is increased (Zayas, 1997).

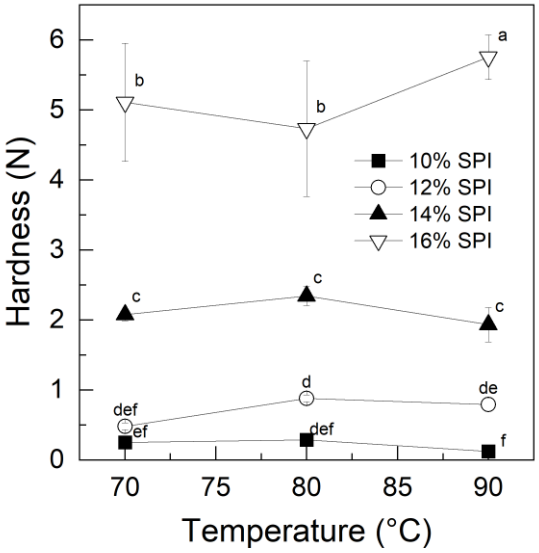



**Figure II.1** Influence of heating temperature on hardness of soy protein gels with 10, 12, 14 and 16% (w/w) SPI at native pH without salt addition. Error bars showing standard deviations. Values followed by different superscripts are significantly different ($P < 0.05$).

The addition of coagulants at various concentrations, except for $FeSO_4$, did not significantly increase the hardness of SPI gels, whereas crosslinking induced by TG distinctly increased gel hardness by ~11 N. Again, between 70 and 90 ºC the temperature did not have a distinct influence on the hardness of the gels, and 85 º was therefore chosen as a heating temperature for later assembly experiments, mainly for practical reasons (comparability to another study and microbial inactivation to facilitate a sufficient shelf life of samples).

The addition of $MgCl_2$ didn't significantly alter the hardness, but a slight trend towards an increasing hardness with increasing concentration was visible (**Figure II.2A**). The results are in line with those obtained by Rui et al. (2016), who had also reported that hardness increased with increasing $MgCl_2$ concentration. However, Rui et al. (2016) performed their experiments at lower salt and protein concentrations (0.25-0.35% w/v $MgCl_2$, 6% w/v SPI). There, a

maximum hardness was reached at 0.3% (w/v) $MgCl_2$ at 6% (w/v) protein, whereas in our experiments a maximum hardness was reached at 5% (w/w) $MgCl_2$. which is likely due to the greater number of protein crosslinking sites requiring more salt molecules to be present. The same trend was observed for the addition of calcium chloride ($CaCl_2$) (**Figure II.2A**). $Ca^{2+}$ ions can counter-balance negative charges on the polypeptide chains of the proteins, favoring protein-protein interactions and lowering protein-water interactions leading to increased aggregate sizes and increased gel network random aggregation with increasing $CaCl_2$ concentration (Maltais et al., 2005). With the addition of calcium sulphate ($CaSO_4$), a maximum hardness was reached upon use of 1% (w/w) of the sulphurous calcium salt (2.70 ± 0.11 N), whereas higher concentrations showed a trend towards decreased hardness (**Figure II.2A**). However, a significant difference wasn't determined. The decreasing hardness may be attributed to increasing aggregate sizes, which led to the syneresis of the gel (Kohyama et al., 1995). With the addition of GDL, a hardness of 2.55 ± 0.10 N was reached (**Figure II.2B**) which was also not significantly higher. Gel formation with addition of GDL to protein suspension occurs since electrostatic repulsion of proteins is minimal allowing for an aggregation of denatured protein by hydrophobic interaction to take place (Campbell et al., 2009). The addition of salts or GDL mainly leads to increased physical interactions, by reducing electrostatic repulsion between proteins, showing a slight trend in increasing the gel strength compared to only heat-induced gels, but statistically not significant. Nevertheless, the use of salts and GDL may be interesting for the manufacture of other vegan meat product alternatives where lower water activities and pH values may be required to prolong shelf life.

Upon addition of iron sulphate ($FeSO_4$), the hardness of the gels significantly increased with increasing concentration to 5.76 ± 2.51 N (**Figure II.2A**). Concluding from the scarcity of publications, $FeSO_4$ is not typically used as a coagulant for soy or other food protein gels, but was included in the study, since it might provide meat analogues with iron, a component that they usually lack in comparison to meat and meat products. It should be noted though that $FeSO_4$-supplemented soy protein gels had an intensive blood-like smell which might pose problems in terms of sensory performance.

With the addition of 25 mg TG per g SPI a hardness of 13.68 ± 1.76 N was reached (**Figure II.2B**), which represented the hardest gel formed in this study. Microbial transglutaminase polymerizes soy protein, which leads to gel formation as the enzyme catalyzes an acyl-transfer reaction between the amino group of lysine and the carboxamide group of glutamine residues

(Nonaka et al., 1989). Additionally, hydrophobic interactions among hydrophobic areas of fragmentary components of SPI contribute to the formation of gel network (Tang et al., 2006)

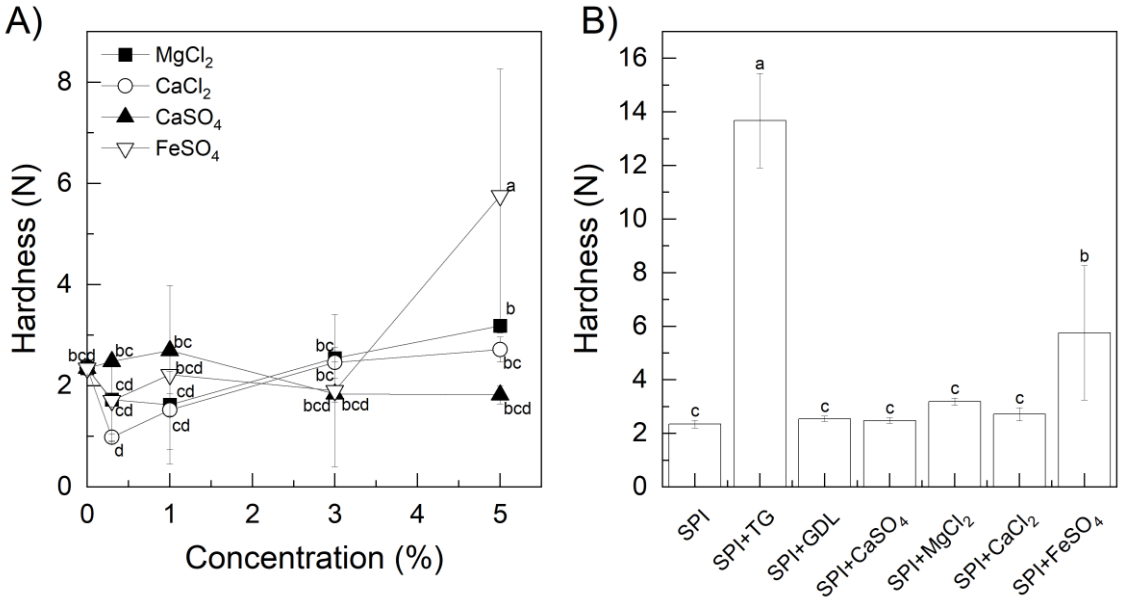



**Figure II.2** Influence of concentration of $MgCl_2$, $CaCl_2$, $CaSO_4$ and $FeSO_4$ on the hardness of gels with 14% (w/w) SPI, heated at 85 °C (A). Comparison of maximum reached hardness of 14% (w/w) SPI gels with different coagulants (0.4% (w/w) TG, 1.3% (w/w) GDL, 1% (w/w) $CaSO_4$, 5% (w/w) $MgCl_2$, 5% (w/w) $CaCl_2$) (B). Error bars showing standard deviations. Values followed by different superscripts are significantly different ($P < 0.05$).

Summarizing, the addition of salts or GDL did not significantly increase the gel hardness (**Figure II.2B**). An increase in protein concentration from 14 to 16% (w/w), the addition of FeSO4 or gelation induced by TG increased the gel strength significantly. However, $FeSO_4$ was not considered as a promising food ingredient because of the intensive blood-like smell it caused. On the basis of these results, assembly tests with SPI suspensions acting as binders upon gelation were carried out only with heat- and TG-induced gels (see below). An increased gel strength is key to the functionality of a binder since the combined product will break at its weakest point. The product may also not be able to withstand later a slicing procedure and disintegrate into its individual components under even weak mechanical loads. Furthermore, the cohesion of the product is also important to generate the desired texture: from a sensory perspective, a binding strength according to the mimicked product is favorable, as too weak as well as too strong a cohesion will lead to an unauthentic texture. In protein extrudates, hardness is mostly caused by the formation of disulphide bonds while in the fat mimetic system, hardness is due to creation of iso-peptide bonds after addition of TG. Furthermore, for the application of SPI gels as binder in complex meat analogues, it should be considered, that further processing

such as drying or frying will lead to further changes in texture of the binder as well as the extrudate and fat mimetic due water loss and increased temperatures.

## Binding behavior of concentrated SPI suspensions

Based on the results above, we then investigated the ability of the SPI suspensions to provide binding between an extrudate and a fat mimetic after a heat or TG treatment to assemble a composite bacon analogue. Initially, the amount of binder applied between extrudate and fat layer was varied but this was found to not play a significant role in the integrity of the assembled bacon analogue. A binder amount of 3 g per sample was the minimum amount of gel required to ensure complete coating of extrudate and fat surfaces. No significant differences were observed when the amount was increased as neither maximum force nor work of adhesion was systematically altered. The maximum forces ranged between 0.79 ± 0.44 N and 1.05 ± 0.67 N and work of adhesion between 0.59 ± 0.31 mJ and 1.06 ± 0.70 mJ (**Figure II.3**). The amount of binder to adhere fibrous protein structures has, to the best of our knowledge, very rarely been studied, and there are only a few studies available that contain results concerning the adhesion of meat pieces or restructured meat. G.H. Lu and T.C. Chen (1999) found a linear correlation between the amount of binder and adhesive strength when applying 0-8 mg/cm$^2$ egg white powder as binder. Kuraishi et al. (1997) used TG and caseinate to adhere meat cubes and found that an excessive amount of caseinate caused the formation of gel pockets, which weakened the binding strength. Based on these results, we postulate that there may be an "amount range" where adhesive strength increases with increasing binder amount, which was likely exceeded by the amount used in our study. Thus, the effect was not seen, and more experiments using lower amounts of binder may have to be carried out at a later point to investigate this further. Likely, the individual hardness of composites in relation to the hardness of the binder may play a crucial role there. However, an in-depth investigation of this would require a tuning of the hardness of all three components, e.g. the extrudate, the fat mimetic and the binder system.

The impact of gelation conditions (heating with or without the prior addition of TG) on ability of SPI to act as a binder was then finally studied. Substantially enhanced adhesion between layers were observed using SPI in combination with TG as a binder as the maximum force increased to 2.70 ± 0.93 N and work of adhesion to 2.44 ± 0.70 mJ (**Figure II.3**). TG has been reported to improve the mechanical properties when used as a binder in restructured fish products (Ramírez et al., 2006). Pictures from samples after the adhesion test additionally show that in samples without TG, rupture occurred almost exclusively in the binder phase, that is

between the extrudate and the fat mimetic (**Figure II.4**). In contrast, upon use of TG, rupture occurred mostly within the fat phase, indicating that the fat mimetic had become the weakest structural entity of the composite samples.

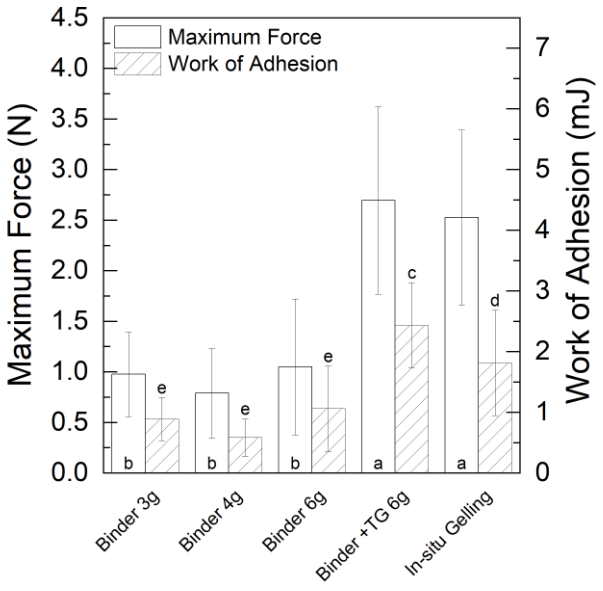



**Figure II.3** Maximum force and work of adhesion of fat-extrudate samples adhered with 3, 4 and 6 g of binder (16% SPI gel), with 6 g of TG-gelled binder and in-situ gelling of fat-mimetic, measured by tensile test. Error bars showing standard deviations. Values followed by different superscripts are significantly different ($P < 0.05$).

At present, there are still many open questions though as it pertains to the function of binders in food systems, an area that has become very relevant as new vegan meat and meat product analogues are being developed. Their design requires such components in order to yield heterogeneous matrices with sufficient integrities and appealing textures. Currently, methylcellulose is being used in many products, but consumer acceptance of this additive is declining. Ultimately, food manufacturers will be required to substitute it for more acceptable alternatives. In light of this, further investigations into functionality of various components to act as binders should be carried out. In a final experiment, we also investigated if the fat-mimetic could be directly used as a binder since its solidification also involved the use of TG possibly providing for adhesion as well. Indeed, results of this in-situ-gelling experiment showed that the adhesion strength remained similar to that of composites made with SPI+TG as a binder, i.e. the maximum force was $2.53 \pm 0.87$ N and the work of adhesion was $1.81 \pm 0.87$ mJ. As such, the emulsion that's serves as a base for the fat mimetic could potentially also be used as an adhesive gluing extrudate layers together. A study on non-meat protein binders between meat pieces showed that the types of molecular interactions stabilizing

a binder network are the most important factor determining their binding ability, and that ideally they should be of the same nature as those of myosin formed gels so that interactions between non-meat protein and myosin take place (Siegel et al., 1979). Our results showed that only the TG-induced gel, with presumably covalent (isopeptide) bonds, led to sufficient cohesion in the assembled bacon analogue system, whereas heat-induced SPI gels with mainly physical bonds were too weak to function as an appropriate binder, especially, when increased mechanical forces, such as slicing were applied to the product. The characteristic firm and fibrous texture of extruded plant protein have been reported to be mainly formed by disulfide bonds (Wall & Huebner, 1981; Lin et al., 2000; Liu & Hsieh, 2008). As gelation in the fat mimetic was induced by TG, the bonds stabilizing the gel were predominately also covalent (isopeptide) bonds.

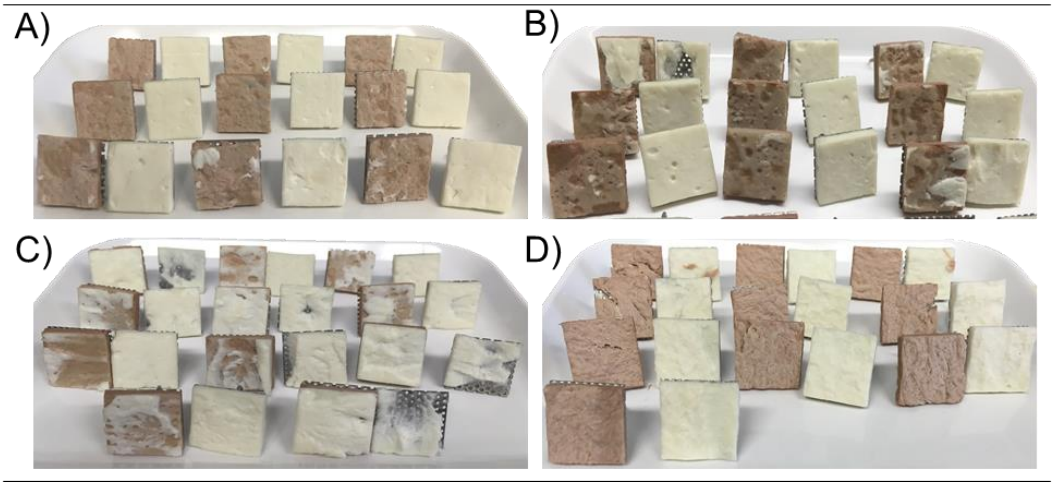



**Figure II.4** Visual appearance of fat-extrudate samples after tensile test, adhered with 39.5 mg/cm$^2$ (4 g) (A), 59.3 mg/cm$^2$ (6 g) (B) pure SPI binder, 59.3 mg/cm$^2$ (6 g) SPI+TG-gelled binder (C) and in-situ gelled fat mimetic (D).

Results of this study shed a first light on the role and importance of binder components to assemble composite systems such as vegan bacon analogues. A protein extrudate and a fat mimetic could be adhered by a soy protein isolate suspension that was subsequently gelled by heating or TG addition. Results show that covalent crosslinking is superior to physically induced network formation to ensure a sufficient product integrity. The outcomes of this study provide a first insight about the range of binder strength that can be achieved with coagulated or TG-cross-linked SPI gels, Furthermore, the application of a tensile test provides an opportunity to assess binder strength and cohesion of a composite meat analogue system.

## Industrial Relevance

Plant-based meat analogues have become a major trend in the food industry, due to consumers' ethical, environmental and health concerns connected with a high meat consumption. While the majority of studies that have been published on fibrous structure formation of plant proteins as well as structuring of plant oils and fats, the number of studies focusing on combining these components to manufacture a variety of assembled products is limited. For manufacturing complex meat analogues, binder functionality is crucial and knowledge is still limited in this area. Our results showed, that the chosen gelation mechanism is key to making it work as an adhesive with covalent crosslinking being a requirement to generate a matrix with sufficient integrity. These insights are of high relevance for developing, manufacturing and improving complex plant-based meat analogues in the food industry.

## Funding

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# III. Chapter

## Influence of Transglutaminase on Glucono-δ-lactone-Induced Soy Protein Gels

**Eva M. Herz[1], Sabine Schäfer[1], Nino Terjung[2], Monika Gibis[1], Jochen Weiss[1*]**

[1]Department of Food Material Science, University of Hohenheim,
Garbenstrasse 25, 70599 Stuttgart, Germany

[2]Product Innovation, DIL German Institute of Food Technology,
Professor-von-Klitzing-Str. 7, 49610 Quakenbrück

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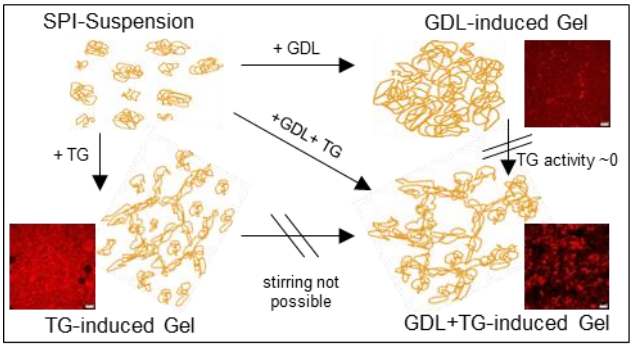
*Corresponding author: Tel: +49 711 459 24415; E-mail address: j.weiss@uni-hohenheim.de

# Abstract

Enzymatic cross-linking of proteins can be used to create a variety of foods with appealing textures, and facilitate further processing such as slicing or packaging. Transglutaminase (TG), a protein-glutamine-γ-glutamyltransferase has been used in the production of processed meat and fish products. However, it has a reaction optimum at pH 7 and is therefore typically unsuitable for application in up-and-coming acidic food products such as fermented vegan meat product alternatives. To determine whether a simultaneous addition of slowly acidifying Glucono-δ-lactone (GDL) and TG can facilitate protein-crosslinking prior to the pH becoming too low, TG and GDL were added separately or in combination to soy protein suspensions composed of 10-15% SPI. Texture analysis showed that SPI-gels at pH 5 - 6, induced by GDL and TG combined, were harder than only GDL-induced gels and comparably hard to TG-induced-gels at the optimum pH of 7. Decreased tan δ values and CLSM images indicated that protein cross-linking had taken place in combined gels. The results suggest an initial network formation induced by TG, that is later in the acidification being supplemented by an agglomeration of proteins due to reduced electrostatic repulsion. Taken together, the combination of slow acidification by GDL and TG-addition leads to acidic gels with enhanced textural properties and shelf life.

**Keywords:** soy protein isolate, gelation, transglutaminase, Glucono-δ-lactone, microstructure, texture

**Graphical Abstract:**



# Introduction

Plant-based food products, especially those that mimic animal-based food products, gain more and more importance due to consumers ethical, environmental and health concerns (Kumar, 2016). The texture of these novel foods plays a critical role in consumer acceptance and designing texture to mimic that of desirable meat or dairy products is, therefore, an important aspect to meet consumers expectations (Hoek et al., 2011; Jeske et al., 2018). In many cases, this requires the creation of a protein network and associated induction of a sol-to-gel transition (Phillips, 2013).

Soy protein is a widely used plant protein due to its good techno-functional properties such as emulsification, foaming and gelling (Kinsella & Melachouris, 1976). Soy protein gelation can be induced by heating, acidification, as well as the addition of salts or specific enzymes to soy protein concentrates or isolates (SPI) (Renkema et al., 2001; Renkema & van Vliet, 2002; Tang et al., 2006; Campbell et al., 2009). Different gel structures can thereby be formed, such as ordered networks or aggregated structures, depending on the heating temperature, the composition of the SPI and the ionic strength (Hermansson, 1986). Salt- or acid-induced gel-formation requires soy protein denaturation in the first step, while in the second step, protons or salt ions neutralize the net charge of the proteins. In turn, hydrophobic interactions become more predominant and aggregation can occur (Kohyama et al., 1995). Gel networks such as these are therefore primarily stabilized by noncovalent linkages such as hydrogen bonds, hydrophobic and electrostatic interactions (Damodaran, 2017).

An acid-induced gel formation caused for example by the production of organic acids such as lactic or acetic acid by bacteria plays a particularly important role in texture formation of shelf-stable food products such as yoghurt or raw-fermented sausages (Katsaras & Budras, 1992; Lucey & Singh, 1997). In this context, cold-set, acid-induced gels can also be formed by the addition of Glucono-δ-lactone to SPI-suspensions (Campbell et al., 2009; Gu et al., 2009), which is an internal ester that hydrolyzes to gluconic acid with a first-order reaction hydrolysis kinetics (de Kruif, 1997). This mimics the slow acidification by lactic acid generating bacteria and leads to a solidification of soy milk (Grygorczyk & Corredig, 2013). Furthermore, GDL is typically used as a coagulant for the production of tofu yielding a smooth texture (DeMan et al., 1986).

Nevertheless, soy protein gels exhibit some textural deficits when they are used alone in food products such as an insufficient hardness (Silva et al., 2019). The addition of transglutaminase (TG), an enzyme that induces protein cross-linking by catalyzing an acyl-transfer reaction between the amino group of lysine and the carboxamide group of glutamine residues (Nonaka et al., 1989), has been shown to lead to the production of very firm gels, also from SPI (Tang et al., 2006), due to the formation of covalent bonds (Damodaran, 2017). The pH-optimum of TG is usually around pH 7 and the enzyme's activity is substantially lowered at decreased pH values (Færgemand & Qvist, 1997; Lerner et al., 2020), resulting in it not being suitable for application in acidic foods. However, GDL acidifies slowly (Kohyama & Nishinari, 1992; de Kruif, 1997), and thus, TG and GDL when added at the same time to a protein suspension, could possibly permit TG to induce crosslinking before the pH becoming too low. As a result, firm gels at low pH-values could potentially be produced. This approach has recently been studied with lactic acid bacteria in a mixture of soy milk and cow milk (Xing et al., 2019; Xing et al., 2020), albeit acid formation of lactic acid bacteria is slower than GDL hydrolysis (Grygorczyk & Corredig, 2013). Zhu et al. (2011) have shown that enzymatic (TG) polymerization of a lower concentrated (6% w/w) soybean glycinin suspension before acidification improved firmness and elasticity of the resulting gels. However, this process is only suitable for lower concentrated systems, where enzymatic polymerization by TG doesn't result in gel formation.

In this study, we hypothesized that the simultaneous addition of GDL and TG would result in the formation of firm SPI-gels at a final acidic pH, compared to only GDL-induced gels, due to protein cross-linking by TG occurring before acidification inhibiting the reaction. To that purpose, GDL-induced, GDL- and TG-induced and pre-acidified TG-induced gels were formed and characterized using texture, rheology, syneresis and microstructure analysis.

# Materials and Methods

## Materials

Soy protein isolate (Supro Ex 37 HG IP) was purchased from Solae Europe S.A. (Geneva, Switzerland), Glucono-δ-lactone (D-(+)-Gluconic acid δ-lactone) from Sigma-Aldrich (Steinheim, Germany), hydrochloric acid (HCl) from Carl Roth GmbH and Co.KG (Karlsruhe, Germany) and transglutaminase (ACTIVA WM from Streptoverticillium mobaraense with an

enzyme activity of 114 U/g, determined by the manufacturer) from Ajinomoto Foods Europe SAS (Hamburg, Germany). Demineralized water was used for the preparation of all samples.

## Methods

*Gel preparation.* Soy protein isolate was used as a model plant protein preparation and dispersed in distilled water with a kitchen aid mini food processor (Whirlpool Germany GmbH, Stuttgart, Germany) for 2 min. Subsequently, GdL and/or TG were added and blended with the protein suspension for an additional 30 s. Afterwards, the protein suspensions were filled into 30 mL containers (Nalgene cups, Nalge Nunc International Corporation, Rochester, NY, USA) and sealed. After incubation for 1 hour at 40 ºC, the samples were stored overnight in a 4 ºC refrigerator. Samples were equilibrated to room temperature before all subsequent measurements.

*Protein charge and isoelectric point.* The ζ-potential of samples was determined using a Zetasizer Nano SeriesZEN3600 equipped with a multipurpose titrator MPT-2 (Malvern Instruments, Herrenberg, Germany), adjusting the pH values between pH 8 and 3 with 0.1 and 0.01 M HCl. Samples were diluted in deionized water to a protein concentration of 0.1% (w/w). The instrument software calculated the isoelectric points of the sample by interpolating the cross-over point between net negative and net positive charges of samples.

*Syneresis.* Water loss of gelled suspensions was determined as follows: approximately 20 g of SPI suspension were filled into 30 mL Nalgene cups, the exact weight was noted, and cups were closed airtight with screw-on caps. After 24 h and 48 h of gel formation, cups were opened and placed upside-down on a filter paper to collect released water. Afterwards, the weight of drained cups was determined. The weight difference between initial and drained weight was then calculated to indicate the amount of spontaneous syneresis that had occurred.

*Texture.* The hardness of gels was measured by a puncture test using an Instron Universal Texture Analyzer (Illinois Tool Works Inc., Darmstadt, Germany). Nalgene cups (V=30 mL) brimmed with SPI gels were penetrated to 75% length scale with a pin geometry (d=13 mm). Each measurement was performed with 10 test samples.

*Rheology.* Gels were incubated between two glass disks set to a gap width of 1.5 mm. After incubation, round samples were cut out and put in an oscillatory rheometer (MCR 300, Anton Paar, Ostfildern, Germany) equipped with a plate-plate geometry (PP25, gap width 1 mm).

Amplitude sweeps were used to determine the linear viscoelastic region (LVR) and were performed with a strain γ ranging from 0.01 to 500% at an angular frequency ω of 1 rad s-1. Frequency sweeps were performed with a strain of γ = 1% at frequencies ranging from 0.1 to 100 rad s-1.

*Microstructure.* The microstructure of gelled samples was analyzed using confocal laser scanning microscopy (CLSM) with a Nikon Eclipse-Ti inverse microscope (Nikon, Düsseldorf, Germany). Samples were stained with Rhodamin B (Carl Roth GmbH and Co.KG, Karlsruhe, Germany), which had been added at a concentration of 0.01% prior to gelation. The samples were protected from light during storage.

*Statistics.* Statistical analysis of variance (one-way ANOVA) and Tukey's post hoc tests were carried out with OriginPro 2020 (OriginLab Corporation, Northampton, USA). Differences at $P < 0.05$ were considered to be significantly different. The assumption of normality and equal variance was tested ($p<0.05$). Experiments were carried out in duplicate and measurements were performed on at least three samples or ten samples for puncture tests. Graphs were also prepared with OriginPro (OriginLab Corporation, Northhampton, USA).

## Results and Discussion

In preliminary experiments, the amount of Glucono-δ-lactone (GDL) required to reach a pH of 5.0, 5.5 and 6.0 in suspensions having SPI concentrations of 10, 12.5 and 15 wt% were determined. Moreover, the general acidification behavior of GDL in these soy protein gels over time was examined. Exemplarily, the acidification behavior of 1.302% GDL in a 15% SPI suspension (target pH 5) can be found in the supporting data (**Figure III.6**): After the addition of GDL to the suspension, the pH decreased from 7.2 to ~ 6.5 after 15 min, to 6.0 after 1 h and 5.5 after ~ 3 h. Furthermore, the amount of TG needed to obtain a maximum hardness in TG induced SPI-gels were determined at pH 7. As shown in the supporting data, with 30 mg TG per g SPI maximum hardness was reached (**Figure III.7**). It should be noted that an initial TG induced gelation followed by later addition of GDL or acids was not investigated since physical incorporation or gelling agents after the onset of gelation would not have been feasible without destroying the formed network. Zeta-potential measurements showed a zero net charge, corresponding to the iso-electric point (pI) of the used SPI at a pH of 4.5, and this would have thus been an interesting gelation condition to include in the study. However, gels having a

pH < 5 were not included in this study because samples with pH values below that were perceived as being too sour in a preliminary sensory test.

## Texture Analysis

The texture of samples was assessed by a puncture test using a texture analyzer since this test allowed for a comparison of all samples despite their different viscosities, ranging from easily pourable to rubber-like. As shown in **Figure III.1A**, the hardness of GDL-induced gels increased with decreasing pH towards the pI from 0.21 ± 0.08 N at pH 6.0 to 0.79 ± 0.39 N at pH 5.0 in samples containing 10% SPI. This can be attributed to enhanced hydrophobic interaction at the iso-electric pH due to an absence of stabilizing electrostatic repulsive forces (Campbell et al., 2009). The increase in hardness was even more pronounced with increasing protein content, with hardness increasing from 1.30 ± 0.08 N at pH 6.0 to 3.54 ± 0.33 N at pH 5.0 in 15% SPI samples. This is indicative of an increased intermolecular interaction between proteins and an increased net matrix area occupied by the protein network (Zayas, 1997).

Gels induced by TG-crosslinking at pH 7 had hardness values of ~ 2.7 N with 10% SPI and ~ 12.9 N at 15% (**Figure III.1C**) and thus were substantially harder than GDL-induced gels. When the pH was adjusted to lower values with HCl prior to TG-addition, gel hardness values were lower (TG pH 6.0, 5.5 in **Figure III.1C**), whereas no visual gelation was observed at pH 5. The hardness decreased with decreasing pH and was eventually similar to GDL-induced gels. Differences were more pronounced at higher protein concentrations.

When GDL and TG were added simultaneously, harder gels at decreased pH values were obtained compared to only GDL-induced gels or HCl-acidified TG-induced gels (**Figure III.1B**). Hardness increased with increasing SPI content, from 2.87 ± 0.58 N with 10% SPI at pH 5.0 to 16.46 ± 4.80 N at 15 % SPI. At all measured pH values, TG+GDL-induced gels were harder than gels only prepared with GDL and gels acidified with HCl before TG-addition. Moreover, gels with TG at pH 5.0 (GDL+TG) were in the same range or even harder than TG-gels at pH 7. This illustrates that the simultaneous addition of GDL and TG led to hard gels even at low pH values which is in contrast to results obtained with suspensions that were pre-acidified with HCl. There, a pronounced decrease in gel hardness was observed even at a mildly acidic pH of 6.0, i.e. hardness decreased by ~ 60% in 15% SPI-gels and ~ 85% in 10% SPI-gels when a pre-acidification with HCl had been taken place. Taking into account the previously observed acidification behavior (**Figure III.6**), it can therefore be concluded that TG induces

cross-linking within less than 30 min after addition to the SPI-suspension. The increased hardness of GDL+TG-gels, compared to GDL-gels, indicates protein cross-linking by TG, while an increasing hardness with decreasing pH may be indicative of a subsequent agglomeration of unpolymerized proteins to the TG-induced network, as previously suggested for sodium caseinate gels by Partanen et al. (2008).

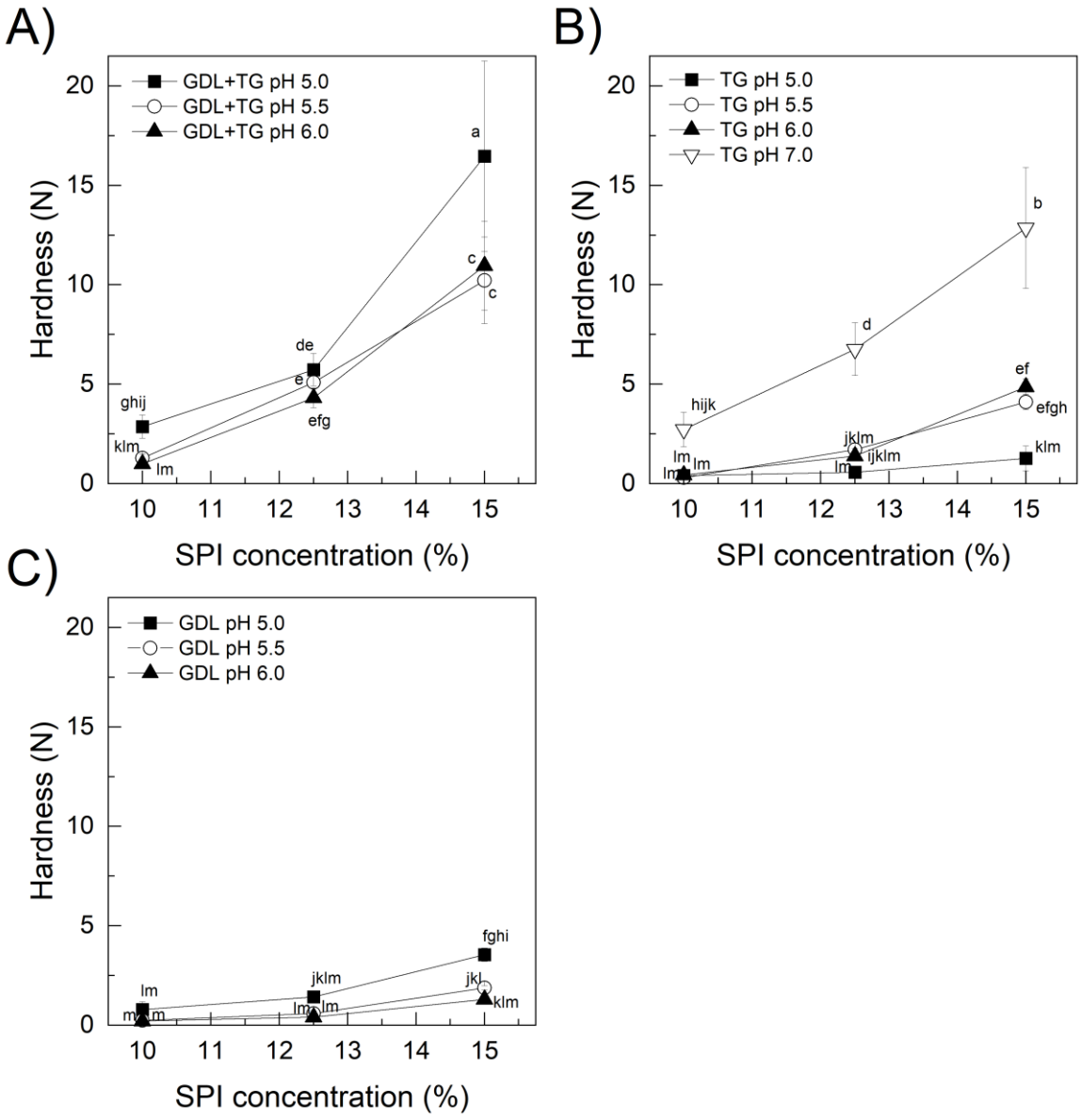



**Figure III.1** Hardness determined by penetration tests of SPI-gels (10, 12,5 and 15% w/w), induced by GDL (A), a combination of GDL and TG (B) and TG-induced gels (C) at pH 5 to 7. Values followed by different superscripts are significantly different ($P < 0.05$).

## Syneresis

Next, the syneresis behavior of the different gels was analyzed to obtain further information about the microstructure and technofunctionality of the analyzed samples (Færgemand & Qvist, 1997). Results of GDL-gels and GDL+TG-gels at pH 5, compared to TG-gels at pH 7 are representative of the generally observed behavior and are shown in **Figure III.2**. The water drainage of TG-induced gels was very low with ~ 0.1%. Reduced syneresis due to enzymatic cross-linking of proteins by TG has also been observed in sodium caseinate gels and has been attributed to a high flexibility of the network (Partanen et al., 2008). In contrast, GDL-induced gels displayed a higher syneresis of ~ 0.8 – 1.7%. Combined GDL+TG-gels had the highest water drainage of ~ 2.4 – 3.7%. Moreover, syneresis increased with increasing protein content. As such, clear differences between samples were observed with respect to syneresis behavior; with combined GDL+TG gels having had the highest water drainage. An increased syneresis has been associated with increased cross-linking in calcium-induced soy protein gels, due to a denser gel matrix (Sun & Breene, 1991). Vice versa, higher syneresis led to increased firmness in salt- and acid-induced soy protein gels (Murekatete et al., 2014). Therefore, the increased syneresis of GDL+TG gels is likely connected with the observed increased firmness, due to water release, and also an indicator of increased network formation. Furthermore, the increased syneresis of the acidic, TG-polymerized gel might be attributed to a lower flexibility and higher rigidity, facilitating water release, as similarly observed and suggested for sodium caseinate gels by Partanen et al. (2008). Under practical aspects, syneresis can be a necessary as well as an unwanted phenomenon. On the one hand, it can be useful to enable water loss and achieve an appropriate moisture content, e.g. in cheese making. Moreover, moisture loss and thus reduction of the aw-value is generally an important approach to increase the shelf life of many foods. On the other hand, it can be considered as a defect, e.g. in yoghurt or mustard where the separating serum phase is perceived as a negative (Lucey, 2001). Knowledge about the syneresis behavior of soy protein gels induced by different gelation mechanisms is therefore of practical interest with regard to manufacturing of various plant-based meat or dairy product analogues.

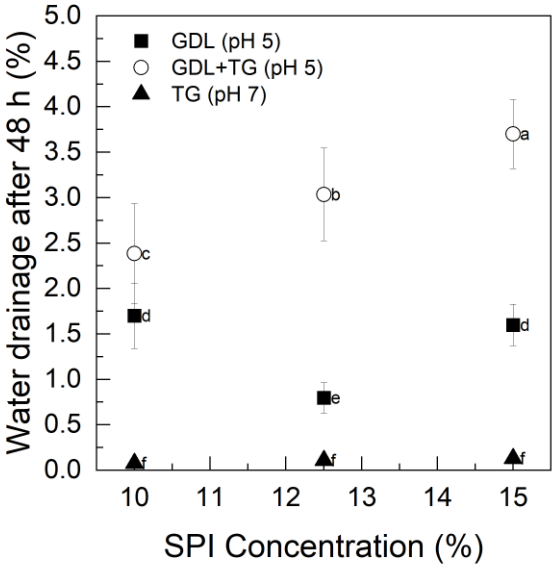



**Figure III.2** Water drainage of 10-15% SPI-gels at pH 5, induced by GDL or GDL+TG and TG-induced gels at pH 7. Values followed by different superscripts are significantly different ($P < 0.05$).

## Rheology

Mechanical properties of gels were additionally investigated by carrying out rheological amplitude and frequency sweeps. Amplitude sweeps showing G' and G'' as a function of strain, indicated that all samples were in gel state with G' being > G'' in the linear-viscoelastic (LVE) region (**Figure III.3**). The LVE region is characterized by a plateau, while a decrease in both moduli indicates irreversible deformation and marks the limit of the LVE region, γL. GDL-only gels had a lower yield point of γL ~ 2 - 3% compared to GDL+TG-gels at pH 5 with γL ~ 3 - 4% and TG-gels at pH 7, which had a γL ~ 8 - 10% (**Table III.1**). The higher γL values showed that samples containing TG could be subjected to larger strains without irreversibly destroying their structure. This is a further indication for an increased number of cross-links induced by TG, which was also observed in studies on cross-linked emulsion gels by Dreher et al. (2020a), Dreher et al. (2020b). Additionally, both gels containing TG had a peak in their G'' progression which indicates cross-linking (Mezger, 2006; Dooling et al., 2016). Amplitude sweep analysis, therefore, suggests that extensive cross-linking of proteins had taken place in both TG-induced gels and gels containing GDL and TG.

As displayed in **Figure III.4**, frequency sweeps yielded, similarly to amplitude sweeps, higher storage moduli than loss moduli values, indicating again a networked structure, as well as an increased magnitude of the moduli at increased protein concentration, indicating a higher

degree of network formation (Schorsch et al., 2000; Mezger, 2006). Samples at pH 5, with and without TG showed a low degree of frequency dependence, whereas, for TG-cross-linked gels pH 7, no frequency dependency was observed, indicating very little to no effect of time on the stability of the gels (Mezger, 2006). The damping factor tan δ is indicative of the ratio between the viscous and the elastic contribution of a material to an oscillatory deformation test (Dickinson & Yamamoto, 1996; Mezger, 2006). Gels that were GDL-induced had higher damping factors of 0.29 at 10% SPI and 0.26 at 15%, compared to combined GDL+TG-gels with damping factors of 0.21 and 0.20 at 10% and 15% SPI and TG-induced gels with a damping factor of 0.1 (**Table III.1**). These results indicate stronger network formation with increasing protein content as well as upon addition of TG.

**Table III.1** Limit of the linear viscoelastic region $\gamma_L$ and damping factor tan δ (at ω = 0.1 rad $s^{-1}$) of 10 and 15% SPI-gels at pH 5 induced by GDL and GDL+TG, compared to only TG-induced gels at pH 7.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **$\gamma_L$ (%)** | | | | | | | | |
| **SPI (%)** | GdL | | | GdL + TG | | | TG | | |
| **10** | 2.09 | ± | 1.40[a] | 2.87 | ± | 1.41[a] | 7.94 | ± | 3.69[b] |
| **15** | 2.92 | ± | 0.84[a] | 3.62 | ± | 1.11[a] | 10.26 | ± | 2.03[b] |
| | **tan δ** | | | | | | | | |
| **10** | 0.29 | ± | 0.02[c] | 0.21 | ± | 0.01[f] | 0.10 | ± | 0.01[e] |
| **15** | 0.26 | ± | 0.00[d] | 0.20 | ± | 0.00[f] | 0.10 | ± | 0.01[e] |

## Microstructure

**Figure III.5** finally shows CLSM images of SPI-gels formed by GDL, TG, and combined treatments. Only images of samples containing 10% SPI are shown since differences in gel structure were more distinctly visible there. Images of GDL-induced gels at pH 5 showed a very dense and evenly distributed structure with small visible accumulations but no strands, as is typical for the microstructure of a gel induced by slow acidification, yielding an evenly distributed structure of aggregated proteins (Partanen et al., 2008; Campbell et al., 2009). In comparison, the images of HCl-pre-acidified suspensions (**Figure III.5C**) contained larger accumulated protein clusters forming a porous structure, caused by the fast acidification. The images of TG-induced gels at pH 7 (**Figure III.5D**) showed similar to **Figure III.5A** a homogeneous and dense structure, without clearly visible strands, but, in contrast, some cavities

and larger accumulations, which might be an indicator for TG-induced cross-linking. In images of gels containing both GDL and TG (**Figure III.5B**), a stranded and porous network was visible. Eissa et al. (2004) and Partanen et al. (2008) observed similar microstructure and textural properties in whey protein gels when GDL-induced or TG+GDL-induced.

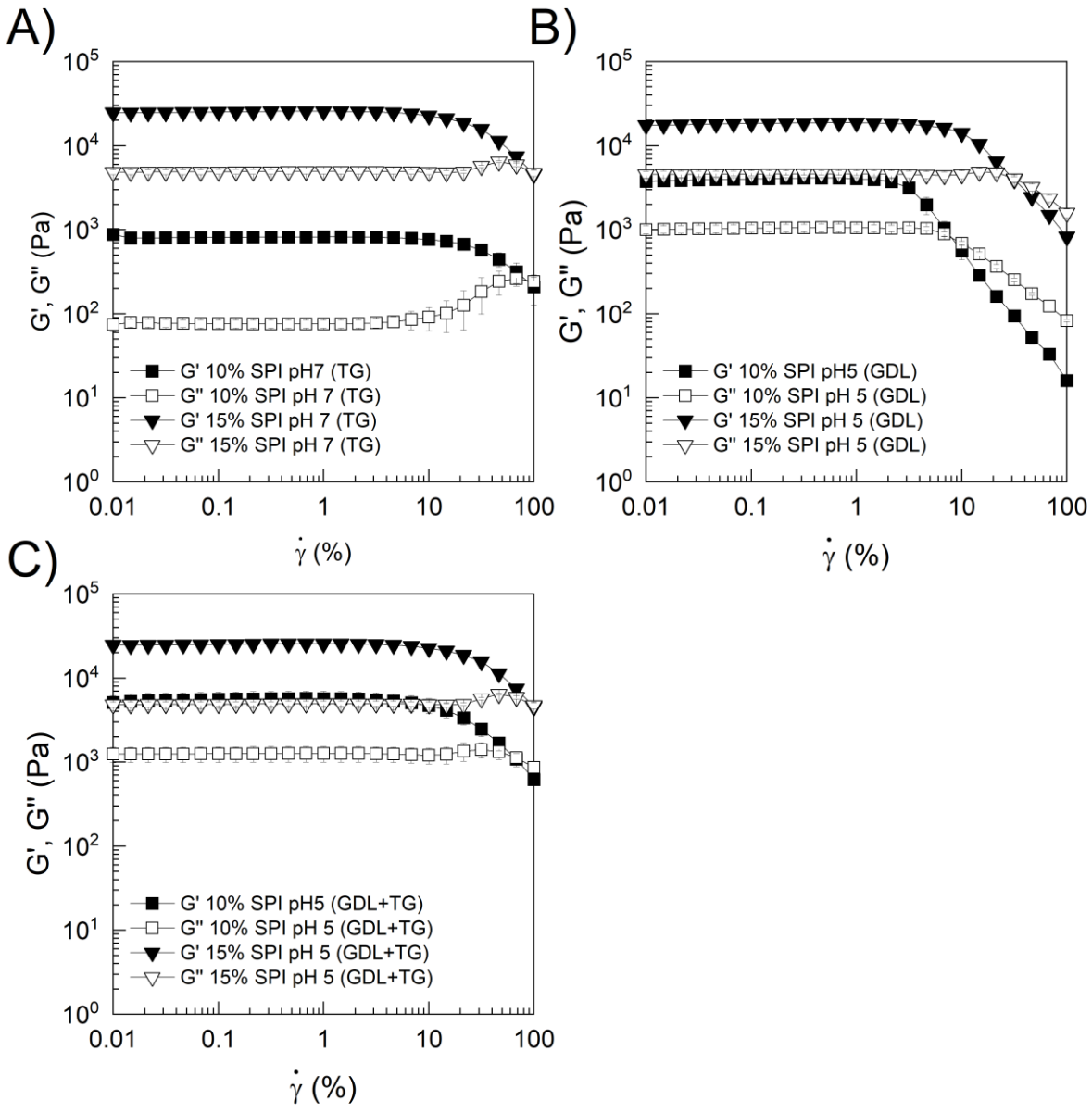



**Figure III.3** Storage modulus G' and loss modulus G'' of 10 and 15% SPI-gels at pH 5 induced by GDL (A), GDL+TG (B) and TG-induced gels at pH 7 (C) as a function of strain (amplitude sweeps).

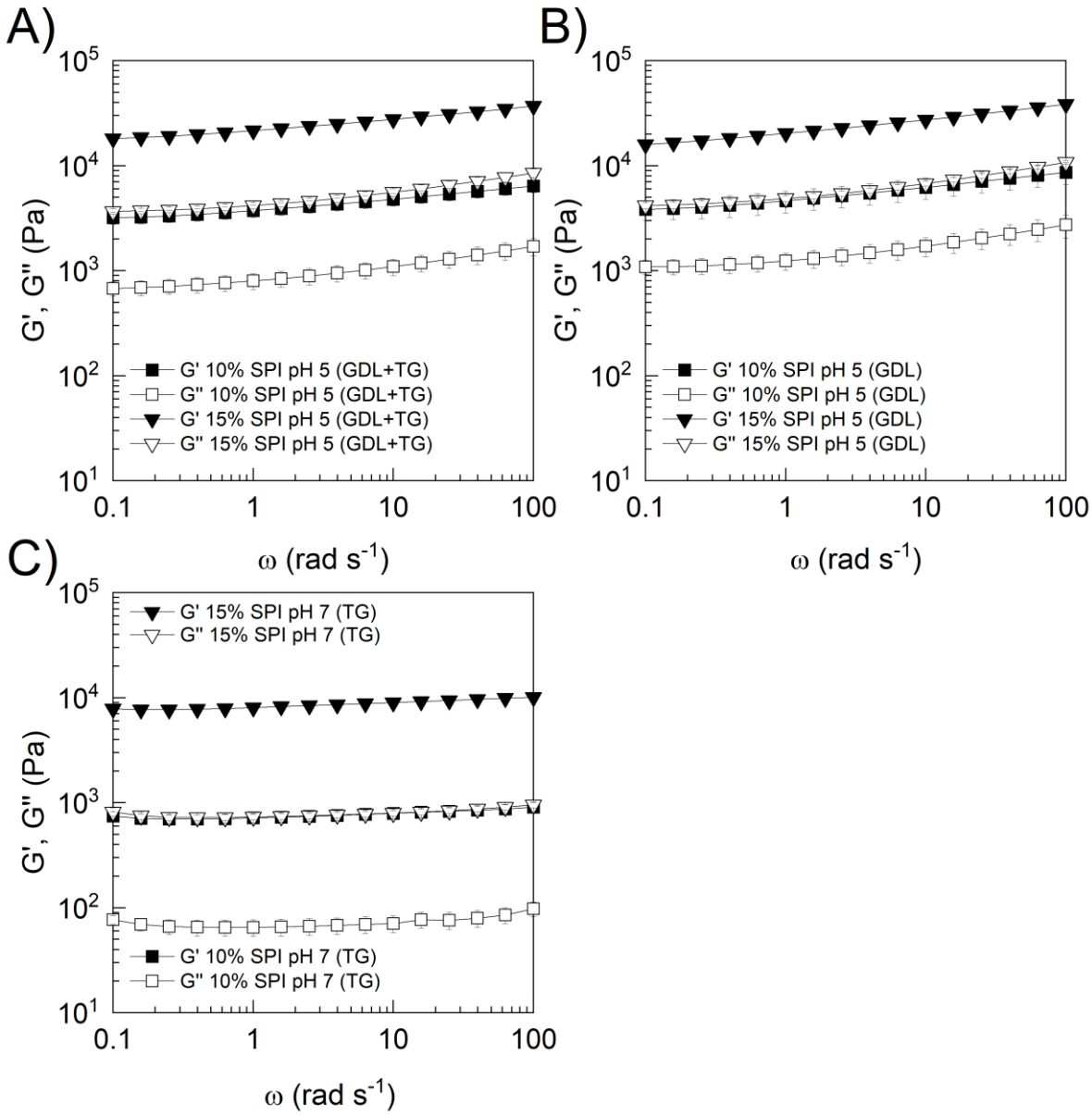



**Figure III.4** Storage modulus G' and loss modulus G'' of 10 and 15% SPI-gels at pH 5 induced by GDL (A), GDL+TG (B) and TG-induced gels at pH 7 (C) as a function of angular frequency.

The microstructure observed in CLSM-images support our previously suggested mechanism of gel formation of GDL+TG-gels: first, protein-cross-linking was catalyzed by TG, generating a covalent "scaffold" throughout the matrix. Gradual acidification by GDL, on the one hand, slowed down cross-link formation by TG, hindering the formation of a complete covalent network. On the other hand, subsequent acidification reduced electrostatic repulsion between the proteins, leading to agglomeration of non-cross-linked protein to the scaffold network, similar to acid-induced gelation.

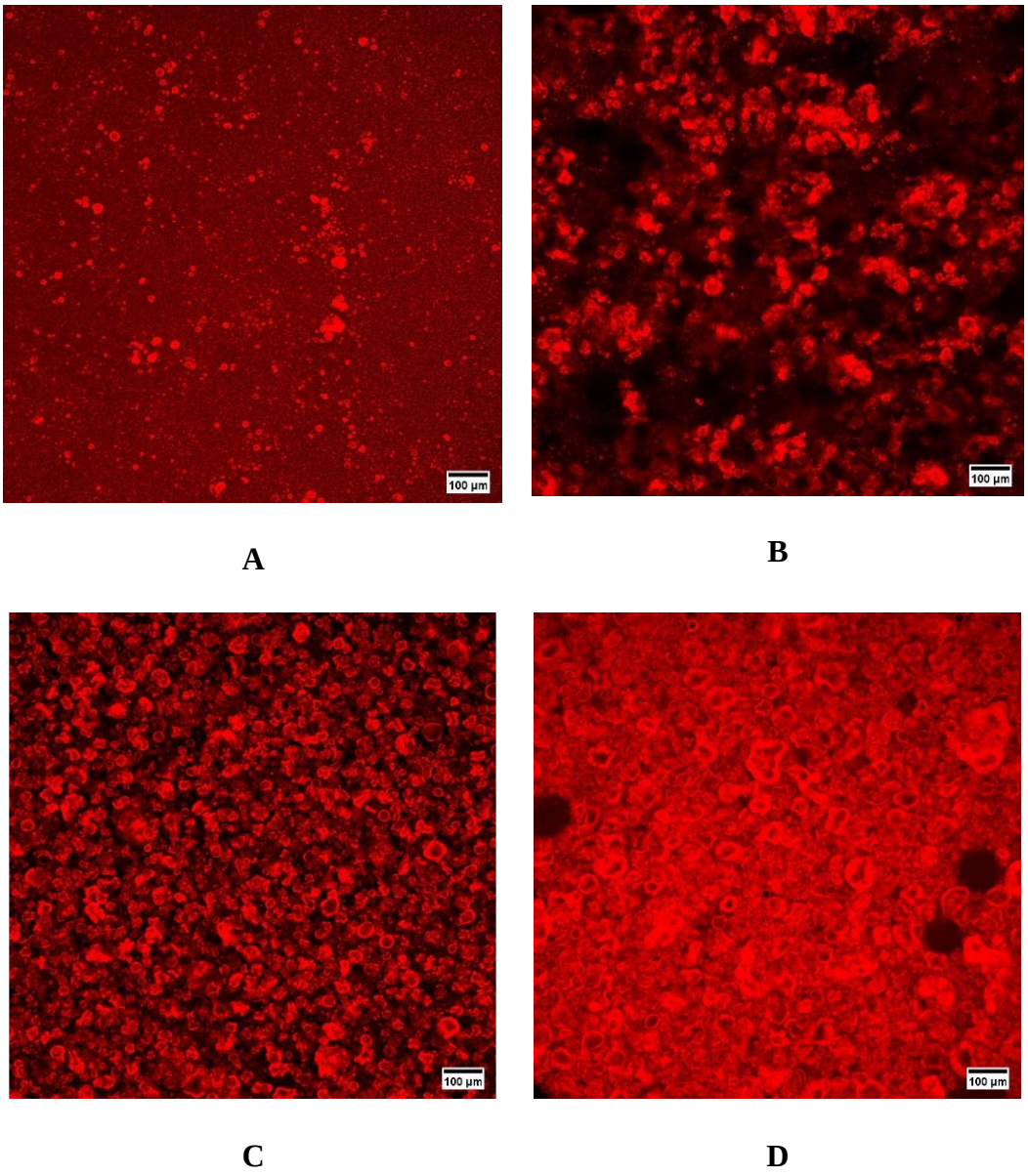



**Figure III.5** Confocal laser scanning microscope (CLSM) images of 10% SPI-gels at pH 5 induced by GDL (A), GDL+TG (B) compared to HCl-acidified (pH 5), TG-added suspension (C) and TG-induced gel at pH 7 (D) stained with Nile red.

To conclude, simultaneous addition of GDL and TG to a SPI-suspension yielded firm gels with comparable hardness to TG-induced gels at higher pH values and a distinct microstructure. In those combined gels, first, protein cross-linking was catalyzed by TG, generating a covalent "scaffold" throughout the matrix. With gradual slightly time-shifted acidification by GDL electrostatic repulsion between the proteins was reduced, leading to agglomeration of non cross-linked protein to the scaffold network. Syneresis and firmness of the gel can be further modulated by pH and protein concentration. We, therefore, suggest that a combined gelation

mechanism of enzymatic cross-linking and acidification might be a good basis for the manufacture of plant-based analogues to dry fermented, shelf-stable sausages, yoghurt, and cheeses, where coherent acidic matrices need to be formed via a protein-based binder. Investigations on the application of the herein investigated systems are currently underway and will be reported in a future publication.

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# Supporting Information

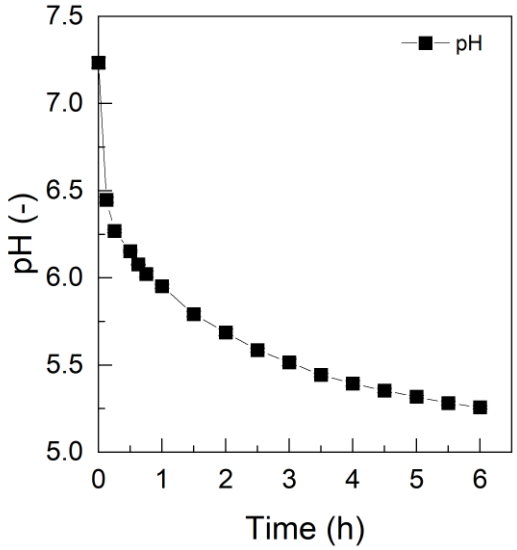


**Figure III.6:** Acidification behavior of 1.302% GDL (w/w) in a 15% (w/w) SPI-suspension.

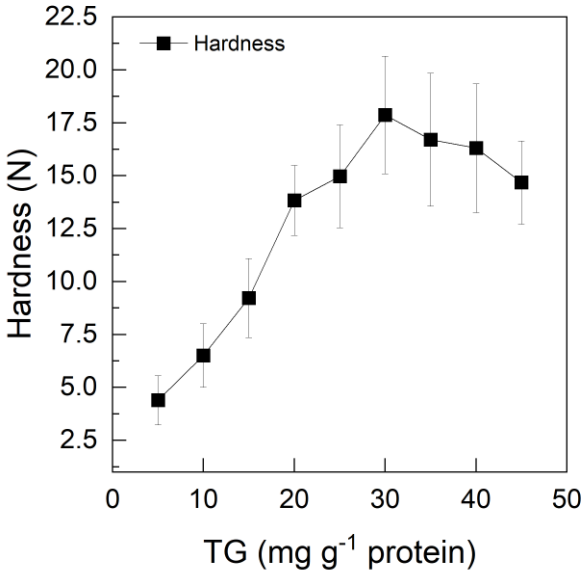


**Figure III.7:** Hardness of SPI-gels (10% w/w) induced by 5 to 45 mg TG per g Protein at pH 7.

# IV. Chapter

# Influence of Extrudate to SPI-Gel-Binder Ratios and Transglutaminase Crosslinking on Texture of a Plant-Based Salami Analogue

**Eva Herz[1], Till Kinne[1], Nino Terjung[2], Monika Gibis[1], Jochen Weiss[1*]**

[1]Department of Food Material Science, University of Hohenheim, Garbenstrasse 25, 70599 Stuttgart, Germany

[2]Product Innovation, DIL German Institute of Food Technology, Professor von Klitzing Strasse 7, 49610 Quakenbrück, Germany

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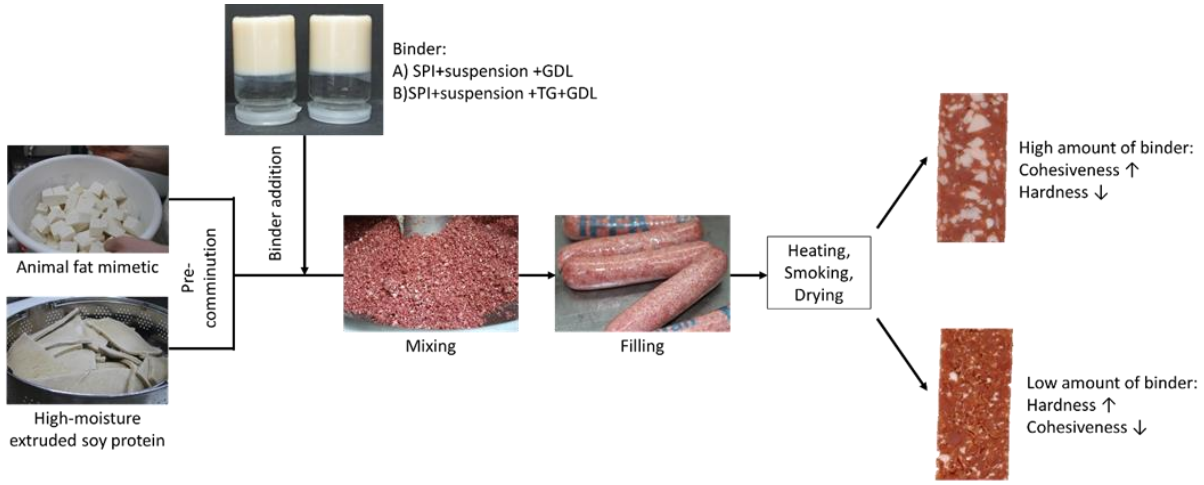
*Corresponding author: Tel: +49 711 459 24415; E-mail address: j.weiss@uni-hohenheim.de

# Abstract

A plant-based salami-type sausage analogue was manufactured with a glucono-δ-lactone- or transglutaminase+glucono- δ -lactone-induced soy protein isolate-gel as binder to hold together fat mimetic particles and extruded proteins with fibrous structure to resemble the structure of classical dry-fermented sausages. The objective was to study the influence of the binder content on the texture of this complex meat analogues. The sausage analogues were processed similarly to traditional sausages. Texture, sensory and composition were analyzed. The hardness of dry-fermented sausages was found to decrease with increasing binder content (e.g. at 45% dry matter from ~ 110 N to ~ 80 N), whereas the cohesiveness increased with increasing binder content (e.g. at 45% dry matter from ~ 0.21 to ~ 0.41). Results from sensory and texture analysis correlated well and showed that both a high firmness and cohesiveness could not be reached in a single formulation. Furthermore, the addition of transglutaminase slightly increased firmness and cohesiveness, however, drying of sausages had a significantly higher impact on increasing hardness. These results indicate that, at lower binder contents, the structure is dominated by the extrudate particles, leading to an increased firmness but a lack of cohesiveness, whereas higher binder contents increased the cohesiveness but decreased hardness.


**Keywords:** Meat product analogue, soy protein, acid-induced gel, transglutaminase, binder

**Graphical Abstract:**



# Highlights

- Traditional sausage production technologies can be used for plant-based analogues
- Low binder contents lead to increased firmness but a lack of cohesiveness
- Higher binder contents resulted in increased cohesiveness but decreased hardness
- Drying had a higher impact on sausage hardness than the addition of transglutaminase

# Introduction

The consumption of meat and especially processed meat products has recently been associated with negative impacts on the environment and human health. The production of animal-derived food has been associated with higher greenhouse gas emissions, land and water use and biodiversity losses than the production of plant foodstuff (Poore & Nemecek, 2018). Moreover, the consumption of processed red meat has been associated with health risks, especially cardiovascular diseases, coronary heart diseases and cancer, potentially linked to its content of saturated fat, cholesterol, iron, phosphatidylcholine and carnitine (Anand et al., 2015). Also the use of nitrite in processed meat products has been considered a health risk, due to the formation of nitrosamine under certain conditions and the report of its carcinogenicity in animal studies, although more recent studies indicated no association between usual nitrite intake and cancer (Bryan et al., 2012). On the other hand, vegetarian diets and diets high in plant foods and plant protein sources have been associated with a lower prevalence for coronary heart diseases and obesity (Anand et al., 2015; Appleby & Key, 2016). Finally, ethical concerns about slaughtering and factory farming of animals are causes for consumers to reduce their consumption of meat (Piazza et al., 2015; Apostolidis & McLeay, 2016; Possidónio et al., 2021). As such, interest in the development of novel plant-based foods has been high.

The term “plant-based food analogues” is used as a category that includes a broad range of products that have been designed to mimic animal-based foods such as meat, fish, eggs, milk, and thereout derived products (McClements & Grossmann, 2021). The subcategory of meat product analogues is usually composed of three basic components to mimic the characteristic texture of their animal-based counterparts: a plant-based fibrous material to mirror the muscle structure of meat, a plant-based fatty material ideally with plastic as well as elastic characteristics to mirror animal fat, and a plant-based binder mirroring solubilized meat proteins

to hold these components together. Since fibrous structures that mimic the characteristic texture of meat do usually not occur naturally in protein-rich plants, they are manufactured through processing approaches using mostly globular plant proteins (Birgit L. Dekkers et al., 2018; McClements & Grossmann, 2021). The most wide-spread process for the production of fibrous material from plant-proteins is extrusion, which can be either performed as high-moisture or low-moisture extrusion. In the extrusion process, a protein-water dispersion is plasticized inside an extruder barrel by a combination of hydration, heating and application of mechanical forces. For high-moisture-extrusion, the product is then conveyed through a cooling die where anisotropic fibrous structures are formed by the inhomogeneous laminar flow, and expansion is prevented (Birgit L. Dekkers et al., 2018). Another technique to produce fibrous structures with plant proteins is by use of a shear cell where intensive shear forces are applied in a rotating cup and bob system, resulting in elongated structures on a micrometer-level (Birgit L. Dekkers et al., 2018).

For plant-based fatty materials, solid fats such as coconut fat or shea butter have been used, sometimes in combination with other oils such as sunflower or canola oil. Those systems - unlike animal fat - do not display any elastic mechanical properties though. An animal fat mimicking system has recently been developed that is composed of plant-based oil or oil-fat-mixtures incorporated in a plant protein network by emulsification and subsequent Transglutaminase-induced covalent network formation (Dreher et al., 2020a). Through the addition of a plant-based organogelator, textural properties of this system can be modulated to meet specific product requirements. For the purpose of this study, this system was employed using a plant oil as lipid phase, which has been shown to be the softest but also the most stable system in terms of resistance to mechanical stresses that may be superimposed during e.g. mixing, forming or filling.

In meat processing, the extraction of salt-soluble myofibrillar protein followed by various processes of gelation is used to provide adhesion between fibrous meat particles and comminuted fat particles (Gordon et al., 1992). In plant-based meat analogues, a binder exhibiting adhesive and cohesive properties has to be added. Currently, mainly hydrocolloids such as methylcellulose, carrageenan, and gums are used for this purpose, but consumer acceptance of those compounds is low (Kyriakopoulou et al., 2021). For example, in the manufacturing of a burger analogue, fat particles and protein extrudates have to be “bound” together to generate a coherent mass that can be formed into patties. Depending on the use

scenario, the binder must not only exhibit gluiness, but must also solidify under certain conditions (e.g. during frying).

While a variety of meat analogues has been established on the market, analogues of dry-fermented sausages are still scarce. As dry-fermented sausages are usually acidified (by lactic-acid bacteria or glucono-δ-lactone, GDL), the analogue product should also have a typical sour taste. In previous studies, we investigated the gelation of soy protein isolate (SPI) by simultaneous addition of an enzymatic crosslinker such as transglutaminase (TG) and GDL to the SPI-suspension, what led to very firm gels (Herz et al., 2021a), as well as the potential of a SPI-gel to work as binder in a complex meat product analogue (Herz et al., 2021b). Therefore, we hypothesized that a raw fermented sausage (aka salami) analogue can be manufactured by mixing of protein extrudates, a fat mimetic and the SPI binder with an acidifier (GDL) and enzymatic crosslinker (TG) and other ingredients such as salt and spices, followed by a filling of masses in casings, incubation and heating, smoking and drying.

Furthermore, we were interested to find out in more detail how different mixing ratios of binder and extrudates influence the texture properties of the product, in particular upon addition of TG. To address this question, the SPI+GDL-binder was added in both the absence and presence of TG at varying mixing ratios to extrudates to manufacture raw fermented sausage analogues. Sample products were then analyzed at various drying stages (undried and 40-55% dry matter) in terms of their texture and sensory properties.

# Materials and Methods

## Materials

High-moisture extrudate, also known as high-moisture meat analogue or HMMA (69.3% moisture content, 21% protein, height ~1,2 cm, width: ~18 cm, length: ~50 cm) from Alpha 8 soy protein concentrate (Solae Europe S.A., Geneva, Switzerland) was provided by Deutsches Institut für Lebensmitteltechnik (DIL e.V., Quakenbrück, Germany). Soy protein isolate Supro EX 37 HG IP was obtained from Solae Europe S.A., Geneva, Switzerland (protein content > 90% on dry matter basis) and canola oil, salt (NaCl) and paprika powder from MEGA eG (Stuttgart, Germany). Spice mix StarFermat was obtained from Frutarom GmbH (Salzburg, Austria). Beetroot powder was purchased from Van Hees GmbH (Walluf, Germany) and Glucono-δ-lactone (D-(+)-Gluconic acid δ-lactone) from Sigma-Aldrich (Steinheim,

Germany). A vegan salami flavor was provided by Givaudan SA (Vernier, Switzerland). Hydrochloric acid (HCl) was purchased from Carl Roth GmbH and Co.KG (Karlsruhe, Germany) and transglutaminase (ACTIVA WM from *Streptoverticillium mobaraense* with an enzyme activity of 114 U/g, determined by the manufacturer) from Ajinimoto Foods Europe SAS (Hamburg, Germany). Cellulose casings (18 mm diameter, CE-FP) were provided by Naturin Viscofan GmbH (Weinheim, Germany).

## Methods

*Preparation of SPI-gels for binder analysis.* 15% (w/w) SPI suspensions were prepared in a Stephan UM 5 Cutter (Stephan Machinery, Hameln, Germany) by mixing water and SPI for 10 s at 500 rpm, followed by 5 min at 1500 rpm under vacuum conditions. If applicable, GDL and/or TG were added for the last 30 s of mixing. GDL was added at a concentration of 0.6% to reach a pH of 5.5 with TG having a concentration of 25 mg/g SPI. For the subsequent texture analysis of the binder system, samples were filled into polyethylene casings, incubated for 1 h at 40 ºC, followed by heating to a core temperature of 85 ºC in an Air Master UK-1800 chamber. For cohesiveness performance tests of the binder system, first, high-moisture extrudate pieces were cut into smaller, uniform pieces of 4 mm thickness (with a commercial meat slicer) and a length of 14 cm and a width of 8 cm. Then, 0.1 g/cm² of SPI dispersion was distributed equally on one piece of extrudate, before a second piece of extrudate was placed on top. The extrudate sample was wrapped in plastic wrap to prevent water loss or uptake during heating and then placed in an aluminum baking tray with another baking tray on top. 8 g/cm² of weight was placed in the second baking tray to press down on the extrudate. A similar incubation and heating step as described above for the binder alone was then carried out.

*Preparation of fat mimetic.* A fat mimetic system was prepared according to the method of Dreher et al. (2020a). Briefly, a 12% (w/w) SPI-suspension was prepared by homogenizing the protein isolate and water for 15 min at 3500 rpm in a MaxxD Lab rotor-stator homogenizer (FrymaKoruma, Rheinfelden, Switzerland). The suspension was then adjusted to pH 7.0. and canola oil was slowly added at a ratio of oil to protein suspension of 70:30 (w/w) during homogenization in the same device for 300 s at a speed of 5000 rpm under vacuum of 450 ± 50 mbar. After homogenization, the temperature was allowed to equilibrate to ~38 ºC and transglutaminase (TG) was added as a 40% (w/w) solution at a concentration of 20 mg/g protein. The emulsion was then filled into 60 mm polyethylene casings (NaloBar APM, Kalle GmbH, Wiesbaden, Germany) using an MWF 591 filler (MADO, Dornhan,

Germany), and incubated in an Air Master UK-1800 chamber (Reich GmbH, Schechingen, Germany) at 40 °C for 60 min to enable protein cross-linking. Afterwards, the fat mimetic system was heated to a core temperature of 85 °C for microbial safety. Samples were then stored at 2 °C until further use after having been showered with cold water (10 °C) for 10 min.

*Preparation of sausage analogue batter.* Plant-based salami-style sausage analogues were prepared with various binder-to-extrudate ratios and additional ingredients as specified in **Table IV**.1. To that purpose, fat mimetics were removed from their casings and pre-cut into pieces with an approximate diameter of 5 cm, and then comminuted in a K64 bowl chopper (Seydelmann GmbH, Stuttgart, Germany) for 20 s at a knife rotational speed of 930 rpm and a bowl speed of 13 rpm. Extrudate pieces that had been pre-cooked for 20 min in 80 ºC hot water and then cooled over night at 2 ºC, were separately comminuted in the bowl chopper at a knife rotational speed of 1500 rpm and a bowl speed of 13 rpm for 65 s to achieve the desired particle size. As a third component, the binder suspension was prepared by mixing SPI (15% w/w) and water at a knife rotational speed of 1500 rpm for 60 s in the bowl chopper, followed by an increase in knife rotational speed of 4000 rpm for 180 s under vacuum. Salt, spice mix, salami flavor, beetroot and paprika powder, Glucono-δ-lactone (GDL) and TG, if applicable, were added to the SPI-suspension shortly before the preparation of the salami analogue mass mixture composed of the three components, i.e. the extrudate, the binder and the fat mimetic. To that purpose, the three components were finally mixed in the aforementioned bowl chopper with a reverse knife setting (knives rotating backwards, contacting the batter with the dull side to prevent further comminution) for 30 s at a bowl speed of 13 rpm. The salami analogue mass was then filled into 28 mm cellulose casings using a VF 610 plus vacuum filler (Handtmann GmbH, Biberach, Germany), incubated in an Air Master UK-1800 chamber (Reich GmbH, Schechingen, Germany) at 40 ºC for 1 h to facilitate cross-linking by TG, and then heated to a core temperature of 85 °C followed by showering with cold water (10 °C) for 10 min for microbial safety reasons. Sausages were smoked twice with beech wood and friction smoke for 5 min followed by drying at 18 °C and 80% relative humidity until dry matter contents of 40 – 55% were reached. The dried salami analogues were stored at 2 °C in sealed bags with modified atmosphere (80% $N_2$, 20% $CO_2$) until further analysis.

*Dry matter, protein content and pH.* The dry matter content was determined gravimetrically in triplicate, using the standard sea sand method (VO(EG)152/2009). 5 g of sample were ground with sea sand and dried in an oven (Type 400, Memmert GmbH, Schwabach, Germany) up to

constant weight loss. The remaining mass represents the dry matter. The protein content was determined by Dumas method, using a Dumatherm N nitrogen determination device (Gerhardt GmbH, Königswinter, Germany). Measurements were performed in triplicate and a conversion factor of 6.25 was used. The pH was measured with a pH-meter 537, WTW, Weilheim, Germany.

*Texture analysis.* The texture of the samples was analyzed by recording the force as function of distance during a double uniaxial compression with an Instron Universal Texture Analyzer (Illinois Tool Works Inc., Darmstadt, Germany). Samples were cut into cylindrical specimens (diameter: 20 mm, height: 15 mm) and compressed to 75% of their original height for the assessment of hardness and to 50% for cohesiveness and springiness, according to Bourne (1968).

*Tensile test.* A previously adapted tensile test (Herz et al., 2021b) to analyze the cohesiveness of binder-extrudate composites as described above was used. After heating and cold storage, the composite samples were cut into squares with a diameter of 2 cm and glued onto stainless steel plates with cyanoacrylate glue. The plates were hooked into an Instron Universal Texture Analyzer (Illinois Tool Works Inc., Darmstadt, Germany) and pulled apart at a constant speed of 1 mm/s. Force versus distance curves were recorded and the maximum force and the area under the curve referring to the work of adhesion were calculated.

*Sensory analysis.* First, a triangle test was carried out with 34 panelists to find out, if a difference between sausages containing SPI+GDL- and SPI+GDL+TG-binder could be detected. Therefore, sausages with 40% and 60% binder were tested. The triangle test and calculation of the result were performed according to (Meilgaard et al., 1999). Furthermore, 20 trained panelists assessed the samples with 45% dry matter among the texture factors hardness, cohesiveness and dryness on a 10-step relative-to-ideal scale, with the midpoint representing the optimum of the attribute. The samples had a diameter of ~ 1,3 cm and were cut into pieces of ~ 3 cm to obtain similar measures to the commercial product. The panel consisted of members of the institute (age range ~ 20 – 60 years), who have received lectures and trainings during their studies, participate regularly in sensory analysis and have received a two product-related trainings before the assessment of the plant-based sausages.

*Statistical analysis.* Statistical analysis of variance (one-way analysis of variance) and Tukey's post hoc tests were carried out with OriginPro2020 (OriginLab Corp., Northampton, MA).

Differences at the $p < 0.05$ level were considered to be significantly different. The assumption of normality and equal variance was tested ($p < 0.05$). Experiments were carried out in duplicate. Graphs were also prepared with OriginPro (OriginLab Corp.).

**Table IV.1: Ingredients and concentrations of plant-based salami-style sausage analogues.**

| Main ingredients | SPI30 | SPI40 | SPI50 | SPI60 | SPI70 |
|---|---|---|---|---|---|
| **SPI Binder (%)** | 30 | 40 | 50 | 60 | 70 |
| **Extrudate (%)** | 55 | 45 | 35 | 25 | 15 |
| **Fat mimetic (%)** | 15 | 15 | 15 | 15 | 15 |

| Additional ingredients | Dosage (g/kg) |
|---|---|
| **NaCl** | 18 |
| **Glucono-δ-lactone** | 6.3 – 9.7 (target pH 5.5) |
| **Spice mix** | 20 |
| **Beetroot powder** | 10 |
| **Paprika powder** | 5 |

# Results and Discussion

## Influence of binder type on mechanical properties of binder systems

First, the behavior of two pure SPI binder systems - one with and one without TG - was analyzed. To that purpose, a 15% (w/w) GDL-induced SPI-gel (pH 5.5), in the following referred to as "SPI+GDL-Binder" and a 15% (w/w) TG- and GDL-induced SPI-gel (TG and GDL were simultaneously added, pH 5.5) in the following referred to as "SPI+GDL+TG-Binder" were prepared, and analyzed using a double-uniaxial compression test to determine the texture properties hardness, cohesiveness and springiness and a tensile test as both described above and in a previous paper (Herz et al., 2021b). Both analyses were also performed with a 15% (w/w) SPI-gel without acidification or TG and a 15% TG-induced SPI-gel without acidification (pH 7).

The results of the texture analysis showed a significantly higher hardness of the SPI+TG gel and SPI+TG+GDL gel compared to the SPI and SPI+GDL samples (**Table IV.2**). The increased hardness can be attributed to protein cross-linking by TG (Tang et al., 2006) and confirmed results from our previous study, indicating that protein cross-linking was catalyzed by TG before the pH became too low by the dissociation of GDL to gluconic acid to be out of the

activity range of the enzyme (Herz et al., 2021a). Cohesiveness and springiness were lower in both acidified samples, but higher for SPI+TG+GDL compared to SPI+GDL. This shows that acidification leads to a less elastic and less cohesive texture, which is in accordance with the results of Renkema and coauthors, who observed stiffer SPI-gels at acidic pH-values in their rheological analyses (Renkema et al., 2000; Renkema & van Vliet, 2002). This was contributed to a lower solubility leading to more protein being incorporated in the network and the formation of thicker gel strands with a lower elasticity. Furthermore, our results indicate that covalent cross-linking induced by TG compensates this effect, which has also previously been reported for lower concentrated soy glycinin gels (6% w/w) where TG-induced cross-linking was applied before acidification (Zhu et al., 2011). Similarly, the maximum force and work of adhesion in the tensile test were lower for the heat-induced SPI-binder without TG or GDL (**Table IV.2**). These results are in accordance with findings of our previous study, where we observed that the addition of TG to an SPI-gel as binder between (another) extrudate and fat mimetic significantly increased the maximum force and work of adhesion (Herz et al., 2021b). This shows that not only covalent, TG-induced cross-linking increases the adhesion properties but increased hydrophobic interactions and decreased electrostatic repulsion from the addition of GDL also play a role.

**Table IV.2: Texture properties of 15% (w/w) soy protein isolate gels, induced by heating or in addition with glucono-δ -lactone or transglutaminase, obtained from a texture (†) and adhesion property test (‡)**

| | heat | heat+TG | heat+GDL | heat+TG+GDL |
|---|---|---|---|---|
| **Hardness (N)** † | 22.45 ± 1.48$^{c}$ | 76.66 ± 9.96$^{a}$ | 15.94 ± 1.43$^{c}$ | 56.46 ± 12.65$^{b}$ |
| **Cohesiveness (-)**† | 0.86 ± 0.03$^{a}$ | 0.87 ± 0.03$^{a}$ | 0.17 ± 0.03$^{c}$ | 0.23 ± 0.06$^{b}$ |
| **Springiness (mm)** † | 6.98 ± 0.95$^{a}$ | 7.90 ± 0.67$^{a}$ | 3.39 ± 0.53$^{c}$ | 5.42 ± 0.92$^{b}$ |
| **max Force (N)** ‡ | 1.80 ± 1.56$^{c}$ | 2.40 ± 2.04$^{bc}$ | 5.33 ± 2.24$^{a}$ | 4.00 ± 1.90$^{abc}$ |
| **Work of Adhesion (J)** ‡ | 0.58 ± 0.47$^{b}$ | 4.60 ± 4.00$^{a}$ | 2.71 ± 1.86$^{ab}$ | 2.73 ± 1.61$^{ab}$ |

*Note*: Values followed by different superscripts are significantly different within the line ($P < 0.05$).

Having looked at the binder system itself, we subsequently wanted to find out if the observed results of the texture analysis and a tensile test could be translated to a more complex meat product analogue matrix in which extrudates and fat mimetics would have to be bound. To answer this and to find out how the binder ratio influences texture properties in such complex matrices, an application test was performed with results being presented in the following.

## Influence of matrix composition and binder type on mechanical properties of wet sausage masses.

Complex sausage matrices containing binder, fat mimetics and extrudates as main components were subsequently prepared by mixing of the individual components. In wet samples, i.e. samples that had been taken from filled and heated, but not yet dried sausages, hardness decreased with increasing binder content (**Figure IV.1A**). Moreover, hardness tended to increase with the addition of TG, especially at higher binder contents. In particular, samples containing above 60% binder were notably influenced by the addition of TG.

For springiness, no systematic impact of binder content was observed, but there were significant increases in springiness found for samples made with the addition of TG at 40 and 50% binder content, samples with SPI+GDL+TG-binder had a significantly higher springiness compared to samples with the according amount of SPI+GDL-binder (**Figure IV.1C**). This effect might be caused by protein crosslinks induced by TG in the SPI-binder matrix itself as well as crosslinking between the SPI suspension and the extrudates and fat mimetic particles.

For cohesiveness, an increase of binder content in uncrosslinked samples tended to increase cohesiveness, but upon addition of TG the influence of binder content that influence disappeared. The addition of TG generally increased the cohesiveness at lower binder contents (30 and 50%) but decreased the cohesiveness significantly at 70% binder content (**Figure IV.1B**). Taken together this indicates that especially the elastic contributions to the mechanical properties of the wet, but heated sausage mass might arise from an interplay between possible internal SPI binder crosslinks as well as possible crosslinks between the binder and the included particles, namely the extrudates and the fat mimetics. In these composite systems, each of the individual structural entities may have a contribution to the behavior of the bulk system, and since the individual components all have different mechanical properties a nonlinear mixing behavior with increasing binder content and decreasing content of fibrous extrudate may emerge. There are not a lot of studies available that have looked at such composite systems, but Kuraishi et al. (1997) observed in a study with caseinate+TG as binder for meat cubes that an excessive application of binder resulted in decreased binding strength. A similar application study of a soy protein binder and TG in a meat analogue has also been done by Lee and Hong (2020) in a plant-based burger patty. Here, the addition of TG to an SPI-binder increased the hardness, springiness and cohesiveness significantly, but variations of compositions especially

those of binder to extrudate ratios were not done. A similar complex behavior of fibrous material – binder content was found by Atilgan and Kilic in a study investigating the effect of addition of microbial transglutaminase, fibrimex (a combination of fibrin and thrombin) and alginate on physicochemical properties of cooked ground meat (Esra Atilgan & Birol Kilic, 2017).

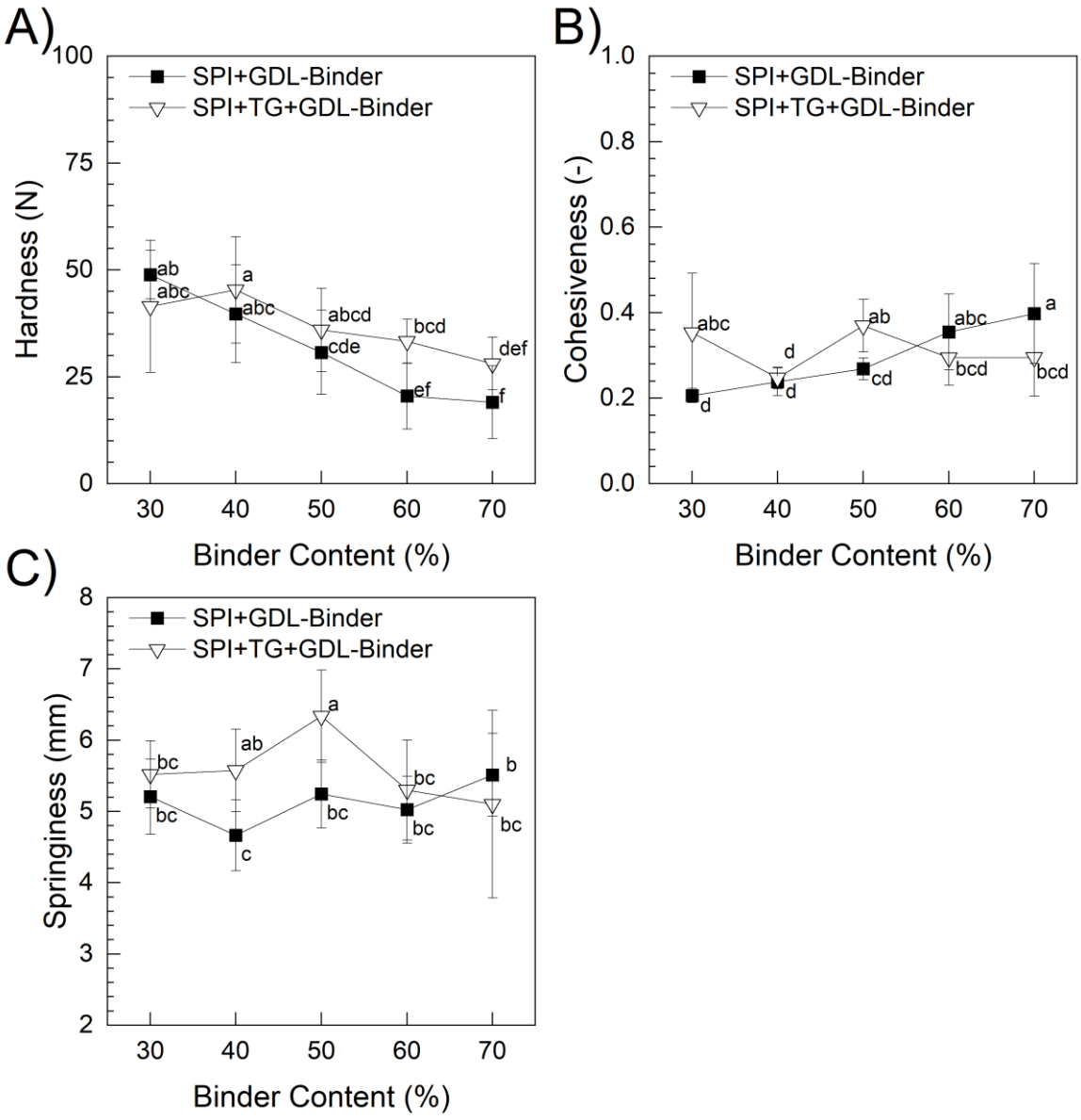



**Figure IV.1:** Influence of TG-addition in the binder on hardness (A), cohesiveness (B) and springiness (C) of undried sausage analogues, analyzed in a double uniaxial compression, at binder ratios between 30-70%. Values followed by different superscripts are significantly different ($P < 0.05$).

## Influence of matrix composition and binder type on drying behavior of sausage masses.

Next, we studied the behavior of the sausage masses during drying. Specifically, the impact of the two different binder systems, i.e. the SPI+GDL-Binder and the SPI+TG+GDL-Binder at different binder-to-extrudate-ratios on drying of the salami analogues were studied. To that purpose, weight loss of the samples was measured throughout the drying and in samples where drying had been completed. The dry matter of the different batters after production ranged from 30.2% for the sample with 70% binder to 37.4% dry matter with 30% binder (see also **Table IV**.3 in the appendix for details). Moreover, the change in dry matter over time during drying is shown representatively for samples with 30, 50 and 70% binder content in **Figure IV.2**.

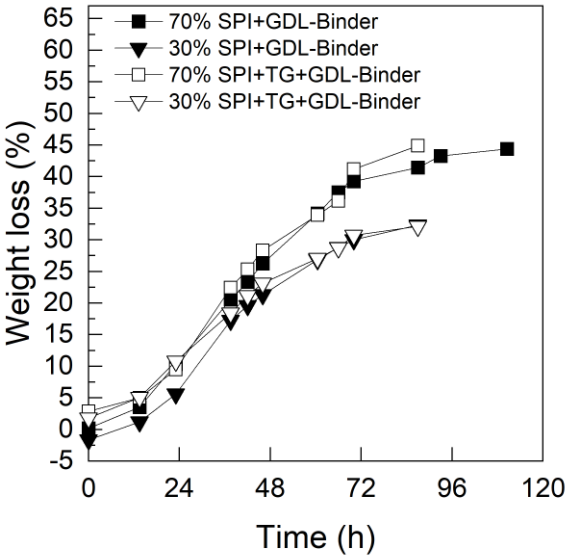



**Figure IV.2:** Weight loss of plant-based sausage with 30 and 70% SPI+GDL-binder and SPI+GDL+TG-binder during 5 days of drying.

The results show that in samples with 30 and 70% SPI+TG+GDL-Binder, water loss during heating occurred, resulting in a higher dry matter at the beginning of drying and more rapid and higher weight losses than samples with the SPI+GDL-Binder. However, the highest dry matter of 55% were texture properties of the samples were studied, was reached within a time frame of 6 h for all binder contents. All samples had a final pH of ~ 5.5, therefore differences in water holding capacity can't be linked to the pH but rather to the results of our previous study where the microstructure of the different SPI-gels used as binder in this study were assessed. Compared to the GDL-induced SPI-gel, the SPI+TG+GDL-induced gel had a more porous

microstructure and showed higher syneresis (Herz et al., 2021a). This porous microstructure might cause faster water migration and faster weight loss of the samples with SPI+TG+GDL-Binder in this study.

## Influence of matrix composition and binder type on texture, sensory properties and appearance of dried sausages

We finally assessed texture and sensory properties of sausages dried to different final dry matter (or moisture) contents. Also the protein content of the sausage analogues has been analyzed and showed similar values independent from binder to extrudate ration, ranging from ~ 18% protein at 40% dry matter to ~ 25% protein at 55% dry matter (**Table IV.4**). Therefore, it can be presumed that differences in texture are to be attributed to macro- and micro-structural differences rather than to chemical composition.

First, we compared the texture properties of sausages with SPI+GDL-Binder and the SPI+TG+GDL-Binder at different dry matter contents (40-55% dry matter). An increased hardness of sausages with TG-crosslinked binder was only significant at 40% dry matter content and 70% binder (i.e. the system that contained the most water and binder), as well as the system with at 45% dry matter and 60% and 50% binder (for further details see appendix, **Figure IV.6**). In all other samples and at higher dry matter contents, no significant difference in hardness was found between samples containing SPI+GDL- and SPI+GDL+TG-binder. Similarly, for cohesiveness, a significantly increased cohesiveness was only detected at 40% dry matter content with 70 and 60%, whereas for lower binder contents and higher dry matter contents at later stages of the drying, differences in cohesiveness between the two different binder systems disappeared. For springiness, no significant differences between dried sausages made with SPI+GDL or SPI+GDL+TG as binder were found. In essence, previous differences in the sausage masses due to use of TG were lost with drying, and TG-crosslinking of the binder thus provided only marginal improvements in mildly dried sausages at high binder contents only. Because of this, the influence of composition and dry matter content on texture of dried salami analogues are only shown below for samples with SPI+GDL-binder. Furthermore, a triangle test comparing sausages with 40% and 60% SPI+GDL- and SPI+TG+GDL-Binder at 45% dry matter was conducted, where no difference on a significance level of 0.05 (see in detail in the supplementary material **Table IV.5**) between the two binders was observed, confirming the findings of the texture analysis, where mostly no difference between dried sausages with TG-

crosslinked and non-crosslinked binder were found. Therefore, a comprehensive sensory analysis on the influence of binder ratio on texture properties was only performed for sausages with SPI+GDL-binder.

Looking at the impact of final moisture content, all samples regardless of their composition and use of TG became harder with increasing dry matter contents (**Figure IV.3A**). Moreover, results of the instrumental texture analysis also showed that hardness decreased with increasing binder content (**Figure IV.3A**). These results can be attributed to the higher hardness (~ 230 N, measured in preliminary experiments in undried state) of the extrudate compared to the hardness of the binder itself (< 10 N (in undried state), **Table IV**.2), which results in a higher hardness at higher extrudate contents and a lower hardness at higher binder contents. The springiness measured by texture analysis increased significantly with increasing binder content from 4.86 ± 0.32 mm with 30% SPI-binder to 5.96 ± 0.57 mm with 70% SPI-binder, but was only little impacted by an increase in dry matter content (**Figure IV.3C**). The springiness of the (undried) binder itself, the GDL-induced SPI-gel, was ~ 3. mm, indicating that drying increases the springiness of the SPI-gel and that there might be a synergistic effect in the mixture with the comminuted extrudate, as outlined above. The cohesiveness measured by texture analysis also increased significantly with increasing binder content, but again only little with increasing dry matter (**Figure IV.3B**). Only at lower binder contents (30 and 40%) did the cohesiveness increase significantly with increasing dry matter content. From a mechanistic point of view, the macrostructure of the sausage analogues can be considered as a particle-filled gel and the texture is therefore influenced by an interplay of the individual texture properties contributing to the overall texture as physical and chemical interactions between binder and particles. The results show overall that the binder content correlates with cohesiveness and springiness, while the extrudate content correlates with hardness. The influence of the amount of binder on binding strength has rarely been studied (Kuraishi et al., 1997; G.H. Lu & T.C. Chen, 1999), but from these results it has been postulated, that there is an "amount range" where binding strength increases with the amount of binder. When considering cohesion as a measure for binding strength, this amount range hasn't been exceeded with the compositions studied. Furthermore, cohesion has been reported to increase with increasing covalent and non-covalent bonds (Azeredo & Waldron, 2016) as well as with decreasing water content (Wall & Huebner, 1981). However, this correlation couldn't be observed this study. We therefore conclude, that in the studied system, the texture is mostly influenced by the amount and texture of the individual

components as well as their interactions rather than cross-links and interactions in the binder itself.

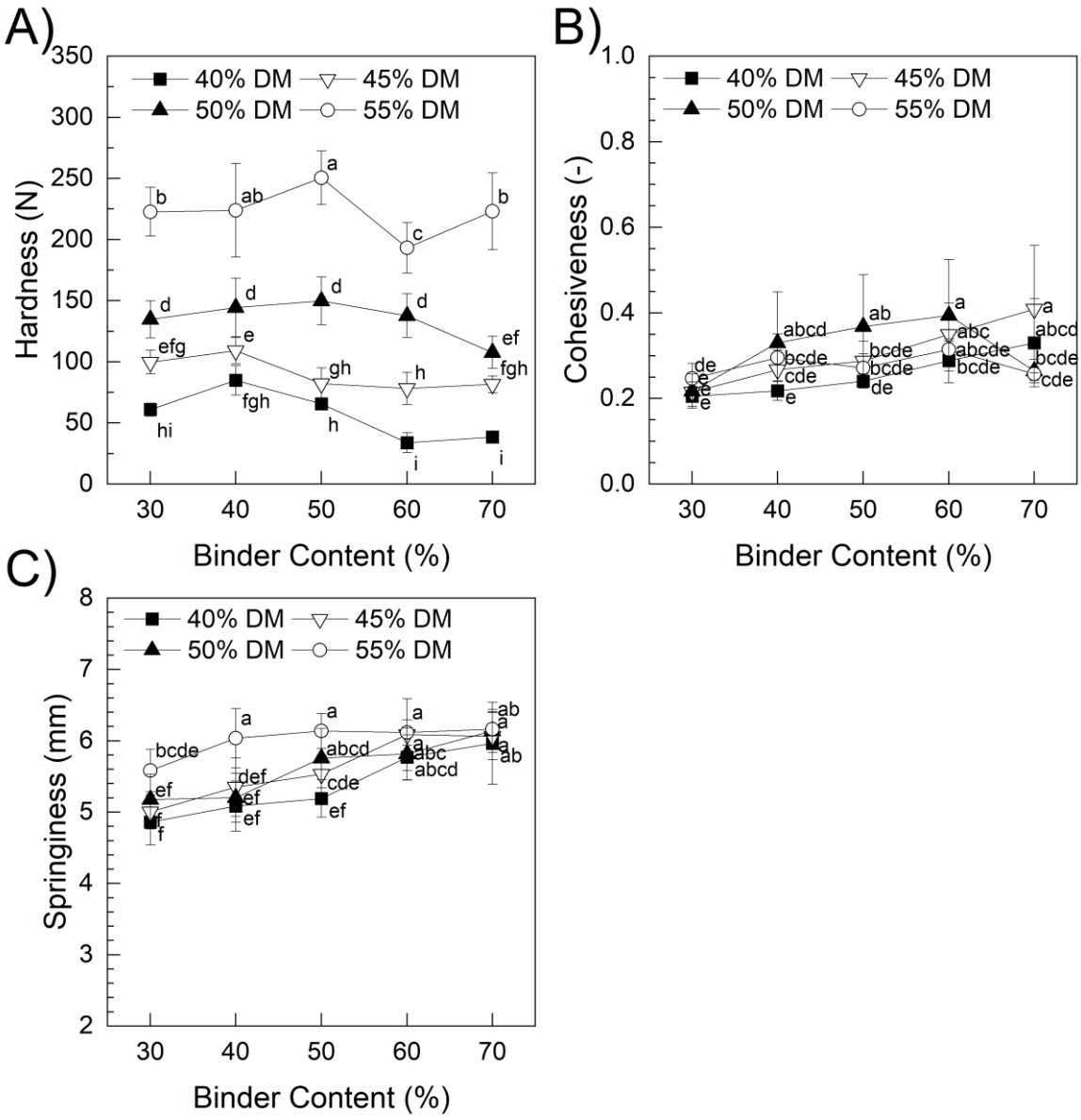



**Figure IV.3:** Influence of binder ratio and drying on hardness (A), cohesiveness (B) and springiness (C), analyzed in a double uniaxial compression, of sausage analogues with 30-70% SPI-GDL-binder, dried to dry matter of 40-55%. Values followed by different superscripts are significantly different ($P < 0.05$).

The effect of a decreased hardness with increasing binder content was also detected in the sensory analysis and therefore correlated well with the texture analysis results. The results of the sensory analysis further showed a significant increase in cohesiveness with increasing

binder content (**Figure IV.4**). Interestingly, results of the sensory analysis indicate that on the one hand an optimal hardness was scored for samples with 30% binder, while, on the other hand, those samples were rated too low in cohesiveness (2.25 ± 1.45) and too dry (6.58 ± 1.55). In contrast, samples with 70% binder were rated close to the optimum in dryness (5.12 ± 1.62) and better in cohesiveness compared to lower binder ratios (3.91 ± 1.46), but were evidently too soft (2.78 ± 1.57). Generally, the dryness ratings decreased with increasing binder content, although all samples had the same dry matter, indicating that the oral perception of dryness can be modulated by different component ratios at the same dry matter and furthermore correlates with the cohesiveness of the samples. To the best of our knowledge, the relationship between texture and dryness perception hasn't been studied so far, and this may be an area worthy of investigations in the future. Dryness, according to ISO 5492:2008, is a "surface textural attribute that describes the perception of water absorbed by or released from a product". In the case of the products studied here, low cohesiveness resulted in a fast break-down of the product into many small particles and therefore probably a fast absorption of saliva, resulting in the perception of increasing dryness in correlation with a decreasing cohesiveness.

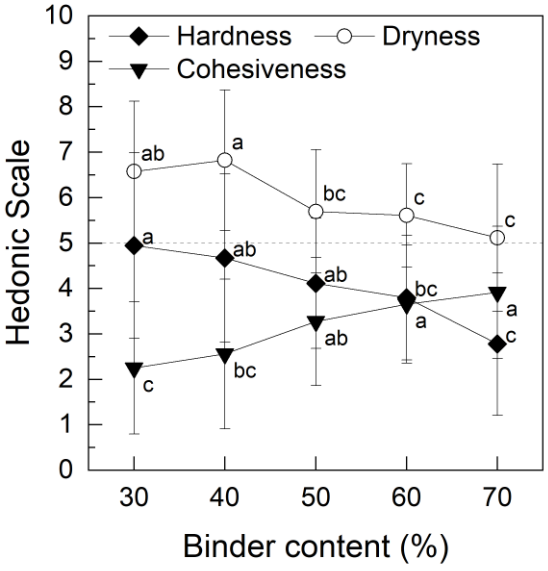



**Figure IV.4:** Influence of binder content (30-70% SPI+GDL-binder) on the sensorial properties hardness, cohesiveness and dryness, assessed by 20 trained panelists on a 10-step relative-to-ideal scale with 5 as optimum value. Values followed by different superscripts are significantly different ($P < 0.05$).

*Optical appearance.* Animal-based salami-type sausages typically have a marbled appearance of small white animal fat particles. In our study, plant-based salami analogues had a similar appearance as shown in **Figure IV.5**. At high binder contents, the optical appearance of the protein phase was coherent, while towards lower binder contents, especially with 30 and 40% binder, the structure appeared incoherent and crumbly. Furthermore, the visible fat particles appeared smaller in size with decreasing binder content, which may be attributed to the decomposition of fat mimetic particles during processing caused by the higher overall hardness of the sausage batter at lower binder contents. Moreover, fat particles appeared less white with increasing dry matter which was also observed by Dreher et al. (2021) in the application of their animal fat mimetic system. The authors explained this effect by a loss of water from the protein network in the emulsion gel during drying. The white appearance of the fat mimetic is caused by light scattering of the emulsified oil droplets in the protein network. When water diffuses out of the network, the volume phase of the oil increases, leading to a less white and more yellow appearance.

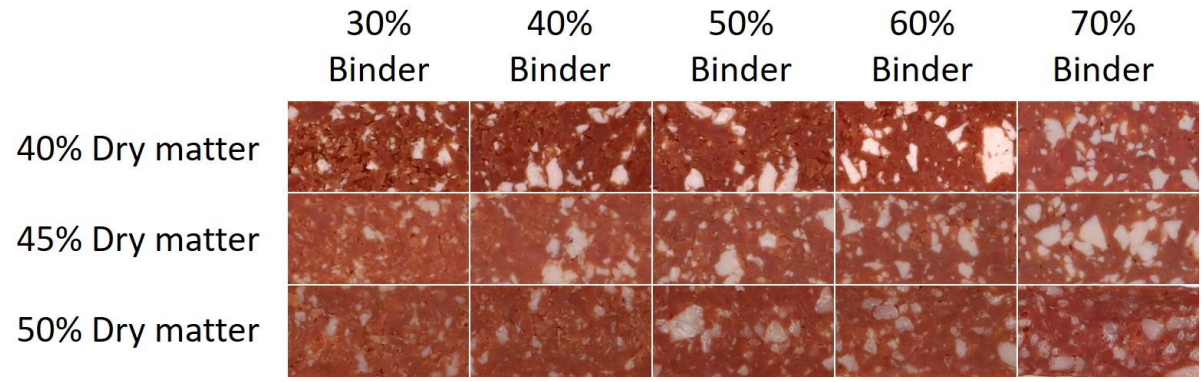



**Figure IV.5:** Images of cross-sections of plant-based salami style sausages analogues with 30-70% SPI+GDL-binder content, dried to dry matters of 45-50%.

# Conclusion

Taken together, our results show that both a GDL-induced SPI-gel as well as a TG+GDL-induced SPI-gel can be used as binder to combine extrudate and fat components into a coherent mass suitable for production of a plant protein-based analogue of a dry-fermented sausage. Using these components, process and technology approaches mirroring that of their conventional meat-based counterparts can be used, i.e. comminution/mixing of components, filling in casings, drying and smoking. Furthermore, the emerging texture of the dried sausages

depends mostly on the amount of binder as well as the degree of drying applied, which offers a formulation- and process-based approach to modulate texture of such products. The use of TG as a crosslinker - in contrast to it being necessary to produce a suitable fat mimetic system – did little to improve the characteristics of the final dried sausage analogues. As such, it is in particular the adhesive properties of a binder that are key to facilitating the manufacturing of a coherent mass that later can be converted into a product with sufficient hardness and cohesiveness that appeals to consumers' preferences.

## Acknowledgements

We would like to thank Daniel Einsiedel (University of Hohenheim) for conducting some experiments and Kurt Herrmann and Johannes Dreher (University of Hohenheim) for ongoing support and fruitful discussions.

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# Supplementary Material

**Table IV.3:** Dry matter content of the raw sausage analogue batters.

| SPI-Binder (%) | Dry Matter (%) |
|---|---|
| **30** | 30.21 ± 0.71 |
| **40** | 32.37 ± 0.01 |
| **50** | 34.27 ± 0.44 |
| **60** | 35.59 ± 0.57 |
| **70** | 37.36 ± 0.38 |

**Table IV.4:** Protein content of salami-type sausage analogues at various dry matter and SPI-binder contents.

| | Protein content (%) | | | |
|---|---|---|---|---|
| **SPI-Binder (%)** | 40 % Dry matter | 45 % Dry matter | 50% Dry matter | 55% Dry matter |
| **30** | 18.02 ± 0.48 | 20.27 ± 0.77 | 22.52 ± 0.46 | 24.78 ± 1.77 |
| **40** | 18.05 ± 0.89 | 19.85 ± 0.14 | 22.11 ± 0.55 | 24.82 ± 0.66 |
| **50** | 18.58 ± 0.98 | 19.97 ± 0.37 | 23.22 ± 0.22 | 25.55 ± 0.22 |
| **60** | 18.93 ± 0.54 | 22.24 ± 0.48 | 23.66 ± 0.33 | 25.55 ± 0.59 |
| **70** | 18.21 ± 0.11 | 21.40 ± 0.55 | 22.77 ± 0.59 | 24.59 ± 0.70 |
| **30 (+TG)** | 18.25 ± 0.88 | 20.03 ± 0.26 | 22.26 ± 0.36 | 24.48 ± 0.08 |
| **40 (+TG)** | 19.33 ± 0.46 | 21.22 ± 0.57 | 23.58 ± 0.57 | 25.94 ± 0.31 |
| **50 (+TG)** | 18.31 ± 0.12 | 20.60 ± 0.10 | 22.89 ± 0.16 | 25.18 ± 0.11 |
| **60 (+TG)** | 18.31 ± 0.57 | 20.59 ± 0.13 | 21.97 ± 0.17 | 25.17 ± 0.16 |
| **70 (+TG)** | 17.51 ± 0.57 | 19.70 ± 0.10 | 21.45 ± 0.14 | 24.07 ± 0.07 |

**Table IV.5:** Results of a triangle test between sausage analogues containing SPI+GDL- and SPI+GDL+TG-binder at 45% dry matter.

| Binder content (%) | Number of panelists | Correct answers | α-level |
|---|---|---|---|
| **60** | 34 | 14 | 0.4 |
| **40** | 34 | 13 | 0.3 |

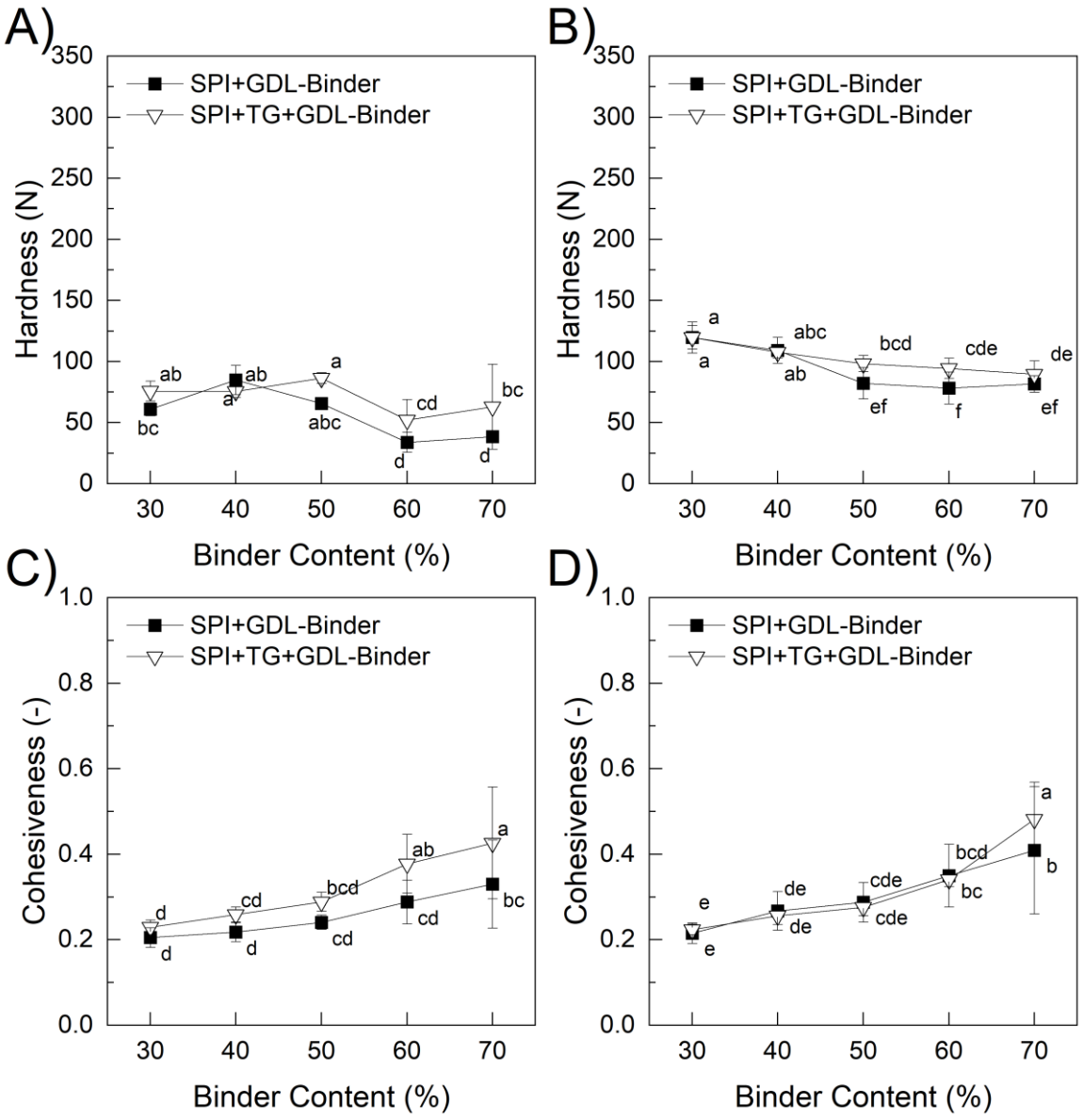


**Figure IV.6:** Influence of TG-addition in the binder on hardness of sausage analogues at 40% dry matter (A), and 45% dry matter (B) as well as on cohesiveness at 40% dry matter (C) and 45% dry matter (D), analyzed in a double uniaxial compression, at binder ratios between 30-70%. Values followed by different superscripts are significantly different ($P < 0.05$).

# V. Chapter

# Functionality of Gluten as Binder in a Plant-Based Salami-Style Sausage Analogue

**Eva Herz[1], Till Kinne[1], Nino Terjung[2], Monika Gibis[1], Jochen Weiss[1*]**

[1]Department of Food Material Science, University of Hohenheim,
Garbenstrasse 25, 70599 Stuttgart, Germany

[2]Product Innovation, DIL German Institute of Food Technology, Professor von Klitzing
Strasse 7, 49610 Quakenbrück, Germany

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# Abstract

Binder compounds are needed to create complex meat analogues from individual components such as plant protein extrudates and fat mimetics, but their functionality have scarcely been studied in detail. Here, we studied the use of gluten as binder due its unique visco-elastic properties and analyzed the impact of hydration and amount of gluten on texture and sensory properties of a plant-based salami-style sausage analogue. While the hardness was not significantly influenced by the binder ratio, springiness and cohesiveness increased with increasing binder content. With increasing drying, the hardness increased, while springiness and cohesiveness didn't change significantly. The results of the sensory analysis correlated with the texture analysis results in terms of cohesiveness, but showed also that an increase in hardness and a decrease in dryness was perceived with increasing binder content. Taken together this study demonstrates that gluten may serve as a suitable binder to modulate the texture of complex meat analogues. Furthermore, it is suggested that hardening after gel formation as well as adhesive and cohesive properties are key factors for binder functionality.


**Keywords:** Meat analogue, Binder, Gluten, Texture, Plant proteins

# Introduction

Plant-based analogues of food that have traditionally been produced from animal-derived raw materials have been becoming more and more popular over the last few years. This is because the production of animal-derived foods, especially from ruminants, is very resource-intensive and leads to higher water use, land use and greenhouse-gas emissions compared to plant foods (Poore & Nemecek, 2018). Furthermore, an increasing number of consumers does not want to eat meat or other animal-derived products, or would like to decrease the overall intake of those products for ethical reasons, such as animal welfare concerns (Apostolidis & McLeay, 2016). Finally, there have been recommendations to reduce the intake red meat specifically due to its consumption having been epidemiologically linked to an increased risk for cardiovascular diseases, coronary heart diseases and cancer (Anand et al., 2015). Vegetarian and vegan diets are often considered healthier since diets high in plant-based foods have lower prevalences for coronary heart diseases and obesity (Anand et al., 2015; Appleby & Key, 2016). Plant-based analogues provide an easy option for consumers to reduce their intake of animal-based foods since consumers do not have to make profound changes to their eating habits and culture (de Bakker & Dagevos, 2012). However, it should be kept in mind that many of the current meat analogues on the market contain high levels of saturated fatty acids and salt that put in question nutritional benefits (Curtain & Grafenauer, 2019). Moreover, various functional ingredients that are not readily accepted by consumers are often needed in their manufacture. One such compound is for example methylcellulose, which is currently one of the most widely used binder compounds in meat and meat product analogues.

Meat analogues are usually composed of three major components: 1) fibrous plant proteins, 2) structured or unstructured plant fats, oils or blends thereof, and 3) a binder that holds together these other components. Globular plant proteins can be brought into a fibrous structure by processing, by for example wet or dry extrusion, or by use of a shear cell (Birgit L. Dekkers et al., 2018). Plant fat or oil can either be directly used, but are more often structured to provide properties more akin to that of animal fat tissue (Devine & Jensen, 2004). For example, a fat mimicking system suited for salami analogues has been developed by Dreher et al. (2020a). It consists of an emulsified and cross-linked fat crystal network that provides elastic and plastic behavior, melting properties and visible fat particles. In contrast to these aforementioned compounds, functionality of binders for meat analogues has hardly been studied (Kyriakopoulou et al., 2021). Binders need to act as a “glue” between the other components,

but also typically harden afterwards in order to yield solid products that can be sliced or shredded. As mentioned above, methylcellulose is often used, but consumer acceptance is declining due to the synthetic nature of the compound. Another often-used binder is egg-white, but because of the aforementioned ethical and environmental concerns, there has been an increasing demand for completely plan-based products.

In previous studies we found that soy protein isolate (SPI) gels, acidified and/or crosslinked by glucono-δ-lactone (GDL) and transglutaminase (TG) can be applied as binder to provide cohesiveness in plant-based salami-style analogues. However, using SPI, no optimal composition could be achieved, since hardness and cohesiveness were oppositely influenced by increasing binder content, i.e. a low binder content led to sufficient hardness but insufficient cohesiveness, while at high binder contents the cohesiveness increased, but hardness decreased. Since gluten is known to be able to form a strong, yet adhesive protein network (Ooms & Delcour, 2019), it might be a promising binder ingredient to create plant-based sausage analogue with appealing textures.

The question, which alternative compounds could work as binders in vegan meat analogues and what properties and functionalities are needed, is still an open one and of interest to researchers and developers of novel plant-based foods. To that purpose we analyzed the texture and adhesion properties of gluten gels, and then investigated the texture properties of salami-style sausage analogues made with different amounts of gluten, fibrous protein extrudates and a fat mimetic.

# Materials and Methods

## Materials

High-moisture extrudates from Alpha 8 soy protein concentrate from Solae Europe S.A. (Geneva, Switzerland) were provided by Deutsches Institut für Lebensmitteltechnik (DIL e.V., Quakenbrück, Germany). Wheat gluten *Weizengluten vital* (protein content 83% on dry matter basis) was purchased from Kröner Stärke GmbH (Ibbenbühren, Germany). A spice mix *StarFermat*, salt (NaCl) and paprika powder were obtained from Frutarom GmbH (Salzburg, Austria). Beetroot powder was purchased from Van Hees GmbH (Walluf, Germany) and Glucono-δ-lactone (D-(+)-Gluconic acid δ-lactone) from Sigma-Aldrich (Steinheim, Germany). A vegan salami flavor was provided by Givaudan SA (Vernier, Switzerland). For

the fat mimetic, canola oil was obtained from MEGA eG (Stuttgart, Germany) and soy protein isolate Supro EX 37 HG IP from Solae Europe S.A., Geneva, Switzerland (protein content > 90% on dry matter basis). Hydrochloric acid (HCl) was purchased from Carl Roth GmbH and Co.KG (Karlsruhe, Germany) and transglutaminase (ACTIVA WM from *Streptoverticillium mobaraense* with an enzyme activity of 114 U/g, determined by the manufacturer) from Ajinimoto Foods Europe SAS (Hamburg, Germany).

## Methods

*Preparation of gluten gels for binder analysis.* 57% Water, 43% gluten powder and 0.3% GDL were mixed for 3 min in a household mixer (KitchenAid 5KSM185PS, Whirlpool Corporation, St. Joseph, MI, USA) with a dough hook and afterwards filled into polyethylene casings, followed by heating to a core temperature of 85 °C in an Air Master UK-1800 chamber. For the tensile tests, extrudates were cut into 4 mm thick slices with a commercial meat slicer having a length of 14 cm and a width of 8 cm. Then, a measured amount of gluten powder was evenly distributed with a sieve onto the extrudate and a defined amount of water was sprayed on with a spray bottle so that the gluten binder formed directly on the extrudate. This was done since an even distribution of pre-hydrated gluten was not possible. Afterwards, a second piece of extrudate was placed on top. The extrudate sample was wrapped in plastic foil to prevent water loss or uptake during heating and then placed in an aluminum baking tray with another baking tray on top. 8 g/cm² of weight was placed in the second baking tray to provide a defined contact pressure. In order for gelation to occur the samples were heated at 85 °C for 1 h.

*Preparation of fat mimetic.* The fat mimetic was prepared according to the method of Dreher (Dreher et al., 2020a). A 12%(w/w) SPI suspension was prepared by homogenizing the protein isolate and water for 15 min at 3500 rpm in a MaxxD Lab rotor-stator homogenizer (FrymaKoruma, Rheinfelden, Switzerland). Subsequently, the suspension was adjusted to pH 7.0. Afterwards, canola oil was slowly added at a ratio of oil to protein of 7:3 (w/w) and homogenized in the same device for 300 s at a speed of 5000 rpm under vacuum of 450 ± 50 mbar. After homogenization, and verification of temperatures being below 38 °C, then transglutaminase (TG) was added as a 40% (w/w) solution at a concentration of 20 mg/g protein. The homogenized emulsion was then filled into 60 mm polyethylene casings (NaloBar APM, Kalle GmbH, Wiesbaden, Germany) using an MWF 591 filler (MADO, Dornhan, Germany), and heated in an Air Master UK-1800 chamber (Reich GmbH, Schechingen, Germany) at 45 °C until they reached a core temperature of 40 °C. Following

that, samples were incubated at 40 °C for 60 min to enable protein crosslinking. Finally, samples were heated at 90 °C until they reached a core temperature of 85 °C. After being showered with cold water (10 °C) for 10 min, they were stored at 5 °C until further use.

**Table V.V.1:** Ingredients and concentrations of the plant-based salami-style sausage analogues with vital wheat gluten (WG) and water in a ratio of 2:1 as binder.

| **Main ingredients** | **WG 7.5** | **WG 15** | **WG 22.5** | **WG 30** | **WG 37.5** |
|---|---|---|---|---|---|
| **Wheat gluten (%)** | 5 | 10 | 15 | 20 | 25 |
| **Water (%)** | 2.5 | 5 | 7.5 | 10 | 12.5 |
| **Extrudate (%)** | 77.5 | 70 | 62.5 | 55 | 47.5 |
| **Fat mimetic (%)** | 15 | 15 | 15 | 15 | 15 |
| **Additional ingredients** | Dosage (g/kg) | | | | |
| **NaCl** | 18 | | | | |
| **Glucono-δ-lactone** | 5 (target pH 5.6) | | | | |
| **Spice mix** | 15 | | | | |
| **Beetroot powder** | 10 | | | | |
| **Paprika powder** | 5 | | | | |
| **Salami aroma** | 5 | | | | |

*Preparation of sausage analogue masses.* Emulsion gels were removed from the casings and pre-cut into pieces with an approximate diameter of 5 cm and afterwards comminuted in a K64 bowl chopper (Seydelmann GmbH, Stuttgart, Germany) for 20 s at a knife speed of 930 rpm and a bowl speed of 13 rpm. Extrudate pieces, pre-cooked for 20 min in 80 ºC hot water and then cooled over night at 2 ºC, were comminuted in the bowl chopper as well at a knife speed of 1500 rpm and a bowl speed of 13 rpm for 65 s to achieve the desired particle size. Salami analogue masses were then produced by combining the comminuted extrudate particles with the powdered components (wheat gluten, salt, spice mix, salami flavor, GDL, beet and paprika powder) in ratios according to **Table V.V**.1, and mixing them in the bowl chopper using a reverse setting (knives rotating backward, contacting the batter with the dull side to prevent further comminution) for 60 s at a bowl speed of 13 rpm. Next, the comminuted emulsion gel was added and mixed for 50 s, and water was then added and mixed for 60 s. The salami analogue masses were then filled into 28 mm cellulose casings using a VF 610 plus vacuum filler (Handtmann GmbH, Biberach, Germany), and heated in an Air Master UK-1800 chamber (Reich GmbH, Schechingen, Germany) at a constant temperature of 92 °C until they reached a core temperature of 85 °C followed by showering with cold water (10 °C) for 10 min. After heating, samples were smoked twice for 5 min in the Air Master UK-1800 chamber, followed by drying at 18 °C and 80% relative humidity until they reached different dry matter contents

ranging between 55 and 65%. To that purpose, samples were taken in regular intervals to determine dry matter content. The dry matter content of undried sausage analogue batters are shown in the supplementary material (**Table V.I**.3). Dried salami analogues were stored at 2 °C in sealed bags under controlled atmosphere until used for further analysis.

*Dry matter content.* The dry matter content was determined gravimetrically in triplicate, using the standard sea sand method (VO(EG)152/2009). 5 g of sample were ground with sea sand and dried in an oven (Type 400, Memmert GmbH, Schwabach, Germany) until a constant weight loss had occurred. The remaining mass represents the dry matter.

*Water activity.* The water activity ($a_w$-value) was determined in triplicate at a temperature of 20 ºC with a Rotronic HygroPalm - HP23-AW-A (Rotronic Messgeräte GmbH, Ettlingen, Germany) device.

*Protein content.* The protein content of the salami analogues was determined with a Dumatherm N nitrogen determination device (Gerhardt GmbH, Königswinter, Germany) using 5.5 as nitrogen conversion factor as suggested for gluten and soy protein (Mariotti et al., 2008).

*Texture measurement.* The texture of the samples was analyzed by recording the force as function of distance of a double uniaxial compression with an Instron Universal Texture Analyzer (Illinois Tool Works Inc., Darmstadt, Germany). The samples were cut into cylindrical specimens (diameter: 20 mm, height: 15 mm) and compressed to 75% of their original height for the assessment of hardness and to 50% for cohesiveness and springiness. The texture parameters were calculated according to Bourne (1968).

*Tensile test.* A previously adapted tensile test (Herz et al., 2021b) to analyze the cohesion of adhered binder-extrudate samples was used: After heating and cooled storage, the extrudate-binder samples were cut into squares with a diameter of 2 cm and glued onto stainless steel plates with cyanoacrylate glue. The plates were hooked into to the Instron Universal Texture Analyzer (Illinois Tool Works Inc., Darmstadt, Germany) and pulled apart at a constant speed of 1 mm/s. Force vs distance curves were recorded and the maximum force and the area under the curve referring to the work of adhesion were calculated.

*Sensory analysis.* 20 trained panelists assessed the samples among the texture factors hardness, cohesiveness and dryness (a) on a just-about-right scale and (b) compared to a commercial vegetarian reference product (vegetarian snack salami “Vegetarische Mühlen Salami Minis”

(Carl Müller GmbH und Co. KG, Bad Zwischenahn, Germany). A 10-step relative-to-ideal scale was used, with the midpoint representing either the reference product or optimal evaluation of the attribute. For example, the attribute hardness was scored between 0 = much too soft, 5 = optimal hardness, 10 = much too hard and compared to the reference product with 0 = much softer than reference, 5 = like reference, 10 = much harder than reference. The panelists were members of the Institute of Food Science and Biotechnology in the age range of ~ 20 – 60 years who have received lectures and training on sensory during their studies and participate regularly in sensory analysis. The panel has additionally received two product-related trainings before the analysis of the plant-based sausages.

*Ethical Statement – Sensory*. The Ethics Committee of the University of Hohenheim has judged that the sensory study has no ethical concerns and therefore no ethical approval was necessary, Reference number 2023/05_Herz, dtd 05/08/23. Each participant gave informed consent via signature before starting the sensory. The panelists participated voluntarily and were able to withdraw from the sensory at any time without consequences. Data were collected anonymously, and the tested food products were prepared in the pilot plant of the Department of Food Material Sciences at the University of Hohenheim which is approved for processing food.

*Statistical analysis*. For statistical analysis and preparation of graphs, the software OriginPro2020 (OriginLab Corp., Northampton, MA) was used. A one-way analysis of variance and Tukey's post hoc tests were carried out and differences at the $p < 0.05$ level were considered to be significantly different. The assumption of normality and equal variance was also tested ($p < 0.05$). Experiments were carried out in duplicate.

## Results and Discussion

*Binder analysis.* First, we analyzed the texture and binder properties of hydrated gluten in a texture analysis and tensile test. In a preliminary test, the hydration range for the used gluten, where no powder clumps where visible and no excess water was present, was determined as 46-43% gluten and 54-57% water, respectively. To ensure full hydration, 43% gluten (57% water) was thus chosen for the texture analysis. The results for hardness, cohesiveness and springiness are shown in **Table V.I**.2. Compared to a TG-induced SPI-gel (15% SPI w/w, pH 7), that were studied similarly in a previous study (currently under review), the hardness of the gluten gel was distinctly higher (~ 324 N vs. ~ 77 N), while cohesiveness and springiness

were in the same range (cohesiveness: ~ 0.87 (TG+SPI-gel) vs. ~ 0.79 (gluten gel), springiness: ~ 7.9 mm (TG+SPI-gel) vs. ~ 7.5 mm (gluten gel) Moreover all texture parameters of the gluten gel higher than the values obtained for heat-, GDL- or TG+GDL-induced SPI-gels. Probably, higher values could be reached with the application of additional mechanical forces, such as during kneading as is typical in bakery applications (Boitte et al., 2013). However, for this study, only minimum shear forces were chosen to obtain transferable results for the following application process, where minimal shearing to prevent smearing of the animal fat mimetic was required.

**Table V.I.2:** Texture properties of a 43% gluten gel from a texture pressure analysis (†) and adhesion properties of a 43% and a 66% gluten assessed in a tensile test (‡).

| | 43% Gluten | 66% Gluten |
|---|---|---|
| **Hardness (N)†** | 323.63 ± 41.50 | |
| **Cohesiveness (-)†** | 0.79 ± 0.05 | |
| **Springiness (mm)†** | 7.48 ± 0.68 | |
| **Break Point (N)†** | 2 869.29 ± 644.11 | |
| **max Force (N)‡** | 5.75 ± 1.89[a] | 4.43 ± 1.39[a] |
| **Work of Adhesion (J)‡** | 18.27 ± 7.64[a] | 9.47 ± 4.13[b] |

*Note*: Values followed by different superscripts are significantly different within one line ($P < 0.05$).

Since preliminary tests had shown that a lower water addition than 54% was possible when the binder was combined with the high-moisture extrudates – likely due to water migration from the extrudate to the binder - a tensile test was also performed with 66% gluten (i.e. 34% water) in comparison. This was also done since water would later have to be removed from sausage masses by drying. Results showed that the maximum force was in the same range, but work of adhesion was higher for 43% gluten, suggesting that a higher water content might lead to increased adhesion properties. The topic of different measures of stickiness and the causes for stickiness have been widely discussed in the field of bakery science and dough formation. Here, it was also found that, among other factors that are more connected with the carbohydrate components, water absorption and an increased protein content correlate positively with the stickiness of wheat doughs (Grausgruber et al., 2003; Yildiz et al., 2012). Compared to the tensile properties of the SPI-binders analyzed in our previous study, the maximum force was in the same range of the SPI+GDL and SPI+TG+GDL binder, while the work of adhesion was more than twice as high as the highest value obtained with an SPI-binder. This can be attributed to covalent disulfide bond formation and hydrophobic interactions (Wang et al., 2017) that are formed during heat-induced gelation of gluten. This is in agreement with the conclusion of our

previous study that indicated a correlation between covalent bonds in the binder and an increase in the work of adhesion (Herz et al., 2021b).

*Physicochemical analysis.* The pH value of all samples of sausage analogues, induced by the addition of GDL, was pH 5.62 ± 0.03 (data not shown). Fermented meat sausages are usually acidified to a pH value of below 5.2 for microbiological safety (Feiner, 2016). However, pH-values in this range were perceived as too sour in preliminary sensory tests. The higher pH has been attributed to the different buffering capacities of plant proteins compared to pork meat (Ebert et al., 2021). The water activity was ~ 0.96 at 55% dry matter of the sausage analogues and decreased to ~ 0.95 at 60% dry matter and ~ 0.93 at 65% dry matter, however, not statistically significantly different. Dried salami usually have $a_w$-values around 0.90-0.88 to be shelf-stable at room temperature (Feiner, 2016). As both microbial growth hurdles, pH and $a_w$ value were insufficient to ensure a sufficient shelf life stability, a pasteurization step and cold storage were applied for microbial safety reasons. The protein content ranged from ~ 26% (7.5% binder) to ~ 32% (37.5% binder) at a dry matter content of 55%, and increased by ~ 2% upon drying to 65% dry matter content. The results of the physicochemical analysis are shown in detail in the supplementary material (**Table V.I**.4).

*Texture analysis of sausage analogues.* Since the results of the binder analysis indicated an increased adhesion with increased water content in the binder, a preliminary experiment with 15% gluten as binder and gluten:water ratios of 4:1, 2:1, 1:1 and 1:2 was performed to determine if the initial moisture content in the binder would lead to differences in the cohesiveness or other texture properties in the final product. Samples were dried to the same dry matter content of 55% and a texture and sensory analysis was performed (data not shown). Here, the results showed no difference in any of the texture properties. Therefore, we concluded that a difference in the initial water content of the binder does not result in a difference in texture properties of the dried product.

To analyze how the amount of gluten binder and the final dry matter content (i.e. the degree of drying) influenced the texture of plant-based salami-analogues, a texture-pressure analysis was performed and the parameters hardness, cohesiveness and springiness were assessed. First, we analyzed the hardness, measured as the force at 75% compression at the first cycle of uniaxial compression (**Figure V.I**.1**A**). At 55% dry matter content, the hardness values ranged from ~ 160 to 195 N and did not differ statistically significant with varying binder content. This was to be expected since the hardness of the gluten gel and the extrudate were in the same range.

An increased hardness of gluten might have resulted from the mechanical forces applied by mixing and filling, since it is known from bakery science that the application of mechanical forces during mixing or kneading can lead to the formation of a gluten network in the dough (Boitte et al., 2013). At 60% dry matter, the hardness values showed a slight trend towards increasing hardness, compared to 55% dry matter, with values in a range of ~ 205 – 290 N, but values were not statistically significant different, probably due to high standard deviations. These values are in the same range as the hardness values obtained for the system with SPI-binder at 55% dry matter (~ 193 – 250 N), which was investigated in a previous study (Herz et al., 2023). At 65% dry matter, the hardness increased significantly, compared to lower dry matter contents to ~ 394 – 525 N. Here, we observed a trend towards a decreasing hardness with increasing binder content, that was significantly different between the two lowest binder amounts of 5 and 10% (5% binder: 525.2 ± 192.0 N, 10% binder: 565.5 ± 148.8 N) and the highest binder amount of 25% (393.8 ± 172.0 N). This indicates that drying might lead to a higher increase in hardness of the extrudate particles than the gluten gel.

Next, we analyzed how the amount of binder and the final dry matter content influenced the springiness and cohesiveness, measured at 50% compression, of the sausage analogues (**Figure V.**I.1). Springiness increased with increasing binder content from 5.49 ± 0.58 mm at 7.5% gluten binder (gluten:water 2:1) to 5.98 ± 0.43 mm at 37.5% gluten binder (at 55% dry matter) (**Figure V.I**.1**C**). With increasing dry matter, the springiness did not change significantly. In comparison, sausage analogues made with 30 - 70% SPI-binder (15% SPI suspension, pH 5.5) in a previous study (Chapter IV) had springiness values ranging from ~ 4.9 to 6.0 mm at 40% dry matter and 5.6 to 6.1 mm at 55% dry matter. For the pure gluten binder, a springiness of ~ 7.5 mm was measured, while the SPI-binder (15% SPI, pH 5.5) had a springiness of 4.4 mm. In the previous study (Herz et al., 2023), we attributed this to the fact that the extrudate particles acted as fillers in the SPI-gel strengthening it synergistically, the results of this study might be attributed to the various binder contents in which gluten was applied. Distinctly lower ratios of gluten-binder (5-25%) are necessary to obtain the same elasticity compared to the SPI-binder (30-70%). This effect can be attributed to the unique elastic properties of gluten, which have been attributed to the "loop-and-train structure" of the high molecular weight (HMW) subunits of glutenin (Belton, 1999). For gluten, it has been suggested that with sufficient hydration, there are both unbound mobile regions ("loops") and inter- and intramolecular bound regions ("trains"). The loops can be extended by stretching and furthermore causing the trains to slide over each other. Elasticity is provided by the restoring force of the loop-train-equilibrium. The

gliadins form a matrix in which the HMW subunits are embedded and therefore also contribute to the visco-elastic properties of the system depending on the molecular interactions.

With increasing binder content, the cohesiveness increased significantly from 0.262 ± 0.023 at 5% binder to 0.499 ± 0.034 (at 55% dry matter). A significant increase can be seen between all samples from 5 to 10% binder and 20 to 25% binder. Between 10 and 20%, the increase in cohesiveness was not significant. Furthermore, an increase in dry matter did not influence the cohesiveness significantly. The cohesiveness values of the gluten binder samples were distinctly higher than those of the previously studied SPI-binder system where the cohesiveness ranged between ~ 0.21 and 0.3 (Herz et al., 2023). According to Kieffer (2007), the cohesiveness of gluten can be attributed to the formation of covalent disulfide bonds and a high number of hydrogen bonds and is therefore strongly connected to the hardness and elasticity of gluten. Furthermore, it should be noted that the analysis of cohesiveness in this complex composite material comprises the cohesiveness of the binder itself as well as the adhesiveness of the binder to comminuted extrudate and fat mimetic particles. In summary, an increase in binder content increased the cohesiveness and springiness of the sausage analogues, while drying did not influence these two texture properties. However, drying did increase the hardness, where the increase was more pronounced at lower binder contents than at higher binder contents.

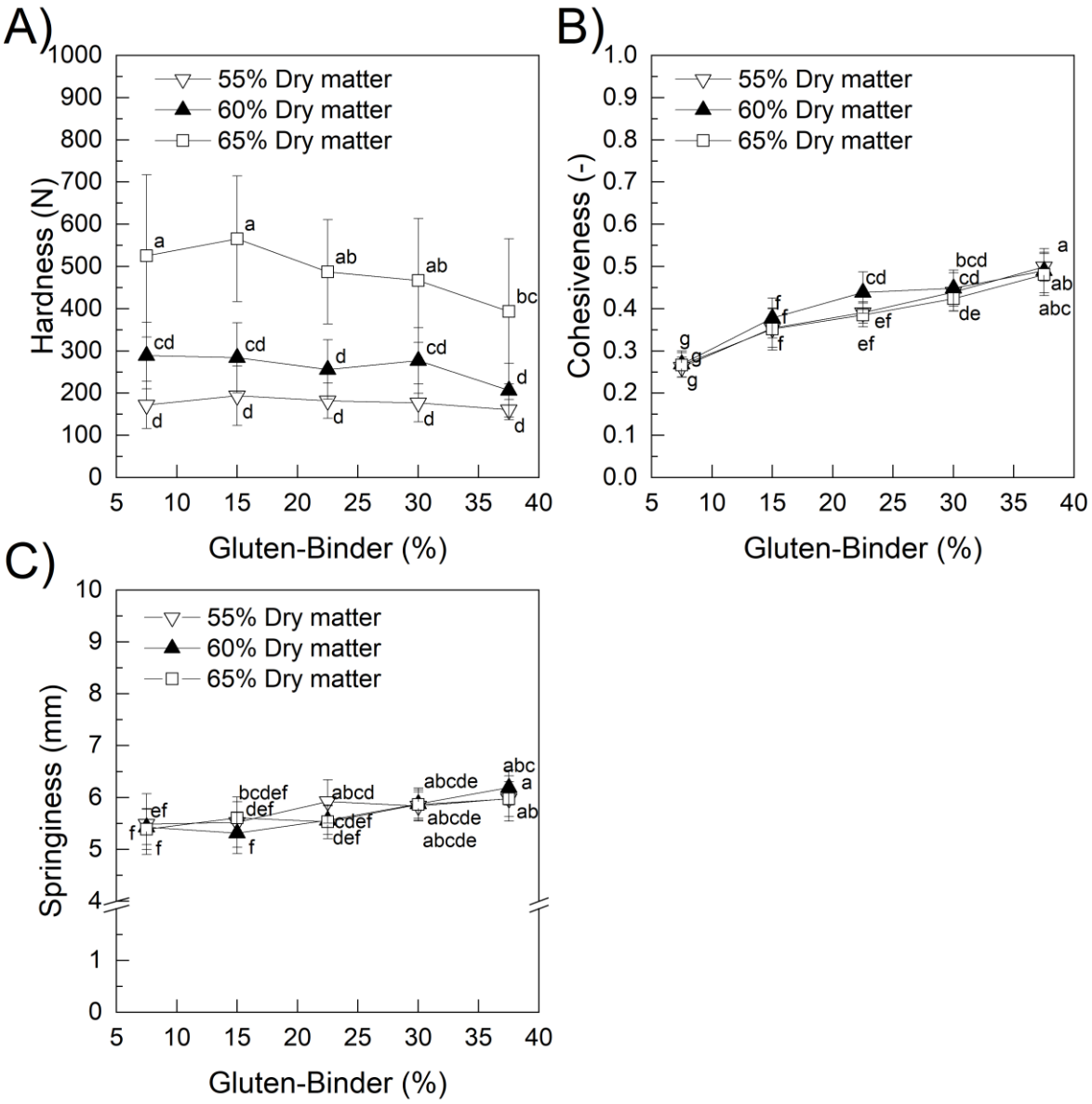


**Figure V.I.1:** Influence of binder content and drying on hardness (A), cohesiveness (B) and springiness (C), analyzed in a double uniaxial compression, of sausage analogues with 7.5 - 37.5% gluten binder (gluten:water 2:1), dried to dry matter content of 55-65%. Values followed by different superscripts are significantly different ($P < 0.05$).

*Sensory analysis of the sausage analogues.* The organoleptic characteristics of the salami analogues were analyzed in a sensory analysis with a trained panel on a 10-point relative-to-ideal hedonic scale and in comparison, to a commercial vegetarian reference product. Here, for optical appearance and taste, no significant differences were found (data not shown). For the attribute hardness, a slight increase with increasing binder content was perceived by the panelists and sausage analogues with 30 and 37.5% gluten binder were rated close to ideal and

significantly higher than samples with 7.5% binder (30% binder: 4.90 ± 1.60, 37.5% binder: 5.28 ± 1.68) (**Figure V.I**.2**A**). The values for cohesiveness increased significantly with increasing binder content from 2.27 ± 1.41 at 7.5% gluten binder, to 4.24 ± 1.10 at 22.55% gluten binder and to 5.43 ± 2.00 at 37.5% gluten binder on the just-about-right scale. A gluten binder content between 30 and 37.5% can therefore be considered to be perceived as ideal in the sensory analysis.

The dryness was ranked significantly higher with 7.5% gluten binder (6.94 ± 1.49 compared to the samples with higher gluten binder content, where the dryness was rated lower (5.95 ± 1.54 – 5.56 ± 1.21), showing that, despite the same dry matter content of the samples (55%), a difference in dryness was perceived. On the one hand, this could be attributed to the binder to extrudate ration and therefore indicate that the difference in dryness perception could have been caused by the high extrudate content. On the other hand, the same correlation of decreasing dryness with increasing cohesiveness was observed in our previously analyzed sausage analogues with SPI-binder, that were rated in dryness from ~ 6.6 with the lowest binder amount (30%) to ~ 5.1 with the highest binder amount (70%) at a dry matter content of 45% (Herz et al., 2023). According to ISO 5492:2008, dryness is a "surface textural attribute that describes the perception of water absorbed by or released from a product". To the best of our knowledge, dryness perception has hardly been studied and the relationship between texture and dryness perception remains to be systematically investigated. Here, we suggest a correlation between cohesiveness and dryness from results of the sensory analysis, as a low cohesiveness, that implies a high crumbliness, means a fast break up of the food material into smaller particles with an increase in interface between food and saliva that might therefore cause a fast absorption of saliva and leading to an increased dryness perception.

Compared to a commercial vegetarian salami analogue containing egg-white as binder, samples with 7.5 – 22.5% gluten binder were rated softer, while samples with 30 and 37.5% gluten binder were rated slightly harder (**Figure V.I**.2**B**). Concerning the cohesiveness, samples with 7.5 – 30% gluten binder were rated less cohesive than the vegetarian comparison sample, while the vegan analogue containing 37.5% gluten binder was rated slightly more cohesive. All samples were perceived as dryer than the commercial comparison sample. As such, a binder content between 30 and 37.5% gluten binder was identified as optimal in the sensory analysis, concerning hardness and cohesiveness. The texture properties of the samples in this binder range are similar to the properties of a commercial vegetarian salami analogue. Only the dryness

was rated higher compared to the commercial sample and dryer than ideal, but in a range of +1 (hedonic scale 0-10) above optimal. Our results therefore show, that gluten could be a suitable replacement for egg-white as binder to produce a plant-based sausage.

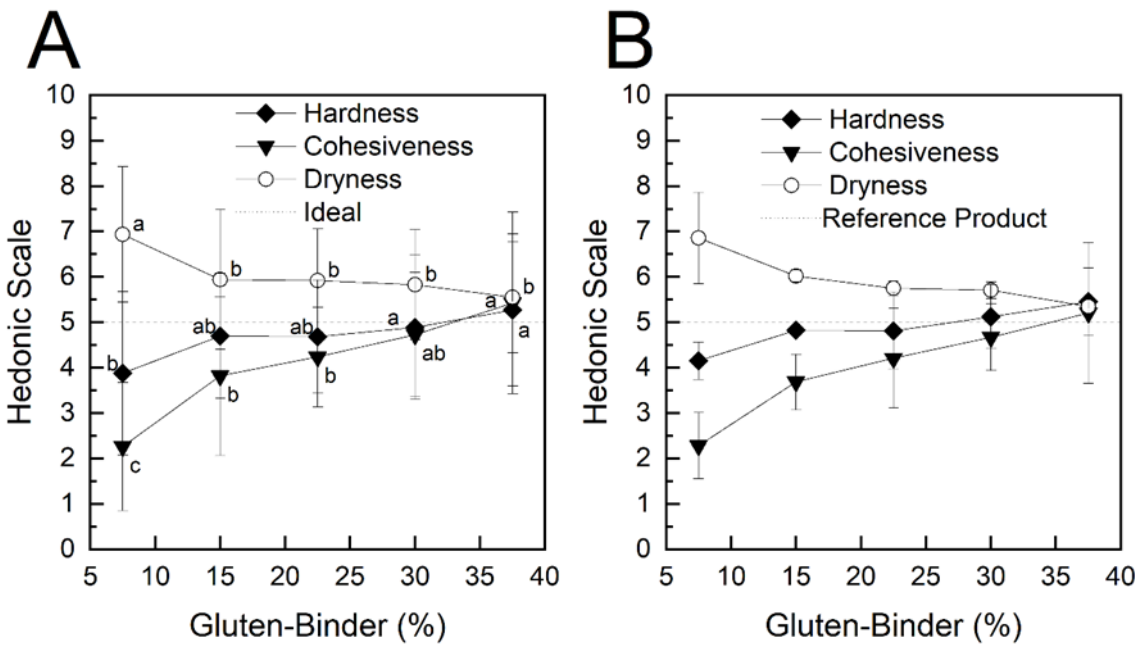


**Figure V.I.2:** Influence of binder content (7.5 - 37.5% gluten binder, gluten:water 2:1) on the sensorial properties hardness, cohesiveness and dryness of a plan-based salami-style sausage analogue at 55% dry matter, assessed by 20 trained panelists (A) on a relative-to-ideal hedonic scale (e.g. 0 = much too soft, 5 = optimal hardness, 10 = much too hard) and (B) compared to a commercial vegetarian reference product(e.g. 0 = much softer than reference, 5 = like reference, 10 = much harder than reference). Values followed by different superscripts are significantly different ($P < 0.05$).

Taken together, results of this study show that gluten was successfully applied as binder in a plant-based salami-type sausage analogue, providing close to ideal cohesiveness and hardness at 30 – 37.5% gluten binder and 55 – 47.5% extrudate after drying to ~ 55% dry matter. Previous texture analyses of a pure gluten gel in a compression and tensile test showed high values for hardness and work of adhesion and can therefore be recommended as an indicator test for the suitability of a material to be applied as binder in a complex meat analogue.

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## Declaration of Competing Interests

The authors declare no conflict of interest.

# Supporting Information

**Table V.I.3:** Dry matter contents of the raw sausage analogue batters with gluten binder (gluten:water 2:1).

| Gluten-Binder (%) | Dry Matter (%) |
|---|---|
| **7.5** | 41.97 ± 0.57 $^{d}$ |
| **15** | 44.23 ± 0.41 $^{cd}$ |
| **22.5** | 46.79 ± 1.06 $^{bc}$ |
| **30** | 49.15 ± 0.07 $^{b}$ |
| **37.5** | 52.22 ± 1.06 $^{a}$ |

*Note*: Values followed by different superscripts are significantly different ($P < 0.05$).

**Table V.I.4:** Water activity ($a_w$) and protein content of sausage analogues with 7.5 - 37.5% Gluten-Binder at dry matter stages of 55 - 65%.

| Binder (%) | | 55% dry matter | 60% dry matter | 65% dry matter |
|---|---|---|---|---|
| **7.5** | $a_w$ | 0.959 ± 0.011 $^{a}$ | 0.952 ± 0.018 $^{a}$ | 0.933 ± 0.014 $^{a}$ |
| | Protein (%) | 25.78 ± 0.07 $^{h}$ | 26.64 ± 0.44 $^{h}$ | 27.46 ± 0.52 $^{gh}$ |
| **15** | $a_w$ | 0.959 ± 0.013 $^{a}$ | 0.947 ± 0.014 $^{a}$ | 0.932 ± 0.001 $^{a}$ |
| | Protein (%) | 26.13 ± 0.29 $^{h}$ | 29.14 ± 0.87 $^{efg}$ | 29.92 ± 0.35 $^{def}$ |
| **22.5** | $a_w$ | 0.959 ± 0.008 $^{a}$ | 0.948 ± 0.010 $^{a}$ | 0.927 ± 0.000 $^{a}$ |
| | Protein (%) | 27.80 ± 1.26 $^{fgh}$ | 30.91 ± 0.11 $^{cde}$ | 31.82 ± 1.23 $^{bcd}$ |
| **30** | $a_w$ | 0.961 ± 0.011 $^{a}$ | 0.953 ± 0.012 $^{a}$ | 0.926 ± 0.008 $^{a}$ |
| | Protein (%) | 29.87 ± 0.33 $^{def}$ | 31.19 ± 1.12 $^{cde}$ | 33.92 ± 0.89 $^{ab}$ |
| **37.5** | $a_w$ | 0.961 ± 0.009 $^{a}$ | 0.953 ± 0.010 $^{a}$ | 0.929 ± 0.011 $^{a}$ |
| | Protein (%) | 32.15 ± 1.13 $^{bcd}$ | 32.49 ± 0.48 $^{abc}$ | 34.46 ± 0.70 $^{a}$ |

# Concluding Remarks and Outlook

## Conclusion

In this thesis, the approach of using plant protein gels, in particular soy protein and gluten, as binders in meat product analogs was investigated. The materials were chosen to mimic the processing and manufacture of meat products, where usually myofibrillar proteins are (partially) solubilized by comminution of meat and the addition of salt. Gelation of the solubilized proteins is later induced by heating or acidification and gives rise to the texture of processed meat products. It was hypothesized that plant protein suspensions can be used as binders for meat analogues, by providing a continuous phase in which comminuted fibrous plant protein particles and fat mimetics are embedded. After gelation of the suspensions a firm and sliceable product may be obtained. Since analogues of meat products in which the different phases are distinctly visible, such as bacon or acidic products, such as salami-style sausages, are still scarce in the market, investigations in binder behavior and properties might provide new approaches for design of such products.

In general, mechanistic approaches to heat-, salt-, acid- or enzyme-(TG)-induced combined gelation were initially of interest for binders in plant-based meat analogue products (**Chapter II & III**), as hardening by gelation was identified as a major characteristic for a binder in the literature review (**Chapter I**). Combined gelation by acid and TG was found to be particularly promising for the development of fermented plant-based meat product analogues (**Chapter III**). The results indicated that with a simultaneous addition of acid-forming glucono-δ-lactone and transglutaminase, cross-links can be catalyzed by TG, before the pH drops below the activity range of the enzyme, resulting in increased hardness of combined gels compared to only GDL-induced gels and similar hardness compared to TG-induced gels at the optimum pH of 7. Decreased tan δ values and images from CLSM supported these findings and indicated, that the formation of a cross-linked protein network was catalyzed by TG. CLSM images provided further insights on the microstructure of the gels, suggesting that after an initial covalent “scaffold” formation induced by TG, a slow acidification by GDL resulted in weakened electrostatic repulsion and agglomeration of non-crosslinked protein to the scaffold network. Hardness as well as syneresis can furthermore be modulated by pH and protein concentration. Furthermore, the influence of the addition of various salts as coagulants ($CaSO_4$, $FeSO_4$, $CaCl_2$, $MgCl_2$) to promote gelation in concentrated soy protein isolate (SPI) suspensions

(10-16% SPI w/w) was tested, but showed no significant increase in gel hardness (**Chapter II**). Furthermore, the binding strength of selected SPI-gels (induced by heating (85 °C) transglutaminase (TG)) when applied in various amounts between a layer of extrudate and a layer of fat mimetic as binder, was analyzed by pulling the composites apart and measuring the force and work of adhesion. The results proved that protein extrudate and fat mimetic could be adhered by a layer of SPI-gel in between, however the gel with covalent cross-links induced by transglutaminase was superior in terms of texture (**Chapter II**).

Moreover, plant-protein gels (SPI and gluten) were applied as binders in salami-type sausage analogues and there, the influence of the binder to extrudate ratio on the texture was studied by texture and sensory analysis. Results indicated that a process similar to traditional sausage production, e.g. comminution and mixing of the components, filling in casings, heating and drying, was suitable for the production of plant-based analogues. Furthermore, results showed that the binder type as well as the binder to extrudate ratio have a substantial influence on the texture of the sausage analogues, especially with respect to hardness and cohesiveness (**Chapter IV & V)**. With the aforementioned GDL-induced SPI-gel and the combined TG+GDL-SPI-gel (**Chapter II**) as binder, the hardness of the salami analogues increased with increasing extrudate content and decreasing binder content, while the cohesiveness changed contrarily (**Chapter IV)**. The addition of transglutaminase increased hardness and cohesiveness of undried sausage masses, but drying had an improving effect, especially on the hardness of the sausages. Results of this study offered on the one hand knowledge and insights into formulation- and process-based approach to modulated the texture of meat product analogues. On the other hand, as sufficient hardness and cohesiveness could not be reached in one formulation with the SPI-binder, the study hinted at the importance that adhesive properties of a binder may play. The latter has so far been neglected but is likely a key property of binders to enable the formation of a coherent mass that can be turned into a cohesive final product.

Therefore, hydrated gluten (gluten:water 2:1 ) was used as binder, as it is known for its adhesive and viscoelastic properties (**Chapter V)**. The gluten binder was applied in a binder to extrudate ratio of 7.5 – 37.5% in a similar sausage composition, employing the above-mentioned manufacturing process. With the gluten binder, it was possible to reach a consistent hardness and increasing cohesiveness and springiness with increasing binder content. Furthermore, the hardness was significantly increased by drying. The sensory analysis showed that close to ideal cohesiveness and hardness were obtained with 30 – 37.5% gluten binder and 55 – 47.5%

extrudate after drying to ~ 55% dry matter. With these formulations, similar texture properties compared to a commercial vegetarian salami analogue with egg white protein as binder were achieved, demonstrating the potential of gluten to replace animal-derived egg-protein as binder in meat product analogues. Furthermore, the results of a pre-trial, the texture analysis of the binder itself, as well as a tensile test between two layers of extrudate, analyzing the work of adhesion, showed good correlation with the texture properties of the final analogue product and can therefore serve as indicator test for future binder ingredients in meat product analogues.

Taken together the results of this thesis showed that, in general, plant protein gels such as soy protein isolate or gluten can be used as binders for composite meat analogue products, but have to fulfill certain key requirements: First, sufficient hardening of the binder has to be induced, which can be tested by measuring the gel hardness of the binder itself. Second, a sufficient binding strength between the binder and other particles of the system is needed, leading to sufficient cohesion. To that purpose, a tensile test was found to be useful as a validation method. The results furthermore indicated that covalent bond formation of the binder system is superior to physical bond formation and that gluten might be a suitable binder to replace animal-derived protein, such as egg-white powder. It might also serve as benchmark for other ingredients as binders in meat product analogues.

# Outlook

The results of this thesis provide new insights in the application of plant protein gels as binders for plant-protein-based meat analogues that are potentially of great interest for commercial application. Three patent applications that are currently pending have made use of this approach for a plant-based ground meat analogue (WO2021009043A1), a vegan bacon analogue (WO2021009075A1) and a vegan fermented peperoni or salami analogue product (WO2022038209A1). However, further considerations should be taken into account:

## Stickiness/Adhesiveness

The results of Chapter IV and V have shown that adhesive properties or stickiness is likely a key property of binders that has so far been neglected. It should therefore be studied in more detail in the future. A first study investigating the stickiness of a potential binder system for meat analogues, a concentrated pea protein - apple pectin mixture, as influenced by biopolymer concentration and pH was published by Moll et al. (2022d). The results showed high stickiness and a viscoelastic character at a concentration of 25 – 30% and pH 6. However, application tests should be performed to confirm the assumption. Furthermore, interaction between the binder and the contact material, such as extrudate or fat mimetic, will also be influenced by the latter due to different surface morphologies and nature of the materials, determining if, for example, covalent or physical bonds may be formed between binder and extrudate. Preliminary studies, where gluten has been applied as binder in sausage analogues with soy-, pea- and gluten-extrudate, showed different cohesiveness values with maximum cohesiveness between gluten-binder and gluten-extrudate (unpublished data). To follow this up, further studies are necessary to determine the binding differences in detail.

## Combination of binders

In this thesis, soy protein isolate and vital wheat gluten have only been studied as single components as binders, but a combination of these two or other proteins could further strengthen the binder network itself, increase binding strength as well as improve the water holding capacity. Preliminary experiments with a combination of SPI and gluten or pea protein isolate and gluten showed promising results (unpublished data), but also here, further studies are necessary.

## Temperature-dependent behavior of binders

In this thesis, the binders have been studied in a layered meat product analogue mimicking a bacon and a salami-type sausage analogue. Bacon is usually consumed in a fried state, which will alter the texture of the binder and the product. Salami is usually eaten in a cold state, but can for example also be consumed as a pizza topping. Depending on the application case, temperature-dependent behavior of the binder should therefore be taken in account and examined.

# References

Adhikari, B., Howes, T., Bhandari, B. R., & Truong, V. (2001). Stickiness in foods: A review of mechanisms and test methods. *International Journal of Food Properties, 4*(1), 1-33. https://doi.org/10.1081/jfp-100002186

Ahmed, J., Ptaszek, P., & Basu, S. (2016). *Advances in Food Rheology and its Applications*. Woodhead Publishing.

Amboon, W., Tulyathan, V., & Tattiyakul, J. (2012). Effect of hydroxypropyl methylcellulose on rheological properties, coating pickup, and oil content of rice flour-based batters. *Food Bioprocess Technology, 5*, 601-608. https://doi.org/10.1007/s11947-010-0327-3

Anand, S. S., Hawkes, C., de Souza, R. J., Mente, A., Dehghan, M., Nugent, R., Zulyniak, M. A., Weis, T., Bernstein, A. M., Krauss, R. M., Kromhout, D., Jenkins, D. J. A., Malik, V., Martinez-Gonzalez, M. A., Mozaffarian, D., Yusuf, S., Willett, W. C., & Popkin, B. M. (2015). Food consumption and its impact on cardiovascular disease: Importance of solutions focused on the globalized food system: A report from the workshop convened by the world heart federation. *Journal of the American College of Cardiology, 66*(14), 1590-1614. https://doi.org/https://doi.org/10.1016/j.jacc.2015.07.050

Andiç, S., Zorba, O., & Tuncturk, Y. (2010). Effect of whey powder, skim milk powder and their combination on yield and textural properties of meat patties. *International Journal of Agriculture and Biology, 12*(6), 871-876.

Anvari, M., Pan, C.-H., Yoon, W.-B., & Chung, D. (2015). Characterization of fish gelatin–gum arabic complex coacervates as influenced by phase separation temperature. *International Journal of Biological Macromolecules, 79*, 894-902. https://doi.org/10.1016/j.ijbiomac.2015.06.004

Apostolidis, C., & McLeay, F. (2016). Should we stop meating like this? Reducing meat consumption through substitution. *Food Policy, 65*, 74-89. https://doi.org/10.1016/j.foodpol.2016.11.002

Appleby, P. N., & Key, T. J. (2016). The long-term health of vegetarians and vegans. *Proceedings of the Nutrition Society, 75*(3), 287-293. https://doi.org/10.1017/S0029665115004334

Arora, B., Kamal, S., & Sharma, V. P. (2017). Effect of Binding Agents on Quality Characteristics of Mushroom Based Sausage Analogue. *Journal of Food Processing and Preservation, 41*(5). https://doi.org/10.1111/jfpp.13134

Atilgan, E., & Kilic, B. (2017). Effects of microbial transglutaminase, fibrimex and alginate on physicochemical properties of cooked ground meat with reduced salt level. *Journal of Food Science and Technology, 54*(2), 303-312. https://doi.org/10.1007/s13197-016-2463-x

Atilgan, E., & Kilic, B. (2017). Effects of microbial transglutaminase, fibrimex and alginate on physicochemical properties of cooked ground meat with reduced salt level. *J Food Sci Technol, 54*(2), 303-312. https://doi.org/10.1007/s13197-016-2463-x

Averina, E., Konnerth, J., D'Amico, S., & van Herwijnen, H. W. G. (2021). Protein adhesives: Alkaline hydrolysis of different crop proteins as modification for improved wood bonding performance. *Industrial Crops and Products, 161*. https://doi.org/10.1016/j.indcrop.2020.113187

Averina, E., Konnerth, J., & van Herwijnen, H. W. G. (2022). Protein-based glyoxal–polyethyleneimine-crosslinked adhesives for wood bonding. *The Journal of Adhesion*, 1-16. https://doi.org/10.1080/00218464.2021.2020111

Azeredo, H. M. C., & Waldron, K. W. (2016). Crosslinking in polysaccharide and protein films and coatings for food contact – A review. *Trends in Food Science & Technology, 52*, 109-122. https://doi.org/10.1016/j.tifs.2016.04.008

Baït, N., Derail, C., Benaboura, A., & Grassl, B. (2020). Rheology and adhesive properties versus structure of poly(acrylamide-co-hydroxyethyl methacrylate) hydrogels. *International Journal of Adhesion and Adhesives, 96*. https://doi.org/10.1016/j.ijadhadh.2019.102449

Bakhsh, A., Lee, S. J., Lee, E. Y., Sabikun, N., Hwang, Y. H., & Joo, S. T. (2021). A novel approach for tuning the physicochemical, textural, and sensory characteristics of plant-based meat analogs with different levels of methylcellulose concentration. *Foods, 10*(3). https://doi.org/10.3390/foods10030560

Baldan, A. (2012). Adhesion phenomena in bonded joints. *International Journal of Adhesion and Adhesives, 38*, 95-116. https://doi.org/10.1016/j.ijadhadh.2012.04.007

Baron, A., Rodriguez-Hernandez, J., Ibarboure, E., Derail, C., & Papon, E. (2009). Adhesives based on polyurethane graft multiblock copolymers: Tack, rheology and first morphological analyses. *International Journal of Adhesion and Adhesives, 29*(1), 1-8. https://doi.org/10.1016/j.ijadhadh.2007.02.008

Belton, P. S. (1999). Mini Review: On the elasticity of wheat gluten. *Journal of Cereal Science, 29*(2), 103-107. https://doi.org/10.1006/jcrs.1998.0227

Beniwal, A. S., Singh, J., Kaur, L., Hardacre, A., & Singh, H. (2021). Meat analogs: Protein restructuring during thermomechanical processing. *Comprehensive Reviews in Food Science and Food Safety, 20*(2), 1221-1249. https://doi.org/10.1111/1541-4337.12721

Bhandari, B., & Roos, Y. H. (2016). *Non-Equilibrium States and Glass Transitions in Foods: Processing Effects and Product-Specific Implications*. Elsevier Science.

Boitte, J. B., Hayert, M., & Michon, C. (2013). Observation of wheat flour doughs under mechanical treatment using confocal microscopy and classification of their microstructures. *Journal of Cereal Science, 58*(3), 365-371. https://doi.org/10.1016/j.jcs.2013.07.009

Boles, J. A., & Shand, P. J. (1998). Effect of comminution method and raw binder system in restructured beef. *Meat Science, 49*(3), 297-307. https://doi.org/10.1016/S0309-1740(97)00132-0

Boles, J. A., & Shand, P. J. (1999). Effects of raw binder system, meat cut and prior freezing on restructured beef. *Meat Science, 53*(4), 233-239. https://doi.org/10.1016/S0309-1740(99)00062-5

Boonyai, P., Bhandari, B., & Howes, T. (2004). Stickiness measurement techniques for food powders: a review. *Powder Technology, 145*(1), 34-46. https://doi.org/10.1016/j.powtec.2004.04.039

Bourne, M. (2002). *Food Texture and Viscosity: Concept and Measurement*. Elsevier.

Bourne, M. C. (1968). Texture profile of ripening pears. *Journal of Food Science, 33*(2), 223-226. https://doi.org/10.1111/j.1365-2621.1968.tb01354.x

Brandner, S., Kratky, T., Holtz, K., Becker, T., & Jekle, M. (2021). Controlling glass bead surface functionality - Impact on network formation in natural edible polymer systems. *Composites Science and Technology, 211*. https://doi.org/10.1016/j.compscitech.2021.108864

Brandt, M. A., Skinner, E. Z., & Coleman, J. A. (1963). Texture profile method. *Journal of Food Science, 28*(4), 404-409. https://doi.org/10.1111/j.1365-2621.1963.tb00218.x

Brenner, T., & Nishinari, K. (2014). A note on instrumental measures of adhesiveness and their correlation with sensory perception. *Journal of Texture Studies, 45*(1), 74-79. https://doi.org/10.1111/jtxs.12050

Brubaker, C. E., & Messersmith, P. B. (2012). Biological Adhesion. In *Polymer Science: A Comprehensive Reference* (pp. 211-229). https://doi.org/10.1016/b978-0-444-53349-4.00235-1

Bryan, N. S., Alexander, D. D., Coughlin, J. R., Milkowski, A. L., & Boffetta, P. (2012). Ingested nitrate and nitrite and stomach cancer risk: An updated review. *Food and Chemical Toxicology, 50*(10), 3646-3665. https://doi.org/https://doi.org/10.1016/j.fct.2012.07.062

Burke, J., & Hartel, R. W. (2021). Stickiness of sugar syrups with and without particles. *Journal of Food Engineering, 290*. https://doi.org/10.1016/j.jfoodeng.2020.110222

Campbell, L. J., Gu, X., Dewar, S. J., & Euston, S. R. (2009). Effects of heat treatment and glucono-δ-lactone-induced acidification on characteristics of soy protein isolate. *Food Hydrocolloids, 23*(2), 344-351. https://doi.org/10.1016/j.foodhyd.2008.03.004

Chen, C. M., & Trout, G. R. (1991). Sensory, instrumental texture profile and cooking properties of restructured beef steaks made with various binders. *Journal of Food Science, 56*(6), 1457-1460. https://doi.org/10.1111/j.1365-2621.1991.tb08615.x

Chen, W. Z., & Hoseney, R. C. (1995). Development of an objective method for dough stickiness. *LWT - Food Science and Technology, 473*, 467-473. https://doi.org/10.1006/fstl.1995.0079

Chen, X., Tume, R. K., Xu, X., & Zhou, G. (2017). Solubilization of myofibrillar proteins in water or low ionic strength media: Classical techniques, basic principles, and novel functionalities. *Critical Reviews in Food Science and Nutrition, 57*(15), 3260-3280. https://doi.org/10.1080/10408398.2015.1110111

Chen, X. D., & Özkan, N. (2007). Stickiness, functionality, and microstructure of food powders. *Drying Technology, 25*(6), 959-969. https://doi.org/10.1080/07373930701397400

Cornet, S. H. V., Edwards, D., van der Goot, A. J., & van der Sman, R. G. M. (2020). Water release kinetics from soy protein gels and meat analogues as studied with confined compression. *Innovative Food Science & Emerging Technologies, 66*, 102528. https://doi.org/10.1016/j.ifset.2020.102528

Cunningham, F. E., & Tiede, L. M. (1981). Influence of batter viscosity on breading of chicken drumsticks. *Journal of Food Science, 46*(1950). https://doi.org/10.1111/j.1365-2621.1981.tb04527.x

Curtain, F., & Grafenauer, S. (2019). Plant-based meat substitutes in the flexitarian age: An audit of products on supermarket shelves. *Nutrients, 11*(11), 2603. https://doi.org/10.3390/nu11112603

Damodaran, S. (2017). *Food Proteins and Their Applications*. Routledge.

de Bakker, E., & Dagevos, H. (2012). Reducing meat consumption in today's consumer society: Questioning the citizen-consumer gap. *Journal of Agricultural and Environmental Ethics, 25*(6), 877-894. https://doi.org/10.1007/s10806-011-9345-z

de Kruif, C. G. (1997). Skim milk acidification. *Journal of Colloid and Interface Science, 185*(1), 19-25. https://doi.org/10.1006/jcis.1996.4548

Dekkers, B. L., Boom, R. M., & van der Goot, A. J. (2018). Structuring processes for meat analogues. *Trends in Food Science & Technology, 81*, 25-36. https://doi.org/10.1016/j.tifs.2018.08.011

Dekkers, B. L., Boom, R. M., & van der Goot, A. J. (2018). Viscoelastic properties of soy protein isolate - pectin blends: Richer than those of a simple composite material. *Food Research International, 107*, 281-288. https://doi.org/10.1016/j.foodres.2018.02.037

DeMan, J., DeMan, L., & Gupta, S. (1986). Texture and microstructure of soybean curd (tofu) as affected by different coagulants. *Food structure, 5*(1), 11.

Desbrières, J., Hirrien, M., & Ross-Murphy, S. B. (2000). Thermogelation of methylcellulose: Rheological considerations. *Polymer, 41*(7), 2451-2461. https://doi.org/10.1016/S0032-3861(99)00413-9

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science, 74*(1), 188-196. https://doi.org/10.1016/j.meatsci.2006.04.014

Devine, C., & Jensen, W. K. (2004). *Encyclopedia of Meat Sciences*. Academic Press.

Dhaliwal, A. S., Mares, D. J., & Marshall, D. R. (1990). Measurement of dough surface stickiness associated with the 1B/1R chromosome translocation in bread wheats. *Journal of Cereal Science, 12*(2), 165-175. https://doi.org/10.1016/s0733-5210(09)80098-2

Dickinson, E., & Yamamoto, Y. (1996). Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. *Journal of Agricultural and Food Chemistry, 44*(6), 1371-1377. https://doi.org/10.1021/jf950705y

Dobraszczyk, B. J. (1997). The rheological basis of dough stickiness. *Journal of Texture Studies, 28*(2), 139-162. https://doi.org/10.1111/j.1745-4603.1997.tb00108.x

Dong, S., Feng, S., Liu, F., Li, R., Li, W., Liu, F., Shi, G., Chen, L., & Zhang, Y. (2021). Factors influencing the adhesive behavior of carboxymethyl cellulose-based hydrogel for food applications. *Int J Biol Macromol, 179*, 398-406. https://doi.org/10.1016/j.ijbiomac.2021.03.027

Dooling, L. J., Buck, M. E., Zhang, W.-B., & Tirrell, D. A. (2016). Programming molecular association and viscoelastic behavior in protein networks. *Advanced Materials, 28*(23), 4651-4657. https://doi.org/10.1002/adma.201506216

Dreher, J., Blach, C., Terjung, N., Gibis, M., & Weiss, J. (2020a). Formation and characterization of plant-based emulsified and crosslinked fat crystal networks to mimic animal fat tissue. *Journal of Food Science, 85*(2), 421-431. https://doi.org/10.1111/1750-3841.14993

Dreher, J., Blach, C., Terjung, N., Gibis, M., & Weiss, J. (2020b). Influence of protein content on plant-based emulsified and crosslinked fat crystal networks to mimic animal fat tissue. *Food Hydrocolloids, 106*, 105864. https://doi.org/10.1016/j.foodhyd.2020.105864

Dreher, J., König, M., Herrmann, K., Terjung, N., Gibis, M., & Weiss, J. (2021). Varying the amount of solid fat in animal fat mimetics for plant-based salami analogues influences texture, appearance and sensory characteristics. *LWT, 143*, Article 111140. https://doi.org/10.1016/j.lwt.2021.111140

Duncan, B., Abbot, S., & Roberts, R. (1999). *Adhesive Tack* (Measurement Good Practice Guide, Issue No. 26).

Ebert, S., Baune, M.-C., Broucke, K., Royen, G. V., Terjung, N., Gibis, M., & Weiss, J. (2021). Buffering capacity of wet texturized plant proteins in comparison to pork meat. *Food Research International, 150*, 110803. https://doi.org/10.1016/j.foodres.2021.110803

Eissa, A. S., Bisram, S., & Khan, S. A. (2004). Polymerization and gelation of whey protein isolates at low pH using transglutaminase enzyme. *Journal of Agricultural and Food Chemistry, 52*(14), 4456-4464. https://doi.org/10.1021/jf0355304

Færgemand, M., & Qvist, K. B. (1997). Transglutaminase: effect on rheological properties, microstructure and permeability of set style acid skim milk gel. *Food Hydrocolloids, 11*(3), 287-292. https://doi.org/10.1016/S0268-005X(97)80058-6

FAO. (2020). *In Brief The State of Food and Agriculture 2020. Overcoming water challenges in agriculture.* Fao.

Feiner, G. (2016). *Salami: Practical Science and Processing Technology*. Academic Press.

Feldstein, M. M., Dormidontova, E. E., & Khokhlov, A. R. (2015). Pressure sensitive adhesives based on interpolymer complexes. *Progress in Polymer Science, 42*, 79-153. https://doi.org/10.1016/j.progpolymsci.2014.10.006

Ferry, J. D. (1948). Protein Gels. In Anson, M. L. & Edsall, J. T. (Eds.), *Advances in Protein Chemistry* (Vol. 4, pp. 1-78). Academic Press. https://doi.org/10.1016/S0065-3233(08)60004-2

Fiszman, S. M., & Damásio, M. H. (2000). Instrumental measurement of adhesiveness in solid and semi-solid foods. A survey. *Journal of Texture Studies, 31*(1), 69-91.

Foegeding, E. A., Bowland, E. L., & Hardin, C. C. (1995). Factors that determine the fracture properties and microstructure of globular protein gels. *Food Hydrocolloids*, *9*(4), 237-249. https://doi.org/10.1016/S0268-005X(09)80254-3

Foegeding, E. A. (2007). Rheology and sensory texture of biopolymer gels. *Current Opinion in Colloid & Interface Science, 12*(4), 242-250. https://doi.org/10.1016/j.cocis.2007.07.001

Foley, K., & Chu, S. (1986). Rheological characterization of elastomer latexes for PSAs. *Adhesives Age, 29*(10), 24-28.

Fox, P. F., McSweeney, P. L., & Paul, L. (1998). *Dairy Chemistry and Biochemistry*. Springer. https://doi.org/10.1007/978-3-319-14892-2

Frabetti, A. C. C., de Moraes, J. O., Jury, V., Boillereaux, L., & Laurindo, J. B. (2021). Adhesion of food on surfaces: Theory, measurements, and main trends to reduce it prior to industrial drying. *Food Engineering Reviews*. https://doi.org/10.1007/s12393-021-09286-9

Fukazawa, T., Hashimoto, Y., & Yasui, T. (1961). Effect of some proteins on the binding quality of an experimental sausage. *Journal of Food Science, 26*(5), 541-549. https://doi.org/10.1111/j.1365-2621.1961.tb00403.x

Furukawa, T., Ohta, S., & Yamamoto, A. (1980). Texture-Structure relationships in heat-induced soy protein gels. *Journal of Texture Studies, 10*(4), 333-346. https://doi.org/10.1111/j.1745-4603.1980.tb00864.x

Ghorbel, D., Launay, B., & Heyd, B. (2003). Improved method for measuring wheat flour dough pressure-sensitive adhesiveness. *Cereal Chemistry, 80*(6), 732-739. https://doi.org/10.1094/CCHEM.2003.80.6.732

Ghorbel, D., & Launay, B. (2014). An investigation into the nature of wheat flour dough adhesive behaviour. *Food Res Int, 64*, 305-313. https://doi.org/10.1016/j.foodres.2014.06.045

Glicerina, V., Balestra, F., Dalla Rosa, M., & Romani, S. (2016). Microstructural and rheological characteristics of dark, milk and white chocolate: A comparative study. *Journal of Food Engineering, 169*, 165-171. https://doi.org/10.1016/j.jfoodeng.2015.08.011

Gordon, A., Barbut, S., & Schmidt, G. (1992). Mechanisms of meat batter stabilization: A review. *Critical Reviews in Food Science and Nutrition, 32*(4), 299-332. https://doi.org/10.1080/10408399209527602

Grausgruber, H., Hatzenbichler, E., & Ruckenbauer, P. (2003). Analysis of repeated stickiness measures of wheat dough using a texture analyzer. *Journal of Texture Studies, 34*(1), 69-82. https://doi.org/10.1111/j.1745-4603.2003.tb01056.x

Gravelle, A. J., Nicholson, R. A., Barbut, S., & Marangoni, A. G. (2019). Considerations for readdressing theoretical descriptions of particle-reinforced composite food gels. *Food Research International, 122*, 209-221. https://doi.org/10.1016/j.foodres.2019.03.070

Grossmann, J. B. (2003). *Looking at Greek and Roman sculpture in stone: a guide to terms, styles, and techniques*. Getty Publications.

Grygorczyk, A., & Corredig, M. (2013). Acid induced gelation of soymilk, comparison between gels prepared with lactic acid bacteria and glucono-δ-lactone. *Food Chemistry, 141*(3), 1716-1721. https://doi.org/10.1016/j.foodchem.2013.03.096

Gu, X., Campbell, L. J., & Euston, S. R. (2009). Influence of sugars on the characteristics of glucono-δ-lactone-induced soy protein isolate gels. *Food Hydrocolloids, 23*(2), 314-326. https://doi.org/10.1016/j.foodhyd.2008.01.005

Hager, D. F. (1980). *Meat analog binder composition and use thereof in making shaped protein food products* 80201215.3).

Hamm, R. (1986). Functional Properties of the Myofibrillar System and Their Measurements. In Bechtel, P. J. (Ed.), *Muscle As Food* (pp. 135-199). Academic Press.

Hartel, R. W., Ergun, R., & Vogel, S. (2011). Phase/state transitions of confectionery sweeteners: Thermodynamic and kinetic aspects. *Comprehensive Reviews in Food Science and Food Safety, 10*(1), 17-32. https://doi.org/10.1111/j.1541-4337.2010.00136.x

Heddleson, S. S., Hamann, D. D., Lineback, D. R., & Slade, S. (1994). Pressure-sensitive adhesive properties of wheat flour dough and the influence of temperature, separation rate and moisture content. *Cereal Chemistry, 71*.

Hermansson, A. M. (1986). Soy protein gelation. *Journal of the American Oil Chemists Society, 63*(5), 658-666. https://doi.org/10.1007/bf02638232

Herz, E., Schäfer, S., Terjung, N., Gibis, M., & Weiss, J. (2021a). Influence of transglutaminase on glucono-δ-lactone-induced soy protein gels. *ACS Food Science & Technology, 1*(8), 1412-1417. https://doi.org/10.1021/acsfoodscitech.1c00102

Herz, E., Herz, L., Dreher, J., Gibis, M., Ray, J., Pibarot, P., Schmitt, C., & Weiss, J. (2021b). Influencing factors on the ability to assemble a complex meat analogue using a soy-protein-binder. *Innovative Food Science & Emerging Technologies, 73*, 102806. https://doi.org/10.1016/j.ifset.2021.102806

Herz, E., Kinne, T., Terjung, N., Gibis, M., & Weiss, J. (2023). Influence of extrudate to SPI-gel-binder ratios and transglutaminase crosslinking on texture of a plant-based salami analogue. *Future Foods, 7*, 100235. https://doi.org/https://doi.org/10.1016/j.fufo.2023.100235

Hilbig, J., Hildebrandt, L., Herrmann, K., Weiss, J., & Loeffler, M. (2020). Influence of homopolysaccharide-producing lactic acid bacteria on the spreadability of raw fermented sausages (onion mettwurst). *Journal of Food Science, 85*(2), 289-297. https://doi.org/10.1111/1750-3841.15010

Hoek, A. C., Luning, P. A., Weijzen, P., Engels, W., Kok, F. J., & de Graaf, C. (2011). Replacement of meat by meat substitutes. A survey on person- and product-related factors in consumer acceptance. *Appetite, 56*(3), 662-673. https://doi.org/10.1016/j.appet.2011.02.001

Hoseney, R. C., & Smewing, J. (1999). Instrumental measurement of stickiness of doughs and other foods. *Journal of Texture Studies, 30*(2), 123-136. https://doi.org/10.1111/j.1745-4603.1999.tb00206.x

Jannin, V., Musakhanian, J., & Marchaud, D. (2008). Approaches for the development of solid and semi-solid lipid-based formulations. *Advanced Drug Delivery Reviews, 60*(6), 734-746. https://doi.org/10.1016/j.addr.2007.09.006

Jeske, S., Zannini, E., & Arendt, E. K. (2018). Past, present and future: The strength of plant-based dairy substitutes based on gluten-free raw materials. *Food Research International, 110*, 42-51. https://doi.org/10.1016/j.foodres.2017.03.045

Kasapis, S. (2004). Definition of a mechanical glass transition temperature for dehydrated foods. *Journal of Agricultural and Food Chemistry, 52*(8), 2262-2268. https://doi.org/10.1021/jf030468w

Katsaras, K., & Budras, K.-D. (1992). Microstructure of fermented sausage. *Meat Science, 31*(2), 121-134. https://doi.org/10.1016/0309-1740(92)90032-Y

Kaur, S., Weerasekare, G. M., & Stewart, R. J. (2011). Multiphase adhesive coacervates inspired by the sandcastle worm. *ACS Applied Materials & Interfaces, 3*(4), 941-944. https://doi.org/10.1021/am200082v

Kazemeini, S. M., Campos, D. P., & Rosenthal, A. J. (2021). Muscle activity during oral processing of sticky-cohesive foods. *Physiol Behav, 242*, 113580. https://doi.org/10.1016/j.physbeh.2021.113580

Kieffer, R. (2007). The role of gluten elasticity in the baking quality of wheat

Kilcast, D., & Roberts, C. (1998). Perception and measurement of stickiness in sugar-rich foods. *Journal of Texture Studies, 29*(1), 81-100. https://doi.org/10.1111/j.1745-4603.1998.tb00155.x

Kinsella, J. E., & Melachouris, N. (1976). Functional properties of proteins in foods: A survey. *C R C Critical Reviews in Food Science and Nutrition, 7*(3), 219-280. https://doi.org/10.1080/10408397609527208

Knipe, C. L. (2014). Sausages, Types of | Emulsion. In *Encyclopedia of Meat Sciences* (pp. 256-260). https://doi.org/10.1016/b978-0-12-384731-7.00143-4

Kohyama, K., & Nishinari, K. (1992). The effect of glucono-δ-lactone on the gelation time of soybean 11S protein: concentration dependence. *Food Hydrocolloids, 6*(3), 263-274. https://doi.org/10.1016/S0268-005X(09)80094-5

Kohyama, K., Sano, Y., & Doi, E. (1995). Rheological characteristics and gelation mechanism of tofu (soybean curd). *Journal of Agricultural and Food Chemistry, 43*(7), 1808-1812. https://doi.org/10.1021/jf00055a011

Kristensen, L., & Purslow, P. P. (2001). The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. *Meat Science, 58*(1), 17-23. https://doi.org/10.1016/S0309-1740(00)00125-X

Kumar, S. (2016). Meat analogs "Plant based alternatives to meat products: Their production technology and applications". *Critical Reviews in Food Science and Nutrition*, 00-00. https://doi.org/10.1080/10408398.2016.1196162

Kuraishi, C., Sakamoto, J., Yamazaki, K., Susa, Y., Kuhara, C., & Soeda, T. (1997). Production of restructured meat using microbial transglutaminase without salt or cooking. *Journal of Food Science, 62*(3), 488-490. https://doi.org/10.1111/j.1365-2621.1997.tb04412.x

Kyriakopoulou, K., Keppler, J. K., & van der Goot, A. J. (2021). Functionality of ingredients and additives in plant-based meat analogues. *Foods, 10*(3), Article 600. https://doi.org/10.3390/foods10030600

Lakemond, C. M. M., de Jongh, H. H. J., Paques, M., van Vliet, T., Gruppen, H., & Voragen, A. G. J. (2003). Gelation of soy glycinin; Influence of pH and ionic strength on network structure in relation to protein conformation. *Food Hydrocolloids, 17*(3), 365-377. https://doi.org/10.1016/S0268-005X(02)00100-5

Laricheva, K., & Mikhailova, O. (2020). Development of scientifically-based recipe and technology for the production of natural honey-based muesli bar. *IOP Conference Series: Earth and Environmental Science, 613*(1). https://doi.org/10.1088/1755-1315/613/1/012067

Laukemper, R., Becker, T., & Jekle, M. (2021). Surface energy of food contact materials and its relation to wheat dough adhesion. *Food and Bioprocess Technology*. https://doi.org/10.1007/s11947-021-02625-y

Lawrie, R. A. (2006). The Eating Quality of Meat. In Lawrie, R. A. (Ed.), *Lawrie's Meat Science (Seventh Edition)* (pp. 279-341). Woodhead Publishing. https://doi.org/https://doi.org/10.1533/9781845691615.279

Lee, E. J., & Hong, G. P. (2020). Effects of microbial transglutaminase and alginate on the water-binding, textural and oil absorption properties of soy patties. *Food Sci Biotechnol, 29*(6), 777-782. https://doi.org/10.1007/s10068-019-00713-6

Lee, H. G., & Yoo, S. (2020). Changes in the physical properties of frozen cooked rice depending on thermal insulation levels of packaging during freeze-thaw. *J Food Sci, 85*(12), 4342-4350. https://doi.org/10.1111/1750-3841.15524

Lennon, A. M., McDonald, K., Moon, S. S., Ward, P., & Kenny, T. A. (2010). Performance of cold-set binding agents in re-formed beef steaks. *Meat Science, 85*(4), 620-624. https://doi.org/10.1016/j.meatsci.2010.03.014

Lerner, A., Ramesh, A., & Matthias, T. (2020). The temperature and pH repertoire of the transglutaminase family is expanding. *FEBS Open Bio, 10*(4), 492-494. https://doi.org/10.1002/2211-5463.12839

Levine, H., & Slade, L. (1990). Influences of the glassy and rubbery states on the thermal, mechanical, and structural properties of doughs and baked products. *Dough Rheology and Baked Product Texture*(January 1990), 157-330. https://doi.org/10.1007/978-1-4613-0861-4_5

Li, L., Thangamathesvaran, P. M., Yue, C. Y., Tam, K. C., Hu, X., & Lam, Y. C. (2001). Gel network structure of methylcellulose in water. *Langmuir, 17*(26), 8062-8068. https://doi.org/10.1021/la010917r

Lin, S., Huff, H. E., & Hsieh, F. (2000). Texture and chemical characteristics of soy protein meat analog extruded at high moisture. *Journal of Food Science, 65*(2), 264-269. https://doi.org/10.1111/j.1365-2621.2000.tb15991.x

Liu, K., & Hsieh, F.-H. (2008). Protein–protein interactions during high-moisture extrusion for fibrous meat analogues and comparison of protein solubility methods using different solvent systems. *Journal of Agricultural and Food Chemistry, 56*(8), 2681-2687. https://doi.org/10.1021/jf073343q

Liu, W., Lanier, T. C., & Osborne, J. A. (2016). Capillarity proposed as the predominant mechanism of water and fat stabilization in cooked comminuted meat batters. *Meat Science, 111*, 67-77. https://doi.org/10.1016/j.meatsci.2015.08.018

Liu, Y.-X., Cao, M.-J., & Liu, G.-M. (2019). Texture Analyzers for Food Quality Evaluation. In Zhong, J. & Wang, X. (Eds.), *Evaluation Technologies for Food Quality* (pp. 441-463). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-12-814217-2.00017-2

Lu, G. H., & Chen, T. C. (1999). Application of egg white and plasma powders as muscle food binding agents. *Journal of Food Engineering, 42*, 147-151.

Lu, G. H., & Chen, T. C. (1999). Application of egg white and plasma powders as muscle food binding agents. *Journal of Food Engineering, 42*(3), 147-151. https://doi.org/10.1016/S0260-8774(99)00112-0

Lucey, J. A., & Singh, H. (1997). Formation and physical properties of acid milk gels: A review. *Food Research International, 30*(7), 529-542. https://doi.org/10.1016/S0963-9969(98)00015-5

Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids, 15*(4), 603-608. https://doi.org/10.1016/S0268-005X(01)00043-1

Macfarlane, J., McKenzie, I., Turner, R., & Jones, P. (1984). Binding of comminuted meat: Effect of high pressure. *Meat Science, 10*(4), 307-320.

Macfarlane, J. J., Schmidt, G. R., & Turner, R. H. (1977). Binding of meat pieces: A comparison of myosin, actomyosin and sarcoplasmic proteins as binding agents. *Journal of Food Science, 42*(6), 1603-1605. https://doi.org/10.1111/j.1365-2621.1977.tb08437.x

Mahato, S., Zhu, Z., & Sun, D.-W. (2019). Glass transitions as affected by food compositions and by conventional and novel freezing technologies: A review. *Trends in Food Science & Technology*, *94*, 1-11. https://doi.org/https://doi.org/10.1016/j.tifs.2019.09.010

Maltais, A., Remondetto, G. E., Gonzalez, R., & Subirade, M. (2005). Formation of soy protein isolate cold-set gels: Protein and salt effects. *Journal of Food Science, 70*(1), C67-C73. https://doi.org/10.1111/j.1365-2621.2005.tb09023.x

Mariotti, F., Tomé, D., & Mirand, P. P. (2008). Converting nitrogen into protein - beyond 6.25 and Jones' factors. *Crit Rev Food Sci Nutr, 48*(2), 177-184. https://doi.org/10.1080/10408390701279749

McClements, D. J., & Grossmann, L. (2021). The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 4049-4100. https://doi.org/10.1111/1541-4337.12771

Means, W. J., & Schmidt, G. R. (1986). Algin/calcium gel as a raw and cooked binder in structured beef steaks *Journal of Food Science, 51*, 60-65. https://doi.org/10.1111/j.1365-2621.1986.tb10836.x

Meilgaard, M. C., Carr, B. T., & Civille, G. V. (1999). *Sensory Evaluation Techniques* (Third Edition ed.). Taylor & Francis.

Melati, J., Lucchetta, L., Prado, N. V. d., Oliveira, D. F. d., & Tonial, I. B. (2021). Physical and sensory characteristics of salty cereal bar with different binding agents. *Food Science and Technology, 41*(suppl 1), 150-154. https://doi.org/10.1590/fst.07820

Mezger, T. G. (2006). *The Rheology Handbook: For Users of Rotational and Oscillatory Rheometers*. Vincentz Network.

Micard, V., & Thibault, J. F. (1999). Oxidative gelation of sugar-beet pectins: Use of laccases and hydration properties of the cross-linked pectins. *Carbohydrate Polymers, 39*(3), 265-273. https://doi.org/10.1016/S0144-8617(99)00012-0

Michalski, M. C., Desobry, S., & Hardy, J. (1997). Food materials adhesion: A review. *Critical Reviews in Food Science and Nutrition*, *37*(7), 591-619. https://doi.org/10.1080/10408399709527791

Mine, Y. (2014). Egg Proteins. In *Applied Food Protein Chemistry* (pp. 459-490). John Wiley & Sons. https://doi.org/10.1016/S0065-3233(08)60504-5

Mitchell, J. R., Buckley, K., & Burrows, I. E. (1976). *Gelling and thickening agents* (USA Patent No. 3,982,003). Office, U. S. P.

Mitchell, J. R. (1980). The rheology of gels. *Journal of Texture Studies*, *11*(4), 315-337. https://doi.org/10.1111/j.1745-4603.1980.tb01312.x

Moll, P., Salminen, H., Schmitt, C., & Weiss, J. (2021). Impact of microfluidization on colloidal properties of insoluble pea protein fractions. *European Food Research and Technology*, *247*(3), 545-554. https://doi.org/10.1007/s00217-020-03629-2

Moll, P., Salminen, H., Rausch, A., Schmitt, C., & Weiss, J. (2022a). Adjusting the stickiness of concentrated pea protein – apple pectin systems via the biopolymer mixing ratio. *Future Foods*, *6*, 100184. https://doi.org/https://doi.org/10.1016/j.fufo.2022.100184

Moll, P., Salminen, H., Seitz, O., Schmitt, C., & Weiss, J. (2022b). Characterization of soluble and insoluble fractions obtained from a commercial pea protein isolate. *Journal of Dispersion Science and Technology*, 1-12. https://doi.org/10.1080/01932691.2022.2093214

Moll, P., Salminen, H., Roeth, C., Schmitt, C., & Weiss, J. (2022c). Concentrated pea protein – apple pectin mixtures as food glue: Influence of biopolymer concentration and pH on stickiness. *Food Hydrocolloids*, *130*. https://doi.org/10.1016/j.foodhyd.2022.107671

Moll, P., Salminen, H., Roeth, C., Schmitt, C., & Weiss, J. (2022d). Concentrated pea protein – apple pectin mixtures as food glue: Influence of biopolymer concentration and pH on stickiness. *Food Hydrocolloids*, *130*, 107671. https://doi.org/10.1016/j.foodhyd.2022.107671

Moll, P., Salminen, H., Stadtmueller, L., Schmitt, C., & Weiss, J. (2023a). Comparison of Binding Properties of a Laccase-Treated Pea Protein–Sugar Beet Pectin Mixture with Methylcellulose in a Bacon-Type Meat Analogue. *Foods*, *12*(1), 85.

Moll, P., Salminen, H., Schmitt, C., & Weiss, J. (2023b). Pea protein–sugar beet pectin binders can provide cohesiveness in burger type meat analogues. *European Food Research and Technology*, *249*(4), 1089-1096. https://doi.org/10.1007/s00217-022-04199-1

Moll, P., Salminen, H., Stadtmüller, L., Schmitt, C., & Weiss, J. (2023c). Solidification of concentrated pea protein–pectin mixtures as potential binder. *Journal of the Science of Food and Agriculture*, *103*(8), 4195-4202. https://doi.org/https://doi.org/10.1002/jsfa.12448

Morel, M.-H., Pincemaille, J., Chauveau, E., Louhichi, A., Violleau, F., Menut, P., Ramos, L., & Banc, A. (2020). Insight into gluten structure in a mild chaotropic solvent by asymmetrical flow field-flow fractionation (AsFlFFF) and evidence of non-covalent assemblies between glutenin and ω-gliadin. *Food Hydrocolloids, 103*, 105676. https://doi.org/10.1016/j.foodhyd.2020.105676

Mühle, R. (2020). Rügenwalder Mühle. https://www.ruegenwalder.de/produkte/vegetarischer-schinken-spicker-mortadella

Mullen, J. D., Smith, D. E., & Alide, O. (1971). *Process for making soy protein more heat coagulable in combination with egg white and compositions containing same* (USA Patent No. 3,594,192). Office, U. S. P.

Murekatete, N., Hua, Y., Chamba, M. V. M., Djakpo, O., & Zhang, C. (2014). Gelation behavior and rheological properties of salt- or acid-induced soy proteins soft tofu-type gels. *Journal of Texture Studies, 45*(1), 62-73. https://doi.org/10.1111/jtxs.12052

Nakayama, T., & Sato, Y. (1971). Relationships between binding quality of meat and myofibrillar proteins: III: Contribution of myosin a and actin to rheological properties of heated minced-meat gel. *Journal of Texture Studies, 2*(1), 75-88. https://doi.org/10.1111/j.1745-4603.1971.tb00274.x

Nijdam, D., Rood, T., & Westhoek, H. (2012). The price of protein: Review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. *Food Policy, 37*(6), 760-770. https://doi.org/10.1016/j.foodpol.2012.08.002

Nonaka, M., Tanaka, H., Okiyama, A., Motoki, M., Ando, H., Umeda, K., & Matsuura, A. (1989). Polymerization of several proteins by Ca2+-independent transglutaminase derived from microorganisms. *Agricultural and biological chemistry, 53*(10), 2619-2623.

Noren, N. E., Scanlon, M. G., & Arntfield, S. D. (2019). Differentiating between tackiness and stickiness and their induction in foods. *Trends in Food Science & Technology, 88*, 290-301. https://doi.org/10.1016/j.tifs.2019.03.014

Nussinovitch, A. (2016). Adhesion Mechanisms and Measurements. In *Adhesion in Foods: Fundamental Principles and Applications*. John Wiley & Sons. https://doi.org/https://doi.org/10.1002/9781118851579.ch2

O'Dea, D., & Hewson, K. (2015). *The Culinary Uses of Eggs - Identification of raw/low-cooked egg dishes that may be of food safety concern.* https://teleskola.mt/wp-content/uploads/2020/04/Culinary-uses-of-eggs-Final-55bb4003a10778ca8cd66c5107b990a5.pdf

Offer, G., & Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Science, 8*(4), 245-281. https://doi.org/10.1016/0309-1740(83)90013-X

Ooms, N., & Delcour, J. A. (2019). How to impact gluten protein network formation during wheat flour dough making. *Current Opinion in Food Science, 25*, 88-97. https://doi.org/10.1016/j.cofs.2019.04.001

Partanen, R., Autio, K., Myllärinen, P., Lille, M., Buchert, J., & Forssell, P. (2008). Effect of transglutaminase on structure and syneresis of neutral and acidic sodium caseinate gels. *International Dairy Journal, 18*(4), 414-421. https://doi.org/10.1016/j.idairyj.2007.10.002

Peleg, M. (1976). Considerations of a general rheological model for the mechanical behavior of viscoelastic solid food materials. *Journal of Texture Studies, 7*(2), 243-255. https://doi.org/10.1111/j.1745-4603.1976.tb01265.x

Phillips, G. O., & Williams, P. A. (2009). *Handbook of Hydrocolloids*. Elsevier.

Phillips, L. G. (2013). *Structure-Function Properties of Food Proteins*. Academic Press.

Piazza, J., Ruby, M. B., Loughnan, S., Luong, M., Kulik, J., Watkins, H. M., & Seigerman, M. (2015). Rationalizing meat consumption. The 4Ns. *Appetite, 91*, 114-128. https://doi.org/10.1016/j.appet.2015.04.011

Pietrasik, Z., Sigvaldson, M., Soladoye, O. P., & Gaudette, N. J. (2020). Utilization of pea starch and fibre fractions for replacement of wheat crumb in beef burgers. *Meat Sci, 161*, 107974. https://doi.org/10.1016/j.meatsci.2019.107974

Poore, J., & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science, 360*(6392), 987-992. https://doi.org/10.1126/science.aaq0216

Possidónio, C., Prada, M., Graça, J., & Piazza, J. (2021). Consumer perceptions of conventional and alternative protein sources: A mixed-methods approach with meal and product framing. *Appetite, 156*, 104860. https://doi.org/10.1016/j.appet.2020.104860

Rahman, M. S., Al-Marhubi, I. M., & Al-Mahrouqi, A. (2007). Measurement of glass transition temperature by mechanical (DMTA), thermal (DSC and MDSC), water diffusion and density methods: A comparison study. *Chemical Physics Letters, 440*(4), 372-377. https://doi.org/https://doi.org/10.1016/j.cplett.2007.04.067

Ramírez, J. A., Del Ángel, A., Velazquez, G., & Vázquez, M. (2006). Production of low-salt restructured fish products from Mexican flounder (Cyclopsetta chittendeni) using microbial transglutaminase or whey protein concentrate as binders. *European Food Research and Technology, 223*(3), 341-345. https://doi.org/10.1007/s00217-005-0210-z

Renkema, J. M. S., Lakemond, C. M. M., de Jongh, H. H. J., Gruppen, H., & van Vliet, T. (2000). The effect of pH on heat denaturation and gel forming properties of soy proteins. *Journal of Biotechnology, 79*(3), 223-230. https://doi.org/10.1016/S0168-1656(00)00239-X

Renkema, J. M. S., Knabben, J. H. M., & Van Vliet, T. (2001). Gel formation by β-conglycinin and glycinin and their mixtures. *Food Hydrocolloids, 15*(4-6), 407-414. https://doi.org/10.1016/S0268-005X(01)00051-0

Renkema, J. M. S., & van Vliet, T. (2002). Heat-induced gel formation by soy proteins at neutral pH. *Journal of Agricultural and Food Chemistry, 50*(6), 1569-1573. https://doi.org/10.1021/jf010763l

Renkema, J. M. S. (2004). Relations between rheological properties and network structure of soy protein gels. *Food Hydrocolloids, 18*(1), 39-47. https://doi.org/10.1016/S0268-005X(03)00040-7

Romero de Ávila, M. D., Ordóñez, J. A., Escudero, R., & Cambero, M. I. (2014). The suitability of plasma powder for cold-set binding of pork and restructured dry ham. *Meat Science, 98*(4), 709-717. https://doi.org/10.1016/j.meatsci.2014.06.014

Rosenthal, A. J., & Thompson, P. (2021). What is cohesiveness?-A linguistic exploration of the food texture testing literature. *J Texture Stud*. https://doi.org/10.1111/jtxs.12586

Rui, X., Fu, Y., Zhang, Q., Li, W., Zare, F., Chen, X., Jiang, M., & Dong, M. (2016). A comparison study of bioaccessibility of soy protein gel induced by magnesiumchloride, glucono-δ-lactone and microbial transglutaminase. *LWT - Food Science and Technology, 71*, 234-242. https://doi.org/10.1016/j.lwt.2016.03.032

Rumpf, H. (1962). The Strength of Granules and Agglomerates. In *Agglomeration* (pp. 379-418). John Wiley.

Sakai, K., Sato, Y., Okada, M., & Yamaguchi, S. (2021). Improved functional properties of meat analogs by laccase catalyzed protein and pectin crosslinks. *Sci Rep, 11*(1), 16631. https://doi.org/10.1038/s41598-021-96058-4

Samejima, K., Hashimoto, Y., Yasui, T., & Fukazawa, T. (1969). Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. *Journal of Food Science, 34*(3), 242-245. https://doi.org/10.1111/j.1365-2621.1969.tb10331.x

Sarkar, N. (1995). Kinetics of thermal gelation of methylcellulose and hydroxypropylmethylcellulose in aqueous solutions. *Carbohydrate Polymers, 26*(3), 195-203. https://doi.org/10.1016/0144-8617(94)00107-5

Schneberger, G. L. (1983). *Adhesives in Manufacturing*. Routledge.

Schorsch, C., Carrie, H., & Norton, I. T. (2000). Cross-linking casein micelles by a microbial transglutaminase: influence of cross-links in acid-induced gelation. *International Dairy Journal, 10*(8), 529-539. https://doi.org/10.1016/S0958-6946(00)00069-8

Schreuders, F. K. G., Dekkers, B. L., Bodnár, I., Erni, P., Boom, R. M., & van der Goot, A. J. (2019). Comparing structuring potential of pea and soy protein with gluten for meat analogue preparation. *Journal of Food Engineering, 261*, 32-39. https://doi.org/10.1016/j.jfoodeng.2019.04.022

Schreuders, F. K. G., Sagis, L. M. C., Bodnar, L., Erni, P., Boom, R. M., & van der Goot, A. J. (2021). Small and large oscillatory shear properties of concentrated proteins. *Food Hydrocolloids, 110*, 106172.

Seideman, S. C., & Durland, P. R. (1983). Restructured red meat products: In review. *Journal of Food Quality, 6*(2), 81-99. https://doi.org/10.1111/j.1745-4557.1983.tb00759.x

Sengsuk, T., Songtipya, P., Kalkornsurapranee, E., Johns, J., & Songtipya, L. (2021). Active bio-based pressure-sensitive adhesive based natural rubber for food antimicrobial applications: Effect of processing parameters on its adhesion properties. *Polymers, 13*(2), 199. https://doi.org/10.3390/polym13020199

Serdaroğlu, M. (2006). Improving low fat meatball characteristics by adding whey powder. *Meat Science, 72*(1), 155-163. https://doi.org/10.1016/j.meatsci.2005.06.012

Shimada, K., & Cheftel, J. C. (1988). Determination of sulfhydryl groups and disulfide bonds in heat-induced gels of soy protein isolate. *Journal of Agricultural and Food Chemistry, 36*(1), 147-153. https://doi.org/10.1021/jf00079a038

Siegel, D. G., & Schmidt, G. R. (1979). Crude myosin fractions as meat binders. *Journal of Food Science, 44*(4), 1129-1131. https://doi.org/10.1111/j.1365-2621.1979.tb03463.x

Siegel, D. G., Church, K. E., & Schmidt, G. R. (1979). Gel structure of nonmeat proteins as related to their ability to bind meat pieces. *Journal of Food Science, 44*(5), 1276-1279. https://doi.org/10.1111/j.1365-2621.1979.tb06418.x

Silva, J. V. C., Cochereau, R., Schmitt, C., Chassenieux, C., & Nicolai, T. (2019). Heat-induced gelation of mixtures of micellar caseins and plant proteins in aqueous solution. *Food Research International, 116*, 1135-1143. https://doi.org/10.1016/j.foodres.2018.09.058

Silvestre, J., Delattre, C., Michaud, P., & de Baynast, H. (2021). Optimization of chitosan properties with the aim of a water resistant adhesive development. *Polymers (Basel), 13*(22). https://doi.org/10.3390/polym13224031

Stading, M., & Hermansson, A. M. (1990). Viscoelastic behaviour of β-lactoglobulin gel structures. *Topics in Catalysis, 4*(2), 121-135. https://doi.org/10.1016/S0268-005X(09)80013-1

Sun, N., & Breene, W. M. (1991). Calcium sulfate concentration influence on yield and quality of tofu from five soybean varieties. *Journal of Food Science, 56*(6), 1604-1607. https://doi.org/10.1111/j.1365-2621.1991.tb08651.x

Sun, P., Zhang, Q., Zhao, Y., Zhao, D., Zhao, X., Jiang, L., Zhang, Y., Wu, F., & Sui, X. (2023). Improving gel properties of soy protein isolate through alkaline pH-shifting, mild heat treatment, and TGase cross-linking. *Food Hydrocolloids, 144*, 108924. https://doi.org/https://doi.org/10.1016/j.foodhyd.2023.108924

Sun, S., Li, M., & Liu, A. (2013). A review on mechanical properties of pressure sensitive adhesives. *International Journal of Adhesion and Adhesives, 41*, 98-106. https://doi.org/10.1016/j.ijadhadh.2012.10.011

Szczesniak, A. S., Loew, B. J., & Skinner, E. Z. (1975). Consumer texture profile technique. *Journal of Food Science, 40*(6), 1253-1256. https://doi.org/10.1111/j.1365-2621.1975.tb01065.x

Szczesniak, A. S. (1987). Correlating sensory with instrumental texture measurements - An overview of recent developments. *Journal of Texture Studies, 18*(1), 1-15. https://doi.org/10.1111/j.1745-4603.1987.tb00566.x

Tabilo-Munizaga, G., & Barbosa-Cánovas, G. V. (2005). Rheology for the food industry. *Journal of Food Engineering, 67*(1-2), 147-156. https://doi.org/10.1016/j.jfoodeng.2004.05.062

Talukder, S. (2015). Effect of Dietary Fiber on Properties and Acceptance of Meat Products: A Review. *Critical Reviews in Food Science and Nutrition, 55*(7), 1005-1011. https://doi.org/10.1080/10408398.2012.682230

Tang, C.-H., Wu, H., Yu, H.-P., Li, L., Chen, Z., & Yang, X.-Q. (2006). Coagulation and gelation of soy protein isolates induced by microbial transglutaminase. *Journal of Food Biochemistry, 30*(1), 35-55. https://doi.org/10.1111/j.1745-4514.2005.00049.x

Tang, C.-H., Chen, L., & Foegeding, E. A. (2011). Mechanical and water-holding properties and microstructures of soy protein isolate emulsion gels induced by CaCl2, glucono-δ-lactone (GDL), and transglutaminase: Influence of thermal treatments before and/or after emulsification. *Journal of Agricultural and Food Chemistry, 59*(8), 4071-4077. https://doi.org/10.1021/jf104834m

Terrell, R. N., Crenwelge, C. H., Dutson, T. R., & Smith, G. C. (1982). A technique to measure biding poperties of non-meat proteins in muscle-juncture formation. *Journal of Food Science, 47*(3), 711-713. https://doi.org/10.1111/j.1365-2621.1982.tb12696.x

Teye, G. A., Sheard, P. R., Whittington, F. M., Nute, G. R., Stewart, A., & Wood, J. D. (2006). Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. *Meat Science, 73*(1), 157-165. https://doi.org/10.1016/j.meatsci.2005.11.010

Tita, A., Campos, D., Sadtler, V., Marchal, P., & Falk, V. (2012). Influence of the binder on the mechanical strength of compacts prepared by modified wet compression method. *Powder Technology, 230*, 86-92. https://doi.org/10.1016/j.powtec.2012.06.058

Toldrá, F., & Hui, Y. H. (2014). Dry-Fermented Sausages and Ripened Meats: An Overview. In *Handbook of Fermented Meat and Poultry* (pp. 1-6). https://doi.org/10.1002/9781118522653.ch1

Totosaus, A., Montejano, J. G., Salazar, J. A., & Guerrero, I. (2002). A review of physical and chemical protein-gel induction. *International Journal of Food Science & Technology, 37*(6), 589-601. https://doi.org/10.1046/j.1365-2621.2002.00623.x

Toufeili, I., Lambert, I. A., & Kokini, J. L. (2002). Effect of glass transition and cross-linking on rheological properties of gluten: Development of a preliminary state diagram. *Cereal Chemistry, 79*(1), 138-142. https://doi.org/10.1094/CCHEM.2002.79.1.138

Trout, G. R. (1988). Techniques for measuring water-binding capacity in muscle foods - A review of methodology. *Meat Science, 23*(4), 235-252. https://doi.org/10.1016/0309-1740(88)90009-5

Tsao, C.-Y., Kao, Y.-C., Hsieh, J.-F., & Jiang, S.-T. (2002). Use of soy protein and microbial transglutaminase as a binder in low-sodium restructured meats. *Sensory and Nutritive Qualities of Food, 67*(9). https://doi.org/10.1111/j.1365-2621.2002.tb09613.x

Tunick, M. H. (2011). Small-strain dynamic rheology of food protein networks. *J Agric Food Chem, 59*(5), 1481-1486. https://doi.org/10.1021/jf1016237

Van Der Leeden, M. C., & Frens, G. (2002). Surface properties of plastic materials in relation to their adhering performance. *Advanced Engineering Materials, 4*(5), 280-289. https://doi.org/10.1002/1527-2648(20020503)4:5<280::AID-ADEM280>3.0.CO;2-Z

Varela, P., & Ares, G. (2012). Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization. *Food Research International, 48*(2), 893-908. https://doi.org/10.1016/j.foodres.2012.06.037

Varvara, R. A., Szabo, K., & Vodnar, D. C. (2021). 3D food printing: Principles of obtaining digitally-designed nourishment. *Nutrients, 13*(10). https://doi.org/10.3390/nu13103617

Vnučec, D., Kutnar, A., & Goršek, A. (2016). Soy-based adhesives for wood-bonding – A review. *Journal of Adhesion Science and Technology, 31*(8), 910-931. https://doi.org/10.1080/01694243.2016.1237278

Verordnung (EG) Nr. 152/2009 der Kommission vom 27. Januar 2009 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Untersuchung von Futtermitteln.

Wagoner, T. B., & Foegeding, E. A. (2018). Surface energy and viscoelasticity influence caramel adhesiveness. *J Texture Stud, 49*(2), 219-227. https://doi.org/10.1111/jtxs.12298

Wall, J. S., & Huebner, F. R. (1981). Adhesion and Cohesion. In *Protein Functionality in Foods* (Vol. 147, pp. 111-130). American Chemical Society. https://doi.org/10.1021/bk-1981-0147.ch006

Wang, G., Zhang, T., Ahmad, S., Cheng, J., & Guo, M. (2013). Physicochemical and adhesive properties, microstructure and storage stability of whey protein-based paper glue. *International Journal of Adhesion and Adhesives, 41*, 198-205. https://doi.org/10.1016/j.ijadhadh.2012.11.010

Wang, K.-Q., Luo, S.-Z., Zhong, X.-Y., Cai, J., Jiang, S.-T., & Zheng, Z. (2017). Changes in chemical interactions and protein conformation during heat-induced wheat gluten gel formation. *Food Chemistry, 214*, 393-399. https://doi.org/10.1016/j.foodchem.2016.07.037

Wang, R., & Hartel, R. W. (2020). Effects of moisture content and saccharide distribution on the stickiness of syrups. *Journal of Food Engineering, 284*. https://doi.org/10.1016/j.jfoodeng.2020.110067

Wang, R., & Hartel, R. W. (2021). Understanding stickiness in sugar-rich food systems: A review of mechanisms, analyses, and solutions of adhesion. *Compr Rev Food Sci Food Saf*. https://doi.org/10.1111/1541-4337.12833

Warner, R. D. (2017). The Eating Quality of Meat—IV Water-Holding Capacity and Juiciness. In Toldra´, F. (Ed.), *Lawrie´s Meat Science (Eighth Edition)* (pp. 419-459). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100694-8.00014-5

Weiss, J., Salminen, H., Moll, P., & Schmitt, C. (2019). Use of molecular interactions and mesoscopic scale transitions to modulate protein-polysaccharide structures. *Advances in Colloid and Interface Science, 271*, 101987. https://doi.org/10.1016/j.cis.2019.07.008

Wijngaards, G., & Paardekooper, E. (1988, 23-25 Nov 1987). Preparation of a composite meat product by means of an enzymatically formed protein gel. Proceedings of the international symposium: trends in modern meat technology. , Den Dolder (Netherlands).

Wikipedia. (2020). https://en.wikipedia.org/wiki/Binder_(material)

Winger, R. J., & Hagyard, C. J. (1994). Juiciness - Its Importance and Some Contributing Factors. In Pearson, A. M. & Dutson, T. R. (Eds.), *Quality Attributes and their Measurement in Meat, Poultry and Fish Products* (pp. 94-124). Springer US. https://doi.org/10.1007/978-1-4615-2167-9_4

Winslow, B. D., Shao, H., Stewart, R. J., & Tresco, P. A. (2010). Biocompatibility of adhesive complex coacervates modeled after the sandcastle glue of Phragmatopoma californica for craniofacial reconstruction. *Biomaterials, 31*(36), 9373-9381. https://doi.org/10.1016/j.biomaterials.2010.07.078

Wittek, P., Zeiler, N., Karbstein, H. P., & Emin, M. A. (2020). Analysis of the complex rheological properties of highly concentrated proteins with a closed cavity rheometer. *Applied Rheology, 30*(1), 64-76. https://doi.org/10.1515/arh-2020-0107

Xing, G., Giosafatto, C. V. L., Rui, X., Dong, M., & Mariniello, L. (2019). Microbial transglutaminase-mediated polymerization in the presence of lactic acid bacteria affects antigenicity of soy protein component present in bio-tofu. *Journal of Functional Foods, 53*, 292-298. https://doi.org/10.1016/j.jff.2018.12.035

Xing, G., Giosafatto, C. V. L., Carpentieri, A., Pasquino, R., Dong, M., & Mariniello, L. (2020). Gelling behavior of bio-tofu coagulated by microbial transglutaminase combined with lactic acid bacteria. *Food Research International, 134*, 109200. https://doi.org/10.1016/j.foodres.2020.109200

Yildiz, O., Meral, R., & Dogan, I. S. (2012). Determination of stickiness values of different flour combinations. *International Journal of Food Engineering, 8*(3), Article 37. https://doi.org/10.1515/1556-3758.2412

Zayas, J. F. (1997). *Functionality of Proteins in Food*. Springer Verlag.

Zhu, J.-H., Yang, X.-Q., Ahmad, I., Zhong, R.-M., Qi, J.-R., & Zhu, D.-H. (2011). Effect of transglutaminase on the functional properties of GDL (glucono-delta-lactone) cold-set soybean glycinin gel. *International Journal of Food Science & Technology, 46*(5), 963-971. https://doi.org/10.1111/j.1365-2621.2011.02566.x

# Eidesstattliche Versicherung

Eidesstattliche Versicherung gemäß § 7 Absatz 7 der Promotionsordnung der Universität Hohenheim zum Dr. rer. nat.

1. Bei der eingereichten Dissertation zum Thema
   PLANT PROTEIN GELS AS BINDERS IN MEAT PRODUCT ANALOGUES
   handelt es sich um meine eigenständig erbrachte Leistung.

2. 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.

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

4. 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.

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