Effects of Non-allergen Ingredients on Functional Properties and Sensory Acceptability of Selected Processed Meat Products

by

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Abstract

Consumer interest in foods with clean labels and ingredients (allergen-free, non-GMO status, recognizable ingredients, minimally processed and natural products that contain no added preservatives or additives) continues to be a major trend within the food industry. Many non-meat ingredients currently used by the meat processors to improve processing functionality are classified by Health Canada as priority allergens (soy, wheat, egg, and milk) and thus, their presence in meat products represents challenges and marketing limitations. However, given the functional properties of these ingredients, finding a replacement that meets consumer's criteria of clean label without sacrificing functionality can be challenging. Although some of novel allergen-free ingredients may have functional attributes of interest to the food industry, there is limited research directly comparing them to competitive ingredients currently used by the meat industry. The objective of the overall research was to evaluate selected non-allergen ingredients as functional, low-cost binders as a potential replacement of current, allergenic ingredients that are largely used by meat processing industry.

A series of experiments was conducted in preliminary trials to screen and select best performing non-allergen binders and establish their appropriate incorporation levels in two meat model systems. Based on initial screening results, potato starch, pea starch, medium/short rice flour and textured pea protein demonstrated the best potential for wheat crumb replacement in beef burgers, whereas hydrolysed collagen, pea starch, potato starch, and white navy bean flour were identified to have the best potential to enhance the processing characteristics of low-fat bologna.

The second experimental study focused on effects of non-allergen binders on functionalities and sensory characteristics of beef burgers. Selected non-allergen plant ingredients (potato starch, pea starch, rice flour and textured pea protein) were applied into beef burgers as alternative binders at 2% and 4%. Colour and oxidative stability of raw burgers, cooking, physicochemical and sensory characteristics of cooked burgers incorporated with non-allergen binders were compared with control treatment containing 5% wheat crumb. Rice flour added at 4% improved colour stability of fresh beef burgers compared to other non-allergen treatments and was comparable to wheat crumb. Burgers processed with 4% textured pea protein delayed lipid oxidation of raw burgers over 4 days of simulated retail display (4 °C). Non-allergen binders incorporated at 4% yielded burgers with similar cooking characteristics to wheat crumb and led to significantly ($p < 0.05$) lower cooking loss and higher moisture retention than those formulated with 2%. Consumer sensory evaluation suggested that burgers with 4% pea starch and textured pea protein had comprehensively higher acceptability. Overall, pea starch and textured pea protein could be potentially utilized as gluten-free alternatives to wheat crumb for meat binder applications.

In the third experimental study the performance of hydrolysed collagen, white navy bean flour, potato starch and pea starch in low fat reduced sodium emulsified pork bologna was evaluated. These non-allergen ingredients were compared to wheat flour and low sodium (LS) and regular salt (RS) bolognas formulated with no binders. Physicochemical and cooking properties, microstructure, and consumer acceptability were evaluated. All non-allergen binders significantly ($p < 0.05$) enhanced the cooking yield compared to LS control and were equivalent to wheat flour. Pea starch decreased redness of interior bologna colour. Amongst the binders tested, potato starch outperformed wheat flour for overall consumer acceptability. Microstructure analysis of potato starch bolognas showed the presence of a starch-protein network. Potato starch had the greatest potential as a substitute for wheat flour without compromising palatability in reduced sodium emulsion type sausages.

In conclusion, replacing high priority allergen containing ingredients with non-allergenic alternatives is feasible and would supply the industry with alternative low-cost ingredients that provide product differentiation and address emerging consumer demand for allergen-free products.

Preface

This thesis is an original work by Tianzhi Yang. The thesis consists of six chapters.

Chapter 1 is a general introduction to current consumer demand for non-allergen meat products followed by hypothesis and objectives of this research. Chapter 2 is the research background and includes the summary of existing studies on applications of allergen binders in different types of processed meat products and the potential non-allergen ingredients. The author has received permissions to reuse all figures in this chapter. Chapters 3 is the preliminary experiments on screening novel non-allergen binders in two meat model systems.

Chapters 4 and 5 constitute the main body of the thesis and focus on effects of selected non-allergen binders on functional characteristics and sensory palatability of two processed meat products. Partial results in Chapter 5 have been published as a short conference paper (Yang, T., Pietrasik, Z., & Betti, M. (2018). Effect of Non-Allergen Binders on Functionalities and Sensory Characteristics of Low Sodium Pork Bolognas. The paper was presented at the 64th International Congress of Meat Science and Technology, August 12-17, 2018. Melbourne, Australia. Source: www.icomst.helsinki.fi/Digicomst.). Chapter 6 is the summarization of outcomes and implications of this research. Brief future perspectives are also included in this chapter.

The present author was responsible for performing major experiments, data analyses and preparation of manuscripts for publication. Dr. Zeb Pietrasik was involved as the supervisory author to provide advisory inputs in the experimental design, manuscript editing and proof reading. Dr. Mirko Betti assisted in proof reading of the manuscripts.

All the sensory evaluations in Chapters 3, 4, and 5 were conducted by sensory scientists and technologists from the Food Processing Development Centre and Consumer Product Testing Centre. The present author was only responsible for data analyses.

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Finally, I would express my profound gratitude to my parents. They always supported me even when I was in the toughest times. I would not achieve this much without their encouragement.

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Abbreviations

$A_{(630nm/580nm)}$: ratio of absorbance at 630nm to 580nm
*a**: redness
AMSA: American Meat Science Association
ANOVA: analysis of variance
APBI: Agrivalue Processing Business Incubator
*b**: yellowness
C*: Chroma
CATA: Check-All-That-Apply
CPTC: Consumer Product Testing Centre
Dm: Detarium microcarpum
FPDC: Food Processing Development Centre
G': storage modulus
G": loss modulus
GMO: genetically modified organism
h: hue angle
HC: hydrolysed collagen
IgE: Immunoglobulin E
IL: interleukin
JAR: Just-about-right
*L**: lightness
LS: low sodium
MDA: malonaldehyde
NB: no binder
NCD: non-communicable disease
OF: Ocean's Flavor sea salt
PCA - principal component analyses
PeS: pea starch
PHR: phosphate replacer
pI: isoelectric point
PoS: potato starch
RBPI: red bean protein isolate
RS: regular salt
SEM: scanning electron microscopy
SP: swelling power
TBA: thiobarbituric acid
TBARS: 2-thiobarbituric acid reactive substance
TCA: trichloroacetic acid
TPA: texture profile analysis
USDA: United States Department of Agriculture
WF: wheat flour
WHO: World Health Organization
WNBF: white navy bean flour

Chapter 1. General introduction and objectives

Red meat as an essential food has been consumed by populations for thousands of years. In some countries, average daily intakes of total meat ranged from 78.8 g/d to 170.4 g/d and from 47.1 g/d to 106.6 g/d, for men and women respectively (McAfee *et al.*, 2010). Red meat is considered as a major dietary source of protein and essential nutrients including iron, zinc, selenium, glutathione, and Vitamin A, B_6, B_{12}, and D. Meat is rich in essential amino acids that human body cannot synthesise and which are beneficial to establishing and rebuilding muscle tissues. Meat contains not only health-related contents of phosphorus, magnesium, copper, cobalt, chromium and nickel, but also a rich source of taurine, which is less able to synthesise in new-born infants (Higgs, 2000).

However, the image of processed meats to consumers is relatively negative on account of their involvement in prevalent diseases (Toldrá & Reig, 2011). Recent reports have established a relationship between consumption of red meat or processed meats and increasing health risk of cardiovascular diseases (coronary heart disease, stroke, hypertension, and myocardial infarction), stomach and colorectal cancer, and obesity (Williamson *et al.*, 2005, McAfee *et al.*, 2010, Hu *et al.*, 2011, Bouvard *et al.*, 2015). Higher intake of meat is also related to higher plasma concentrations of total cholesterol, low-density lipoprotein cholesterol and triglycerides (Li *et al.*, 1999, McAfee *et al.*, 2010). Therefore, the general public has already generated abundant confusion and fear to the safety of processed meats (Oswell, Thippareddi & Pegg, 2018). More recently, an increasing trend towards “clean label” for food products has emerged in North America and the use of term “clean-label” has dramatically exploded over the past decade (Tarté, 2009, Asioli *et al.*, 2017).

Clean label products usually refer to those less processed or processed with natural or organic ingredients without artificial additives. Ingredient declaration lists of such products are short, simple and free from "chemical-sounding" additives or components causing food allergy and intolerance (Jayasena & Jo, 2013, Asioli *et al*., 2017, Aschemann-Witzel, Varela & Peschel, 2019). Ingredients that do not meet clean-label requirements include but are not limited to chemically synthesized antioxidants and preservatives, nitrites, phosphates, chemically modified starches, and food allergens (Oswell, Thippareddi & Pegg, 2018, Petracci *et al*., 2013, Tarté, 2009). Amongst them, phosphates, modified starches, and some food allergens are functional ingredients in processed meat products: improving the water holding capacity, processing yield, and eating quality (Petracci *et al*., 2013). Therefore, addressing consumer demand for clean label while maintaining production goals and product attribute requirements can be challenging and costly.

Extenders, fillers, and binders have been widely used in processed meat products to improve hydration and textural characteristics. They are protein or carbohydrate-based ingredients derived from animals, plants, or even microorganisms, such as isolated or textured proteins, flours, starches, fibres, and gums. Chemically modified starches and typical food allergens can be considered as binders. Binders play roles in not only replacing fat and meat, but also contributing improved nutritional values, overall cooking and textural functionalities, and sensorial characteristics to processed meats due to their water and fat binding capacity and interactions with meat myofibrillar proteins (Petracci *et al*., 2013, Bolger *et al*., 2017, Beriain *et al*., 2018). They provide more flexibility for meat producers to broaden the categories of products for meeting the consumer requirements and demands (Petracci *et al*., 2013).

In the past few decades, binders containing soy, wheat, egg, and milk have been extensively utilized in meat industry (Ensor *et al*., 1987, Leduc *et al*., 1999, Day *et al*., 2006, Singh *et al*., 2008). However, these substances are considered as high-priority food allergens in Canada (Health Canada, 2011) and thus their presence in processed meat products is problematic for growing number of consumers concerned about health and wellness as well as the impact of diet on overall wellbeing. However, due to their importance for functionality in processed meats, simply removing current binders to produce allergen-free products is not viable. Therefore, studies on replacing traditional allergen binders in processed meat products with allergen-free ingredients are becoming vital.

Replacing commonly used non-meat binders is even more challenging in reduced sodium products. Approaches to reducing salt content in processed meat products include: direct reduction of salt based on sensory acceptance of products; the use of salt substitutes associated with masking agents; the incorporation of flavour enhancers (enhancing the perception of saltiness when combined with salt); alteration of physical form of salt (increasing taste bioavailability); introducing high-pressure or ultrasound processing technology (improving salt diffusion) (Desmond 2006, Inguglia *et al*., 2017). Directly reducing salt can negatively affect functionality and palatability of processed meat products such as the decrease of protein extraction, water binding and strength of meat gels due to the fact that low ionic strength can limit the functionality of the traditional myosin heat-set matrix. Potassium chloride is the most commonly used sodium chloride replacer in meat industry. However, the metallic flavour of potassium-based salt substitutes limits the application of potassium chloride (Inguglia *et al*., 2017). Some modifications on traditional NaCl-KCl mixes need to be studied further. Addition of non-allergen binders should compensate adverse effect that reducing sodium results in. The removal of allergenic binders and the incorporation of useful but unappreciated ingredients can satisfy both producers and customers (Tarté, 2009).

Potential non-allergen binders may be obtained from legumes/pulses, cereals, fruits and vegetables, and animal collagens (Asgar *et al*., 2010, Petracci *et al*., 2013). Although some of novel allergen-free ingredients may have functional attributes of interest to the food industry, there is limited research directly comparing them to competitive ingredients currently used by the meat industry.

The following hypothesis was tested:

Reformulating selected meat products to replace high priority allergen containing ingredients with non-allergenic alternatives will not negatively impact the functionality and consumer acceptability of developed products.

The general objective of this thesis is to evaluate novel non-allergen ingredients as functional, low-cost as a potential replacement of current, allergenic ingredients that are largely used by industry.

The first experimental study of this thesis (described in **Chapter 3**) focused on screening and selection of various commercially available ingredients for their effectiveness in two meat model systems. Sensory science methodology was used as a main tool to establish an acceptable incorporation level of the best performing non-allergen ingredients.

The second experimental study of this thesis (described in **Chapter 4**) was designed to evaluate the best performing non-allergen ingredients as a replacement of wheat crumb in regular fat beef burgers. The specific objectives of the study were:

a) to explore the effects of selected binder treatments on colour and fat oxidation stability of fresh beef burgers stored under the simulated retail display and frozen burgers stored in dark;

b) to explore the effects of selected binder treatments on cooking, physicochemical, and sensorial properties of cooked beef burgers;

c) to compare the impacts of different addition levels of the same binder and compare all the non-allergen binders to wheat crumb allergen control in burgers.

In the third experimental study of this thesis (described in **Chapter 5**) the performance of most promising non-allergen binders in low fat reduced sodium pork bologna sausages was evaluated. The specific objectives of the study were:

a) to explore the effects of different treatments on viscoelastic properties of raw bologna batter;

b) to explore the effects of different treatments on cooking, physicochemical, and sensorial properties of vacuum-packed cooked pork bolognas;

c) to explore the effects of different treatments on microstructure of cooked bolognas.

Chapter 2. Research background

2.1. Health concerns associated with consumption of processed meat products

2.1.1. Food allergy

Food allergy is an inflammatory disease and it causes serious food safety problems. In the last few years, the incident rate of food allergy and other relevant diseases has been increasing rapidly, with 1% to 2% adults and 4% to 8% children worldwide having high allergy reactivity mediated by Immunoglobulin E (IgE) (Huang *et al*., 2018). In Canada, 6.7% of the population (7.1% for children and 6.6% for adults) were suffering food allergy based on a self-report survey (Soller *et al*., 2012). More than 40% of Canadians read food labels and look for allergen information for themselves or families when shopping (Allergy, Genes and Environment Network, 2015). In the United States, estimated range of prevalence was from 1% to 10%, mostly on the basis of self-report or parent-report of allergy (Sicherer & Sampson, 2014). Another statistic from 2009 to 2010 in the U.S. revealed 8% of children had a food allergy, and 30.4% of these children had multiple food allergies. In 2012, 5.6% or 4.1 million children in the U.S. reported food allergies (Bloom, Cohen & Freeman, 2013). In Europe, overall self-reported food allergy prevalence was 5.9% from 2000 to 2012 (Nwaru *et al*., 2014, Savage & Johns, 2015).

IgE-mediated type I hypersensitivity is one of the major food allergies (Huang *et al*., 2018). After food allergens are ingested into the body, IgE antibody is produced in B cells. This progress is triggered due to the activated cells secreting interleukin (IL)-4, IL-5, IL-13 and other cytokines. The interaction of IgE and mast cells, basophil granulocyte cells or other target cells induces the body hypersensitiveness. When the same allergen is taken into the human body, IgE molecules on target cells are activated by the allergen and the bridging reaction is mediated, thereby the downstream signal pathway is activated, which results in the degranulation and release of the mediators including histamine, 5-hydroxytryptamine, and leukotriene in target cells, leading to an allergic reaction (Sicherer & Sampson, 2010, Huang *et al*., 2018). Huang *et al*. (2018) interpreted the mechanism of food allergy as shown in Figure 2.1. Due to allergic reaction boosting the permeability of intestinal epithelial cells to the antigen, the pathway of some specific cytokines is influenced, which induces a Th2-type inflammatory response.

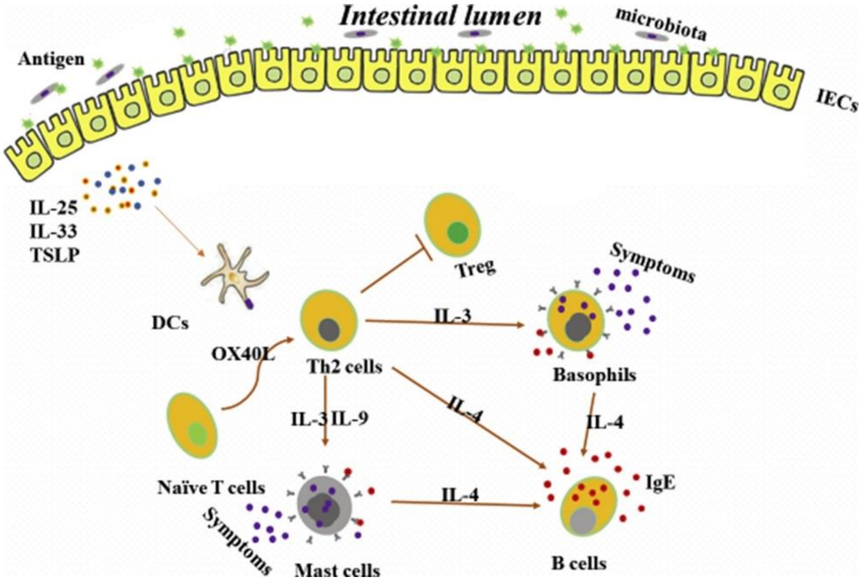


Figure 2.1. Diagram of food allergy mechanism (Huang *et al*., 2018). Copyright © 2018, Elsevier Ltd.

Although Hefle, Nordlee & Taylor (1996) organized the food allergy information in detail, different countries may have various allergen lists. Health Canada has identified priority food allergens in Canada, including eggs, milk, mustard, peanuts, crustaceans and molluscs, fish, sesame seeds, soy, sulphites, tree nuts, wheat and triticale (Health Canada, 2011). Some of them, including wheat flour, soy protein, and whey protein, have been widely formulated in processed meat products as binders. This is attributed to their unique properties in meat matrix to enhance the texture, flavour, cooking characteristics, and reduce processing cost (Lauck, 1975, Bejosano

& Corke, 1998). The information on common allergen binders and their functions will be introduced in detail in the section ***2.2.4***. Substitution of these common allergen binders is considered as priority due to public health concerns. For example, it is estimated that the prevalence of allergy to wheat was about 0.3% to 0.6% of the population around the world (Lupi *et al*., 2014).

2.1.2. High sodium intake

In North America, Europe, and Australia, about 70% of consumed salt is derived from processed foods, and meat products account for 20% among them (Inguglia *et al*., 2017). Processed meat consumption has been criticized because processed meats contain high levels of sodium (Horita *et al*., 2011). Capuano *et al*. (2013) investigated the sodium content of 1016 packaged foods in the Netherlands, and found that the processed meat group had on average the highest sodium content (1030 mg/100 g). Among them, bacon contained the highest amount of sodium (1370 mg/100 g), followed by salami (1330 mg/100 g). Sodium content in smoked sausage and ham shoulder were similar (1050 mg/100 g). Sodium in Knakworst (frankfurter sausage) was the lowest (759 mg/100 g) amongst 10 types of meats. In another study, Kameník *et al*. (2017) measured the sodium content of 5 cooked meat products from Czech Republic and Germany. The means of these Czech/Germen products are listed as followed: speckwurst/Knackauer: 824/936.5 mg/100 g; Gothaj sausage/Schinkenwurst: 857.3/810.7 mg/100 g; ham sausage/Bierschinken: 933.4/881.3 mg/100 g; cooked ham: 980.9/1054.6 mg/100 g; frankfurters: 975.9/781.7 mg/100 g. Additionally, based on the database of Food Standards Agency and USDA, average sodium ranges of common processed meats in U.K. and U.S. are shown as follows: beef burgers: 290–590 mg/100 g; sausages: 433–1080 mg/100 g; frankfurters: 720–920 mg/100 g; cooked ham: 900–1220 mg/100 g; bacon 1000–1540 mg/100 g; breaded chicken: 200–420 mg/100 g; chicken nuggets: 510–600 mg/100 g (Desmond, 2006, Inguglia *et al*., 2017).

In Canada in 2010−2011, research data showed that the average sodium levels of packaged meats were as followed: sausages and wieners: 912 mg/100 g; deli meats: 1092 mg/100 g; fresh and frozen meat and poultry: 554 mg/100 g (Arcand *et al*., 2014). Health Canada (2012) published a *Guiding Benchmark Sodium Reduction Levels for Processed Foods* and aimed to reduce sodium intake to average goal by Phase 3 (the end of 2016), and the proposed sales-weighted average sodium levels/maximum sodium levels of each meat product category were partially listed below: uncooked fresh sausage: 660/690 mg/100 g; fully cooked smoked or unsmoked sausage and wieners: 830/870 mg/100 g; fully cooked packaged deli meats: 850/890 mg/100 g; burgers, meatballs, meat loaf, and breaded meat and poultry: 450/470 mg/100 g. Comparing the data (Arcand *et al*., 2014) when Canada's Sodium Reduction Strategy was implemented to the above sodium benchmark targets, it was indicated that 30.5% of meat and meat substitutes had already met the goal of Phase 3, while 61.2% of meat and meat substitutes exceeded maximum levels. The latest measurements of sales-weighted average sodium levels in 2017 (Health Canada 2018) suggested that progress in sodium reduction of foods under categories of uncooked fresh sausage, fully cooked smoked or unsmoked sausage and wieners, and fully cooked packaged deli meats were still in the interim phase, and salt reduction in burgers, meatballs, meat loaf, and breaded meat and poultry did not make meaningful progress. This revealed that the Canadian meat industry still had long-way responsibilities to tackle the issues.

According to the World Health Organization, populations around the world are consuming more sodium than is physiologically necessary in recent years (WHO, 2012). The risks associated with high intake of sodium have been widely studied and it is proved relative to a number of noncommunicable diseases (NCDs) including hypertension, cardiovascular disease and stroke (Aburto *et al*., 2013). Decreasing sodium intake may reduce blood pressure and the risk of associated NCDs (WHO, 2012). Hence, the World Health Organization (WHO) recommended a sodium reduction to <2 g/day sodium or 5 g/day salt for adults (Inguglia *et al*., 2017).

2.2. Functional binders and salt in processed meat products

2.2.1. Meat protein classification

Based on their solubility, meat proteins are grouped as sarcoplasmic, stromal and myofibrillar proteins (DeFreitas, 1994). Sarcoplasmic proteins are composed of soluble proteins at low ionic strength in the sarcoplasm, accounting for approximately 30% to 34% of the total proteins. They are very similar to the proteins in the cell cytoplasm except for the presence of myoglobin and glycolytic proteins (Morrissey, Mulvihill & O'Neill, 1987, Asghar *et al*., 1985). Stromal proteins, also referred as connective tissue, consist of three major fibrillar proteins: collagen, reticulin and elastin, accounting for 10% to 15% of the total proteins. The function of stromal proteins is to connect the muscle organs and other tissues to the skeleton as well as cover the body (Morrissey, Mulvihill & O'Neill, 1987, Asghar *et al*., 1985). Myofibrillar proteins consist of contractile elements of muscle in the myofibrils, accounting for 50% to 55% of the total proteins. Myofibrillar proteins could be extracted at high ionic strength but become soluble in low ionic strength solutions once extracted. The major proteins in this salt-soluble portion are myosin, actin, tropomyosin, troponin, and α-actinin (DeFreitas, 1994). Myosin and actin are directly responsible for contraction-relaxation cycle of muscle and they are the major components of the thick and thin filaments respectively. Myosin proteins occupy 52−53% of the total myofibrillar proteins and consist of two large chains: heavy chains and light chains (Eskin & Shahidi, 2012, Asghar *et al*., 1985). Figure 2.2 represents a schematic structure of myosin. Myosin is the major myofibrillar protein related to protein gelation or binding meat pieces together. Therefore, it is widely studied for textural properties and water holding capacity of meat products (DeFreitas, 1994, Fukazawa, Hashimoto & Yasui, 1961, Yasui *et al.*, 1979, Ishioroshi *et al.*, 1980, Dudziak, Foegeding & Knopp, 1988).

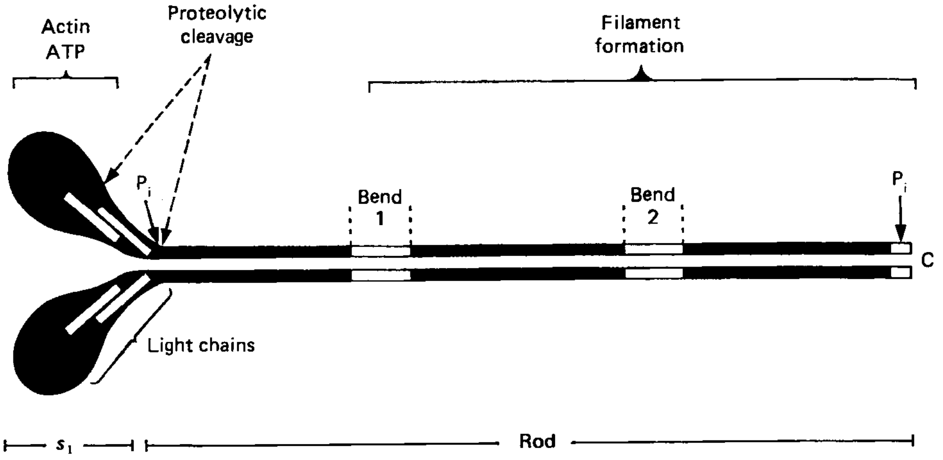


Figure 2.2. Diagrammatic representation of a myosin molecule (Citi & Kendrick-Jones, 1987). Copyright © 1987, WILEY Periodicals Inc.

2.2.2. Meat products and meat protein matrix

According to Petracci *et al.* (2013) processed meat products could be classified into 4 types on the basis of the degree of muscle size reduction after manufacturing and the final destination of muscle: i) whole-muscle products, including marinated whole carcass or cut-ups, where the distribution of both intra- and extra-cellular water remains intact cyto-architecturally and geometrically; ii) restructured products, which are processed with bonding chunks or pieces of meat together, including rolls and restructured hams; iii) coarse ground products, which are processed with minced meat and where fibrous muscle structure is still maintained to some extent, including burgers, meatballs, and breakfast sausages; iv) emulsified/comminuted products, which are processed with finely comminuted meat batter or slurry and where muscle fibre structure is undetectable, including hot dogs, frankfurters and bolognas (Figure 2.3).

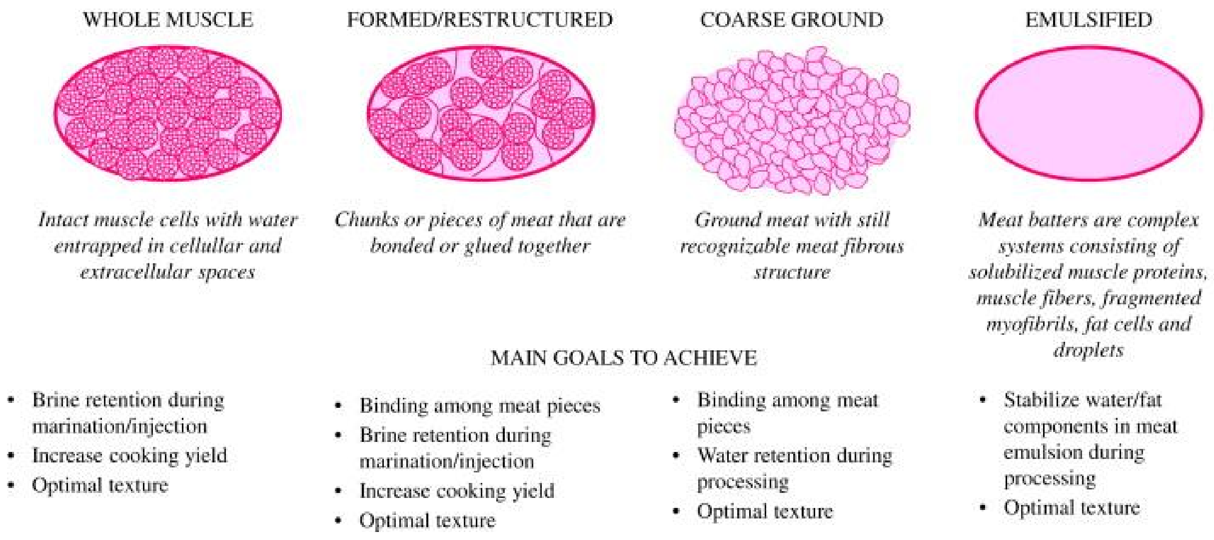


Figure 2.3. Classification of meat products based on raw meat materials of manufacturing and different functionalities of functional ingredients (Petracci *et al*., 2013). Copyright © 2013, Elsevier Ltd.

The principal components in the meat system are water, fats and proteins. Among these, proteins are the major structural components owing to the function of holding the components together by means of binding water and fats (Comer, 1979). In meat processing, comminution contributes to reducing the meat size into a fine particulate phase. Comminution is performed in the presence of sufficient level of salt to provide an ionic strength to induce swelling of muscle fibres, and water binding with partially extracted myofibrillar proteins. This results in the formation of a fibrous and tacky protein suspension where protein-water interaction and protein-lipid association occur and it functions as a water binder and a fat stabilizer. Fat is dispersed within the protein sol matrix. Figure 2.4 shows the complex structure of finely comminuted meat products. After the utilization of thermal energy during cooking, salt-soluble protein-protein aggregation happens. The structure of aggregated filamentous network helps suitably entrap both water and fat. These processes lead to higher moisture and fat retention, and lower fat granule coalescence (Acton *et al.*, 1983).

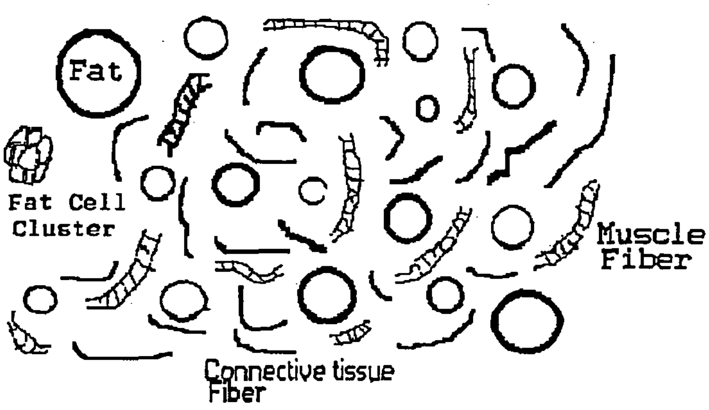


Figure 2.4. Schematic illustration of a meat batter (Gordon, Barbut, & Schmidt, 1992). Copyright © 1992, Taylor & Francis.

Additionally, environmental conditions surrounding the myofibrillar proteins during the whole manufacturing, which include salt concentration (such as sodium chloride), pH, and the temperature profile that continually increases from approximately 0 °C to 66 °C to 71 °C, could affect the interrelated functional responses (Acton *et al.*, 1983).

2.2.3. Function of salt in meat products

Salt (sodium chloride) is one of the most extensively used and multi-functional ingredients in meat processing (Ruusunen & Puolanne, 2005). For thousands of years, it has been used for the preservation of processed meat products. The preservative property of NaCl primarily comes from its capacity to reduce the water activity which ameliorates microbiological stability and extends the shelf life (Sofos, 1984). In the modern meat industry, salt is also used as a seasoning or flavour enhancer (Desmond, 2006). Except for the perceived saltiness, NaCl contributes to the characteristic taste of processed meat products and enhances the flavour (Gillette, 1985). Salt also provides the desired textural properties of processed meat products which could be attributed to solubilisation of myofibrillar proteins. Salt improves several functionalities: increasing cooking yield and juiciness (due to the fact that salt can increase protein hydration and water holding capacity, and increase the binding properties to other proteins); enhancing the viscosity of meat batter (favouring formation of heat-stable emulsion); facilitating the incorporation of fat (fat-binding) to stabilize meat batter, which cause the formation of a desirable gel after cooking (Terrell, 1983, Inguglia *et al*., 2017).

NaCl dissociates into sodium (Na^+) ion and chloride (Cl^-) ion in a meat matrix. However, Cl^- ions have stronger binding capacity to positively charged groups of myosin than Na^+ ions. Absorption of Cl^- ions to myosin and actin filaments facilitates the electrostatic repulsive forces between muscle fibres, resulting in unfolding of the protein structure matrix, exposing more side groups and enlarging the spaces between actin and myosin (Petracci *et al*., 2013, Hamm, 1986). Additionally, the binding of Cl^- ions with positively charged myosins induces the shift of the isoelectric point (pI) to a more acidic pH, and thus leading to the increase of a gap between pI and the pH of meat. As a result, the increasing gap between these two pH values causes the increase of the capillary effect of muscle fibres to improve water binding capacity (Feiner, 2006, Puolanne and Halonen, 2010, Petracci *et al*., 2013).

2.2.4. Function of non-meat binders, fillers and extenders

Utilization of non-meat proteins and carbohydrates, referred as binders, fillers or extenders, to optimize the functional characteristics of processed meat products could not only reduce the cost of the formulations (replacing meat with non-meat ingredients), but also address the variability of natural quality in raw meat and increase flexibility for meat producers to broaden the categories of products and meet consumer demands (Petracci *et al*., 2013). Their role is not only to replace higher cost meats with lower cost non-meat ingredient but also to improve textural and flavour properties of processed meat products (Beriain *et al*., 2018).

Extenders are defined primarily as plant proteins from legumes and animal proteins such as whole milk and eggs. Textured vegetable proteins, with soybean as the most common source, are common meat extenders. Fillers mostly refer to plant ingredients with low protein and high carbohydrate content including cereals, roots, tubers and vegetables and some refined starches and flours. They are capable of absorbing excessive amount of water. Binders are considered as non-meat ingredients derived from animals or plants, with high level of proteins that favour both water and fat binding, such as high-protein soy, wheat and milk products, including soy isolate, wheat gluten, and caseinate. The addition quantities of binders are lower than extenders, but they contribute to water binding and protein network structuring. Some fillers like starches and flours could be used as binders due to physical entrapment of water and fat in meat products (Gunter & Peter, 2007). In many studies, these three terms are used interchangeably.

In comminuted meat products, a gel structure is formed with protein and insoluble carbohydrate components during cooking, and this gelation process contributes to the formation of a stable, structured, and homogeneous meat system (Comer, 1979). Only a few polysaccharides are able to gel at the critical concentration, which is relatively lower than that of proteins, while others play roles as thickeners and stabilizers in different meat systems (Nazir, Asghar & Maan, 2017).

Binders and fillers could also play a role of fat substitute (Amini *et al*., 2015) and alleviate the limitation of reduced sodium products due to the low ionic strength and insufficient extraction of proteins. In restructured products such as sausages or deli meats, the structural functions of salt-soluble proteins are substituted by the addition of binders and fillers (Inguglia *et al*., 2017).

2.2.4.1. Interactions between meat myofibrillar proteins and non-meat binders

Charged polysaccharides have the possibility to interact with other polysaccharides, proteins and lipids in order to change the properties of food (DeFreitas, 1994). Three kinds of interactions between proteins and polysaccharides could occur during meat gel formation: i) positively charged protein interacting with sulfated or carboxylated polysaccharide; ii) interaction between two polymers with the same net charge; iii) highly selective linking (covalent bonding) between proteins and polysaccharides (Stainsby, 1980).

More specifically, two polymers with opposite net charges, for example, a cationic protein below its pI and an anionic polysaccharide at or above its pI, would interact primarily electrostatically in nature during exothermic changing (Ledward, 1994). A negatively charged sulfated or carboxylated group of polysaccharides tends to directly interact with a positively charged protein residue including guanidinium and imidazole. However, sulfated polysaccharides are negatively charged within a wide range of pH (Glicksman, 1983). When the pH is above the pI and proteins are negatively charged, proteins indirectly interact with polysaccharides by polyvalent metal ions acting as cation bridges between the negatively charged carboxyl groups on proteins and the negatively charged polysaccharides (Lin, 1977, DeFreitas, 1994). The distribution of ionizable groups and charge density on the surface proteins, the ease of unfolding original structures, and the backbone flexibility and overall charge of the polysaccharides could affect the strength of the electrostatic interactions (Ledward, 1994, Samant *et al*., 1993, Dickinson, 1998).

Covalent interaction is another protein–polysaccharide conjugation formed in Maillard-type reactions (Kasran, 2013).

The interactions in the meat matrix are complex and not limited to protein-polysaccharides electrostatic linkage. Researchers have investigated several physical and chemical interactions in meat systems. Electrostatic interactions and hydrogen bonds were determined as the major forces involved in the calcium alginate/pork myofibrillar protein gel systems (Ustunol *et al*., 1992). Li *et al*. (2017) reported that the over-drying potato starch might stabilize the network structure of surimi protein gel by increasing hydrogen bonds and non-disulfide covalent bonds and decreasing ionic bond. These interactions contribute to the alteration of functionalities, texture and sensory attributes of different types of meats.

2.2.4.2. Non-meat proteins

Non-meat proteins derived from animal or plant sources were extensively utilized in a variety of meat products in the last century. Vegetable proteins can be processed in coarse ground meat products in order to bind fat, in finely comminuted systems in order to stabilize emulsion, or in whole muscles to increase water holding capacity and structural integrity (Petracci *et al*., 2013).

Collagen and gelatin extracted from pork, beef and poultry by products have better water holding ability and gelling ability compared to most starches and soluble fibres. As a consequence of particular hydration characteristics (swelling and solubility), collagen is added in raw minced products to increase moisture retention and brines of injected/tumbled whole muscle products to retain juiciness (Petracci *et al*., 2013). Gelatin is derived from collagen by heating. Gelatin can form two kinds of gel melting between 27 °C and 34 °C: physical gel (transparent, elastic, and thermally reversible) and chemical gel (stiff and thermostable) at low concentration (0.5% to 1%

w/w) (Nazir, Asghar & Maan, 2017). It is added to canned meat products, emulsified low fat products and jellied products (Petracci *et al*., 2013).

Milk protein contains two major proteins: whey protein and casein (mainly sodium caseinate). Whey protein can form thermally irreversible gel with a complex network of protein aggregates, strings and clusters after a series of transitions including unfolding/denaturation of native structure followed by aggregation and the strand formation, and association of strands (Nazir, Asghar & Maan, 2017, Banerjee & Bhattacharya, 2012). In contrast, native casein is highly hydrophobic and micelles aggregate together (Banerjee & Bhattacharya, 2012). It cannot form gel matrix and has relatively lower moisture retention ability than whey (Petracci *et al*., 2013), unless acidification or enzymatic hydrolysis are implemented (Nazir, Asghar & Maan, 2017, Banerjee & Bhattacharya, 2012). However, sodium caseinate is able to bind fat and favours higher viscosity in emulsions. Therefore, whole milk, whey, or casein can act as binders or extenders in both coarse ground and emulsified systems, and soluble proteins such as whey protein concentrate and Hydrolysed casein can be used in marinated or injected products (Petracci *et al*., 2013). Youssef & Barbut (2010) reported that 2% meat protein substitution (sodium caseinate, milk protein isolate or whey protein isolate) reduced cooking loss of emulsified beef batter compared to whole meat batter. Whey protein isolates provide higher moisture retention due to the formation of distinct gel regions in meat batter matrix. Different types of dairy proteins were also successfully applied in low-fat emulsified beef sausages by Marchetti, Andrés & Califano (2013) and chicken frankfurters made from mechanically deboned meat by Barbut (2007).

Soy protein isolates (high dry-weight protein content), composed of two major proteins: β-conglycinin and glycinin, are the most commonly used plant proteins in meat industry. β-conglycinin is a trimeric globular glycoprotein with three subunit types (α′, α, and β) in seven combinations (Petracci *et al*., 2013). Glycinin is a polymer with an acidic and a basic polypeptide linked by a disulphide bound (Renkema, Knabben & Van Vliet, 2001). The popularity of soy isolates can be interpreted by their high nutritive value and increase of the protein level in meat products. They reduce the formulation costs by replacing lean meat, and enhance sliceability and consistency in restructured and emulsified products (Petracci *et al*., 2013). Soy protein isolates combined with carrageenan were used in ground pork patties by Gao, Zhang & Zhou (2015). The soy/carrageenan mixture resulted in a harder and chewier texture, lower cooking loss and higher thermal stability due to lower exudation rate of water and fat. Incorporation of protein and polysaccharide induced smooth and compact structure with a continuous protein matrix. Soy protein has potential as binder or extender in beef patties (Kassama, Ngadi & Raghavan, 2003) and dehydrated chicken rings (Mishra *et al*., 2015).

Both albumen (in egg white) and yolk in egg are pable to gel upon heating (Banerjee & Bhattacharya, 2012). Egg albumen can be described as an aqueous solution with plentiful globular proteins (Nazir, Asghar & Maan, 2017). The denaturation temperature of egg albumen is around 60 °C, lower than that of egg yolk (around 70 °C) (Petracci *et al*., 2013). Due to the non-reversible gel that albumen can form, egg white is used as binding agent in restructured meat products, and fat emulsifier, contributing to the firmness (Teye, Teye & Odoi, 2012).

2.2.4.3. Starches and flours

Native starch granules are composed of amylose with a linear molecular structure and amylopectin with a branched molecular structure. Amylose is responsible for gel strength owing to the formation of hydrogen bonds after heating, swelling, rearranging, and cooling process. Amylopectin provides viscosity and elasticity for the gel matrix and lowers the gelatinization temperature. Figure 2.5 illustrates the gelatinization mechanism of starch. Starches have various functionalities thanks to the differences of amylose/amylopectin ratio, types of monomers and side-chain radicals, and molecular weights (Petracci *et al.*, 2013, Feiner, 2006).

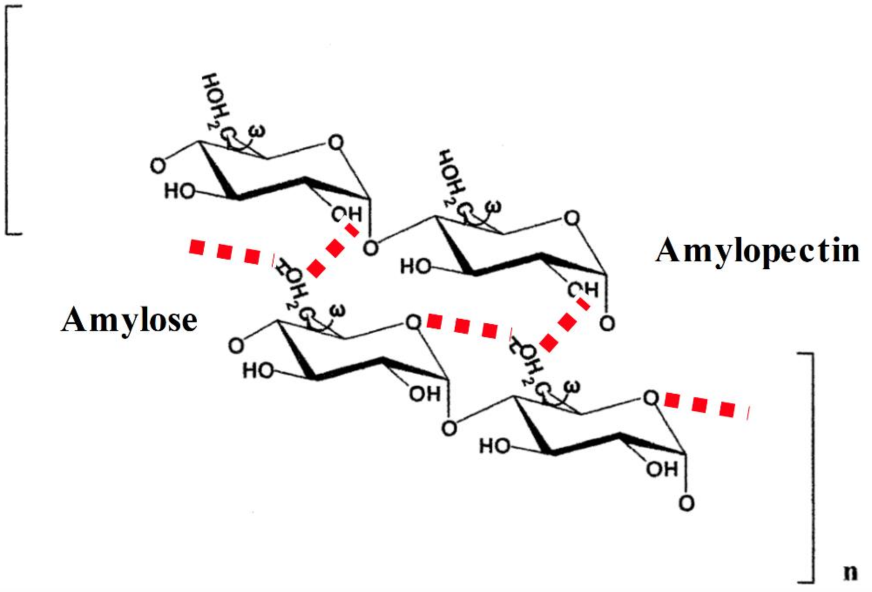


Figure 2.5. Gelatinization mechanism of starch. The dotted lines represent hydrogen bonding (Tako *et al.*, 2014). Open access.

Basically, starches play roles as thickener and gelling agent on account of their bulking and moisture retention properties. Physical and chemical modifications of starches have been applied to simulate fat-like mouth feeling and enhance freeze/thaw stability in meat products. Chemical modification can be achieved via oxidation, cationization, grafting, and derivatization including etherification, esterification and crosslinking, sometimes assisted with microwave, radiation and extrusion (Kaur *et al.*, 2012). However, chemically modified starches are treated as non-clean label ingredients. Pre-gelatinized starches and instant swelling starches obtained or produced by physical modifications are used to eliminate this concern. They have higher water binding capacity during cooking or in cold batter before heating (Petracci *et al.*, 2013, Feiner, 2006).

Flours from crops like pulses and cereals contain both starches and proteins, which are exploited to improve the cooking and textural properties in meat batter and bind meat pieces together in minced meat products. Fine wheat flour with gluten is mostly selected as binder because of low cost and improvement of firmness and cooking loss (Ahamed *et al*., 2007). Pre-jellified cereal flours can also optimize the swelling and hydration properties by inducing complete gelation of starch/protein matrix even at low ultimate cooking temperatures (Petracci *et al*., 2013). Ganie, Kumar & Tanwar (2017) evaluated the effects of replacing 10% lean meat with different combinations of barley flour and pea flour on qualities of low-sodium emulsified fish balls. Incorporation of barley flour and pea flour in 1:3 ratio resulted in optimum cooking yield, physicochemical quality, emulsion stability, and sensory attributes. Low sodium fish balls were safe (lipid oxidation and microbiological profile) for consumption for 2 weeks of refrigeration storage without compromising sensory qualities. Devadason, Anjaneyulu & Babji (2010) investigated the qualities of comminuted buffalo meat nuggets processed with four binders: corn starch, wheat flour, wheat semolina, and tapioca starch at 2.5% respectively. Although no significant difference was observed in frying loss, and moisture and protein content of nuggets, corn starch and refined wheat flour provided harder texture than the other binders. Products with corn starch had higher fat content and emulsion stability. Products formulated with corn starch also had higher sensory scores for overall acceptability and all other attributes. This could be attributed to the microstructure with dense protein network, uniform fat globules, and less vacuoles that corn starch facilitated.

2.2.4.4. Fibres and gums

Fibres are added to meat products as dietary fortification nutrients, and have resistance to digestion and absorption in gastro-intestinal tract without noticeable negative effect on sensory attributes (Toldrá & Reig, 2011). Consumption of foods with higher dietary fibre content favours reducing the risk of cardiovascular diseases, obesity, colon cancer, and other disorders (Talukder, 2015).

Plant fibres are classified into non-carbohydrate lignin, carbohydrate cellulose, and carbohydrate non-cellulose (such as hemicellulose, pectin, gums, mucilage, algal polysaccharide, and resistant starch), differing in structure and in physiological effects. Fibrous cellulose has a higher degree of polymerization and less solubilization in alkali than non-fibrous hemicellulose. Common plant fibres are from whole grains, cereals, pulses, vegetables, fruits, and nuts. Animal and microbial-origin fibres include chitin, chondroitin, yeast glucan, and xanthan gum (Sharma *et al*., 2016, Talukder, 2015). Addition of fibres to meat products not only benefits human health but also has technological use (Talukder, 2015).

Fibres can be obtained as by-products from various potential sources: pomace, peel and pulp refuse, seed, oilcake, stem, hull, husk, and pod, bran, algae and seaweed (Sharma *et al*., 2016). The functional properties of fibres (such as reducing cooking loss, modulating texture, stabilizing emulsion, increasing freeze/thaw stability, replacing fat in reduced-fat meat products) mostly come from their water and oil holding capacities. Fibres provide water holding capacity for coarse systems like burgers, meatballs, and sausages either in cold raw meats during processing and shelf-life or during heating. On the basis of solubility, some soluble fibres can be exploited in marinated lean meats to retain tenderness and juiciness, or in finely comminuted systems to emulsify fat and increase cooking yield, whereas insoluble long fractions fibres in emulsified system prevent fat coalescence. Fibres also contribute to harder texture and give a nice bite for mechanically deboned meats. In pre-dusted systems, fibres can reduce oil absorption and maintain moisture during freezing/reheating by retarding water migration from the inner meat matrix to the coating batter

(Amini *et al*., 2015, Petracci *et al*., 2013). Li, Aliani & Holley (2013) used uncooked dry-fermented sausages adding ground deodorized yellow mustard. Mustard inhibited the growth of *Staphylococcus* and accelerated pH reduction without affecting water activity and instrumental texture in 28 days. When the amount of mustard was above 3%, overall acceptability, flavor, texture scores reduced though consumers liked the appearance and colour. Sausages with 1% mustard were the most acceptable and had similar sensory characteristics to the no mustard control. Álvarez *et al*. (2011) studied functional properties of frankfurters adding 2.5% rice bran or walnut paste as macronutrients and canola-olive oil as pork fat replacer. Frankfurters with canola-olive oil had higher emulsion stability (less water and fat exudates) than regular ones, and combination of walnut paste significantly decreased cooking loss and enhanced emulsion stabilization compared to the incorporation of rice bran and non-binder. In vegetable oil emulsions, products with walnut paste had less lipid oxidation than ones with rice bran at the end of refrigerated storage (21 days).

Gums favour increasing cooking yield, improving texture, and offsetting syneresis without affecting meat protein functionalities due to their gelling properties in meat products (Petracci *et al*., 2013, Feiner, 2006). Gums go through complete hydration in meat batter, followed by the polymer strands crosslinking with each other and forming junction zones. The gum dispersion ultimately transforms into a gel network structure at a critical concentration and a degree of crosslinking (Nazir, Asghar & Maan, 2017). Figure 2.6 shows different types of junction zones. Gels formed after heating and subsequent cooling are termed heat set gels, whereas others formed at room temperature are termed cold set gels.

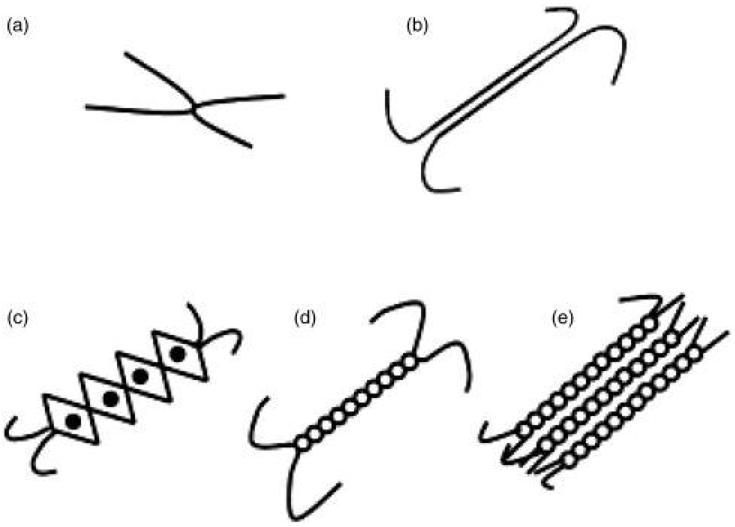


Figure 2.6. Idealized junction zones in polysaccharide gels. (a) Point crosslink, (b) extended block-like junction zone, (c) egg-box model for the junction zones in alginate and pectin gels [the calcium ions (eggs) link the blocks of the polysaccharide chains (egg-boxes) together], (d) double-helical junction zone, and (e) junction zone formed by aggregation of helical segments of the polysaccharide chains (Nazir, Asghar & Maan, 2017). Copyright © 2017 Elsevier Ltd.

Carrageenans are frequently introduced in meat products owing to their water binding capacity, cold stability, and freeze/thaw stability (Talukder, 2015). Only κ- and ι-carrageenans can gel so they can be used in injected or tumbled meats at low percentage to reduce purge loss, maintain juiciness, and enhance sliceability, whereas λ-carrageenan cannot form a gel and is used as a thickener (Petracci *et al.*, 2013, Nazir, Asghar & Maan, 2017). The presence of K^+ helps strong gel formation of κ-carrageenan, whereas Ca^{2+} favours soft gel formation of ι-carrageenan, owing to reducing electrostatic repulsion between anionic polymer chains and producing linkages from disordered coils into the helical state and further incorporating into double helices (Banerjee & Bhattacharya, 2012, Nazir, Asghar & Maan, 2017). Without K^+, κ-carrageenans induce formation of a firm but brittle gel that tends to undergo syneresis, while ι-carrageenans form an elastic gel that resists syneresis. Consequently, κ- and ι- fractions are usually blended in commercial carrageenan to mediate texture and control purge loss in meat products (Petracci *et al*., 2013, Feiner, 2006).

2.3. Research on salt and allergen replacement in processed meat products

2.3.1. Sodium reduction in meat products

Developing salt reduced products should tackle the effects that salt may have on functionalities as water-holding and fat-binding capacity, stability, texture, sensory and shelf life (Desmond, 2006). Several strategies have been applied to reduce sodium content in processed meats by meat processors.

The direct approach is to partially or completely replace sodium chloride with other chloride salt (potassium, magnesium, and calcium chloride) or non-chloride salt (phosphate, lactate, citrate, ascorbate, and sulphate) either separately or in combination (Petracci *et al*., 2013). Potassium chloride is the most common replacer applied in low sodium meat formulations. For example, Pietrasik & Gaudette (2015) successfully applied salt replacer Ocean's Flavor—OF60 (sea salts with 60% less sodium than sodium chloride, replaced by potassium, sulfate, and magnesium, etc.) in smoked turkey sausages without negatively affecting their water binding, texture, and shelf life up to 60 days of refrigerated storage. However, even though some other aspects have been modified with a blend of sodium and potassium chloride, substitution of sodium chloride by potassium chloride or magnesium chloride causes a bitter and metallic aftertaste (Terrell & Olson, 1981). Some researchers suggested that it is possible to apply potassium chloride up to 30% to 40% as salt replacer in processed meat products and retain the functional and sensorial properties, but in order to maintain equvalent protein solubility, 15% more potassium chloride than sodium chloride should be used to alleviate the molecular mass differences between KCl and NaCl

(Paulsen *et al*., 2014, Petracci *et al*., 2013). Another problem of adding potassium chloride compared to sodium chloride is lower microbiological stability at certain concentrations. High level of potassium chloride intake could also be detrimental to heart disease, type I diabetes, and renal disease (Lee *et al*., 2012, Sinopoli & Lawless, 2012, Khaw & Barrett-Connor, 1984). Bitter/metallic aftertaste could be masked by adding some flavourful ingredients such as onion, garlic, and pepper (Petracci *et al*., 2013). In addition, Gaudette & Pietrasik (2017) pointed out that natural salt replacers containing potassium chloride could potentially replace sodium chloride in processed meats with complex flavor profiles such as spices and smoke due to their ability to mask the bitterness elicited by salt replacers, while meats with simple flavor profiles might require further flavor optimization.

Some novel modified potassium chlorides were produced for solving the problems associated with bitter taste of natural potassium chloride. Stanley, Bower & Sullivan (2017) investigated sodium replacement in pork sausage with modified potassium chloride-based salt, bound with citric acid and maltodextrin after spray drying, containing 85% potassium chloride. It was indicated that there was no significant effect of salt replacement on major physicochemical properties including contents of moisture, protein and fat, texture, lipid oxidation, and redness, similar to results published by Zhao & Claus (2013). Sausages made with modified potassium chloride had higher acceptability than those using standard potassium chloride. Furthermore, sensory characteristics of sausages with modified potassium chloride-based salt were similar to those with equal molar sodium control.

Another approach to reducing sodium in meat products is the addition of flavour enhancers and naturally salty tasting products (monosodium glutamate, alapyridain, alkyldienamides, yeast extract, seaweed, vegetable proteins and dehydrated milk) that mask undesirable taste and increase the perception of salt. For example, yeast autolysates allow up to 20% of NaCl reduction by masking the metallic flavour of KCl (Santos *et al*., 2014, Inguglia *et al*., 2017, Desmond, 2006, Busch, Yong & Goh, 2013, Petracci *et al*., 2013). Glutamate enhances both salty and umami flavour mutually (Keast & Breslin, 2003). Potential application of other umami substances such as ribonucleotides might be performed (Dötsch *et al*., 2009). Mycoscent, derived from mycoprotein, can also impart saltiness and enhance flavour without NaCl through delivering natural ribonucleotides and glutamic acid (Verma & Banerjee, 2012).

Additionally, modifying physical forms and status of salt (i.e. reducing particle size through micronization/encapsulation or changing crystal shape) might achieve the alteration of taste bioavailability and further change perceived saltiness (Busch, Yong & Goh, 2013, Rama *et al*., 2013). Smaller salt particles dissolve more rapidly than larger crystals which results in greater saltiness perception (Busch, Yong & Goh, 2013, Shepherd, Wharf, & Farleigh, 1989). Rios-Mera *et al*. (2019) reported that reducing salt from 1.5% to 1.0% using micronized salt had no detrimental effect on the pH, colour, cooking loss and some sensory attributes of beef burgers, including saltiness and juiciness. However, some researchers believe changing size is more suitable for products with original physical crystal salts (such as dry cured meats), whereas it is less effective in meat products where salt is solubilized (Petracci *et al*., 2013). Change in salt shape from granular crystal to flaked increases solubility, blendability and adherence in the saliva and improves fat and water binding properties in red meat batter. Free-flowing crystalline microspheres of salt have maximum surface area to volume ratio which provides more salty flavour (Inguglia *et al*., 2017, Desmond, 2006). The benefit of this solution is meeting clean-label demand of consumers by using pure NaCl without chemical aftertaste. Nevertheless, relatively higher cost and limited commercial application remain to be considered. Figure 2.7 demonstrates some shapes of commercial modified salts.

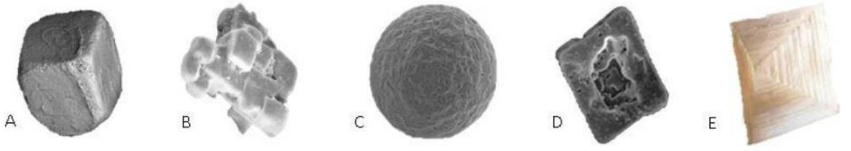


Figure 2.7. Modified shape of salt crystal. A) Normal shape table salt. B) Cargill Alberger Fine Flake Salt. C) Tate & Lyle SODA-LO Salt Microspheres. D) Cargill Star Flake Dendritic Salt. E) Cargill, Alberger Flake Salt (Inguglia *et al*., 2017). Copyright © 2017, Elsevier Ltd.

2.3.2. Replacement of allergen binders in meat products

There has been a lot of research showing that some plant-based binders could replace traditional allergen binders and contribute to comparable or even better functional and sensory properties when used alone or in combination in regular or low-fat restructured, coarse, and comminuted systems.

Ergezer, Akcan& Serdaroğlu (2014) added 10% bread crumbs, and 10% and 20% potato puree in low fat meatballs as extenders. Addition of 20% potato puree increased water holding capacity, moisture and fat retention, and resulted in the highest penetration values of meatballs in comparison of 10% bread crumbs. Incorporation of 10% potato puree contributed to the highest overall sensory acceptability score, thus potato puree could replace bread crumb meatballs. Colle *et al*. (2019) investigated the effect of potato extracts and textured soy protein flour on shelf stability and cooking yield of beef patties. Addition of two binders delayed discoloration and lipid oxidation of patties stored in a retail display. Patties formulated with 2% potato extracts had higher cooking yield that those with 2% soy flour. Der (2010) processed fresh burgers with toasted wheat crumb, green lentil and red lentil flours. Incorporation of 6% green lentil and red lentil flour resulted in significantly ($p < 0.05$) higher redness of burgers compared to products containing 6% wheat crumb. Pietrasik & Janz (2010) reported that processing with 4% pea starch increased the redness of low-fat beef bolognas compared to a no binder control, and there were no significant colour changes between the potato starch and wheat flour treatments. Cooking yields of bolognas with 4% pea starch and wheat flour were comparable. Onweluzo *et al*. (2003) reported that emulsified buffalo loaves processed with 0.5% or 1.0% seed flour of *Detarium microcarpum* (Dm) presented similar ($p > 0.05$) cooking yield, consumer shrink, and water holding ability to control products where 3% wheat semolina was added as a binder. Replacing 3% wheat semolina with 1.0% Dm seed flour in comminuted buffalo loaves with 10% fat could lead to more tender texture. Shand (2000) indicated that there were no differences ($p > 0.05$) in colour, cohesiveness, and springiness of low-fat pork bolognas with 4% potato starch or wheat flour.

2.3.3. Other applications of non-allergen binders in meat products

Studies directly comparing allergen and allergen-free binders are limited. However, extensive publications on application of non-allergen binders in meat products can provide guidance for further research.

Selected vegetative extenders including lentil flour, sorghum flour, boiled and mashed potato, and water chestnut flour mixed into different blends were tested in restructured chicken meat blocks by Malav *et al*. (2015). Chicken meat blocks with 5% lentil flour, 5% sorghum flour, and 5% potato presented higher cooking yield, harder texture, and higher overall acceptability score than products with other blends or control. After 15 days storage at refrigeration temperature under aerobic conditions, products were still acceptable in terms of textural, microbiological and sensory properties.

Al-Juhaimi *et al*. (2016) utilized Moringa seed flour as a potential binder and meat substitute in beef patties. Adding Moringa seed flour to beef patties was reported to improve cooking properties and decrease thiobarbituric acid value and aerobic plate counts during the storage period. Increasing the level of flour led to higher lightness and yellowness but lower redness. Although patties formulated with Moringa seed flour had lower sensory acceptability compared to non-flour control, the sensory stability of flour-formulated patties was higher during 21 days storage and sensory acceptability of non-formulated patties declined significantly during longer storage. Akwetey, Oduro & Ellis (2014) developed meatloaves with 0% to 20% whole cowpea flour in place of ground beef and proved that cowpea flour could decrease cooking loss and increase moisture content of meatloaf compared to the one formulated with no binder. Relatively high overall acceptability scores of meatloaves with up to 15% cowpea flour were obtained, and most of sensory properties were evaluated as “like very much” by consumers for products with up to 10% flour. Replacing meat with 10% flour could save about 27% of production costs. Naveena *et al*. (2006) applied finger millet (ragi) flour in chicken patties and found that diameter and thickness shrinkage was successfully alleviated and addition of 5% contributed to the optimal sensory characteristics. Although pH and lipid oxidation increased, and yellowness and sensory attributes decreased, lightness and redness of chicken patties were maintained during 21 days cold storage. Cha *et al*. (2015) substituted meat with 0% to 30% white jelly mushroom in pork patties. With increase of white jelly mushroom percentage in patties, moisture content, cooking yield, yellowness and lightness increased in comparison of control groups. Patties with 10% mushroom had the highest overall acceptablility while the control had the lowest sensory acceptance. Akwetey & Yamoah (2013) produced low-fat pork patties with solar-dried plantain flour at different levels: 3%, 6%, and 9%. As the plantain flour concentration increased, cooking yield, water holding capacity, and moisture retention increased as well. Patties with 3% plantain flour had similar crude protein percentage to control with no binder and 9% plantain flour led to higher tenderness.

Zargar *et al*. (2014) explored the incorporation of pumpkin at different levels (6%, 12%, 18%) in emulsion type chicken sausages. The authors suggested that although some physicochemical properties such as pH, emulsion stability, cooking yield, and protein content decreased as addition of pumpkin increased, moisture and crude fibre content increased. Sausages with 12% pumpkin presented the optimal comprehensive sensory attributes. Furthermore, fibre enriched sausages could be stored at refrigeration temperature for two weeks without adversely affecting quality. Jang, Lee & Chin (2016) incorporated 1% red bean protein isolate (RBPI) in extracted pork myofibrillar protein gel and concluded that the addition of protein isolate resulted in increased cook yield by approximately 9% compared to non-RBPI control. Shan *et al*. (2015) investigated shaddock albedo as emulsifier in frankfurters at up to 12.5%. Shaddock albedo in frankfurters caused increased lightness, yellowness and hardness, and decreased redness and chewiness. Addition of 5% albedo addition resulted in the highest emulsion capacity of meat batter, while 7.5% concentration brought about the lowest cooking loss, fat content in expressible fluid, and total expressible fluid in cooked products. Gravelle, Barbut & Marangoni (2017) compared effects of native and modified potato and tapioca starch on texture and stability of chicken myofibrillar gels. It was revealed that native potato starch increased moisture retention more effectively than native tapioca starch. Modified potato starch reduced liquid retention while modified tapioca starch increased liquid retention. Native potato and modified tapioca starches caused harder gels as well. Potato starches were swollen and hydrated to a specific extent during protein network formation, but native tapioca starch required higher temperatures to gelatinize, and the modified tapioca was hard to swell. Sanjeewa *et al*. (2010) screened six high-yielding chickpea varieties and two kinds of chickpea flour were reported to improve instrumental and sensory texture properties of low-fat pork bolognas as extended at 2.5% and 5.0%.

Although incorporation of these novel non-allergen ingredients contributes to the improvement of functional properties in processed meats, direct comparision of their efficacy in the same and consistent meat system needs to be further expolored.

Chapter 3. Preliminary screening trials

Studies conducted in the initial phase of this thesis research focused on evaluation of commercially available non-allergenic binders/fillers/extenders and tested their cooking and sensory performance in two different model systems (ground beef and emulsion type pork product).

3.1. Identifying and selection of non-allergen binders for replacement of wheat crumb in beef burgers

3.1.1. Preliminary screening of non-allergen binders for beef burgers

In the coarse ground beef system, the first phase was a general screening of a wide array of different ingredients to shorten the list for internal sensory panels. Regular fat beef burgers were chosen as a model system and initially the burgers were formulated with a 5% inclusion level of tested ingredients. Throughout the screening process, these levels were further adjusted on the basis of sensory and cooking characteristics to determine an acceptable incorporation level.

Initially, locally available Canadian pulses and pulse fractions such as yellow pea, faba bean, chickpea and lentils were incorporated. Among considered pulse ingredients, different flours (red lentil flour, navy bean flour, black bean flour, chickpea flour, yellow pea flour, garbanzo bean flour, and faba bean flour) and pulse fractions (two types of insoluble pea fibre, soluble pea fibre, pea starch, pea protein, faba bean protein) were evaluated. In addition to pulse ingredients, selected non-pulse ingredients that could be used as potential wheat crumb replacers including potato flour or fractions, tapioca starch, rice flour, orange peel fibre, plum and citrus extracts and flax seed meal were tested.

3.1.1.1. Bench top development for preliminary screening

Fresh regular ground beef (25% fat) was purchased from local grocery stores and stored in the refrigerator (4 °C) until use. Beef, spice mix (0.8% salt, 0.15% onion powder and 0.1% black pepper) were blended in a Hobart mixer (N-50, Hobart, Trot, OH, US) with a paddle attachment at low speed for 15 seconds. The respective binders and 12% water were added and mixed for an additional 30 seconds. The meat mixture was formed into patties with 130 mm diameter and 15 mm thickness (around 160 g) using a manual patty press (GVPP50, General Food Service, Weston, FL, US) between two sheets of patty paper, then frozen and stored at -20 °C until cooking. Frozen burgers were placed on a preheated (190–200 °C) grill (Garland ED-42B electric broiler, Russell Food Equipment Ltd, Edmonton, AB, Canada). Burgers were cooked and flipped every 2 minutes, until the internal temperature reached 71 °C. Cooked patties were placed in trays and allowed to cool for about 5 min and weighed. Cooking loss of each treatment was calculated by the following formula: cooking loss = (raw weight - cooked weight) / (raw weight) × 100%.

3.1.1.2. Sensory reference to preliminary screening

The preliminary screening evaluations were used to obtain guidance to narrow down the list and select the best performing ingredients for next phase. Preliminary screening was performed informally by the project team members (n = 6). Cooked beef burgers were individually evaluated for appearance, flavour and textural properties. Flavour and textural attributes were considered as major screening criteria. Each participant described the flavour and textural properties they were perceiving, and assigned a liking, disliking or neutral rating for each burger. Participants identified which addition level they would eliminate based on strong unpleasant binder flavours or pasty/rubbery texture or objectionable colour. Comments on flavour and texture were collected and liking frequency was tallied. Preliminary screening was used to determine the amount of binders that would be evaluated in a larger internal screening panel.

3.1.1.3. Preliminary screening results

Results of preliminary screening showed that at 5% incorporation level, various ingredients contributed different flavour and texture to beef burgers. While some non-allergen ingredients such as flours from faba bean, chickpea, black bean, and garbanzo bean caused undesirable flavours even at reduced incorporation levels in burgers, some others showed a potential for wheat crumb replacement when used at lower addition level. Burgers incorporated with some pulse ingredients such as pea starch and textured pea protein usually exhibited mushy/pastry or incompact (easy to fall apart) texture, whereas pea fibre caused rubbery and tough texture. The textural performance of red lentil flour and white navy bean flour incorporated at 5% was acceptable. Among the non-pulse ingredients, tapioca starch, two types of rice flour, two types of potato starch tapioca starch, two types of rice flour, two types of potato starch also provided soft texture to burgers. Off-flavour and dry texture were observed in burgers with plum powder and PROSUR® PHR (a commercial clean label phosphate replacer). With the reduction of amount added, the negative aspects were alleviated. Based on initial screening results, five pulse ingredients (pea starch, red lentil flour, white navy bean flour, textured pea protein, pea fibre) and eight non-pulse ingredients (tapioca starch, two types of rice flour, two types of potato starch, and plum, citrus and potato extracts) had the best potential for replacement of wheat crumb.

3.1.2. Scale-up evaluation of selected non-allergen binders for beef burgers

In the second phase of preliminary trials small consumer sensory panels (n~30) were employed for further screening and selection of only the best performing candidates to be tested in a full-scale study to determine their effect on meat quality attributes.

Out of over 20 ingredients tested in preliminary, the best performing binders listed in Table 3.1 were selected for further evaluations. The goal of these evaluations was to refine the list and verify their performance and acceptability using a series of small sensory panels.

3.1.2.1. Bench top development for scale-up evaluation

Fifteen different burger treatments incorporated with binders (Table 3.1) were manufactured according to the procedure described in the section ***3.1.1.1***.

Table 3.1. Binder source information and percentage used in burger formulations.

Binder	Source	Content in the formulation
Wheat Crumb	Breader B34216 White #50, Newly Weds Foods, Edmonton, AB, Canada	5%
Native Pea Starch (Accu-Gel)	Nutri-Pea Limited, Portage la Prairie, MB, Canada	3%
Tapioca Starch	Pacific Blends Ltd., Port Coquitlam, BC, Canada	3%
Potato Starch (Ingredion)	Ingredion Canada Inc., Mississauga, ON, Canada	1%
Native Potato Starch	Manitoba Starch Products, MB, Canada	3%
Potato Extract (IQA5038)	Basic American Foods, Walnut Creek, CA, United States	3%
PROSUR® PHR*	Wenda Ingredients, Naperville, IL, United States	0.25%
Plum Powder	Sunsweet Growers, Inc., Yuba City, CA, United States	1%
Medium/Short Rice Flour	PGP International Inc., Woodland, CA, United States	3%
Long Rice Flour	PGP International Inc., Woodland, CA, United States	3%
Red Lentil Flour (Homecraft Pulse 5101)	Ingredion Canada Inc., Mississauga, ON, Canada	5%
White Navy Bean Flour	Infra-Ready Foods, Saskatoon, SK, Canada	5%
Textured Pea Protein (24/30)**	Sotexpro, La Croix Forzy, Bermericourt, France	3%
Pea Fibre (Centara 3)	Nutri-Pea Limited, Portage la Prairie, MB, Canada	2%

*PHR is a commercial clean label phosphate replacer.

**Textured pea protein contains 24% of pea protein.

3.1.2.2. Sensory evaluation of internal panel

Participants were recruited from the Food Processing Development Centre (FPDC) (Leduc, AB, Canada), Agrivalue Processing Business Incubator (APBI) (Leduc, Alberta), or residents of Leduc who frequently consume beef burgers. The evaluation of the patties was conducted in the dedicated sensory evaluation lab at the FPDC. All burger treatments were grouped into 3 sets according to similarities of the binders and each set was evaluated on a particular day. A wheat crumb control was included in the each set as a reference to assure the consistency of results. Three-digit blinding codes were used to label each treatment during panel set up, and all burgers were cut into thirds immediately after being removed from the grill and weighed for cooking loss, individually wrapped in aluminium foil, and placed into a 60 °C chamber (LHU-113, ESPEC Corp., Osaka, Japan) until prepared for serving. All sensory panel responses were collected using a computerized program specific for sensory evaluation (Compusense Cloud, Compusense Inc., Guelph, Ontario). A complete block design was used for each day's panel to conduct sensory evaluations. Samples were presented one at a time. Each panellist evaluated six (Day 1 & 2) or five (Day 3) treatments of burgers. Ninety-eight panellists over 18 years of age (47 males and 51 females) received verbal instructions upon arrival at the FPDC, and were seated at individual testing booths lit with white lighting where written instructions were integrated into electronic ballot presentation. Samples were placed on a 6-inch white coded styrofoam plate (Genpak) and passed through serving hutches to each panellist in a sequential, monadic manner. A forced 90 sec break was administered and room temperature water and unsalted crackers were provided for palate cleansing between samples. Panellists were asked to rate overall acceptability and the acceptability of the appearance, flavour, texture, and aftertaste using a 9-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely. The Check-All-That-Apply (CATA) method was used to further define the flavour attribute with the supplied terms: grilled flavour, cereally, beany, bitter, metallic, off flavour, savoury, tangy, bland, beefy, salty, fruity, potato; and textural attribute: rubbery bite, tough/leathery, crumbly, mushy/pasty, chewy, granular texture, greasy, leaves a mouth coating, nice bite, chalky texture. A 7-point Just-about-right (JAR) scale was used to further describe appearance (colour), texture (firmness and juiciness), and aftertaste (lingerer and pleasantness) characteristics. The JAR scale was anchored with 1 = too pale/soft/dry or no aftertaste/very unpleasant, 2 = moderately pale/soft/dry, 3 = slightly pale/soft/dry, 4 = just-about-right, 5 = slightly dark/firm/juicy, 6 = moderately dark/firm/juicy, and 7 = too dark/firm/juicy or lingering/very pleasant. A 5-point hedonic scale was also used to evaluate purchase intent of each sample, where 1 =definitely would not purchase, 2 = probably would not purchase, 3 = might/might not purchase, 4 = probably would purchase, 5 = definitely would purchase.

Internal sensory data were analysed using two-way ANOVA with treatment as fixed effect and panellist as random effect using Compusense Cloud, and Fisher's LSD was used to test for least squared mean differences ($p < 0.05$). Principle Component Analysis (PCA), Penalty Analysis, Correspondence Analysis, Principal Coordinate Analysis, and Cluster Analysis were generated by XLStat 2016 (Addinsoft, Paris, France) and Origin 2017 (OriginLab, Northampton, MA, USA).

3.1.2.3. Scale-up evaluation results

3.1.2.3.1. Cooking loss of burgers

Cooking loss of burgers is shown in Figure 3.1. Burgers with PHR and plum powder had the highest ($p < 0.05$) cooking loss among all the treatments, similar to the no binder control, and higher than wheat crumb control. This might be caused by the lower amount of binder used. However, higher percentage of PHR and plum powder in burgers was unacceptable because of the rubbery texture or strong plum flavour and colour. The rest of binders resulted in significantly lower cooking loss than those three treatments. Native pea starch resulted in lower cooking loss than the potato starch from Ingredion. The cooking loss of burgers formulated with potato starch (Ingredion), rice flour (both long and medium/short), textured pea protein, and pea fibre were significantly higher than that of ones formulated with pea starch, tapioca starch, potato extract, red lentil flour, white navy bean flour, and even wheat crumb burgers at selected levels.

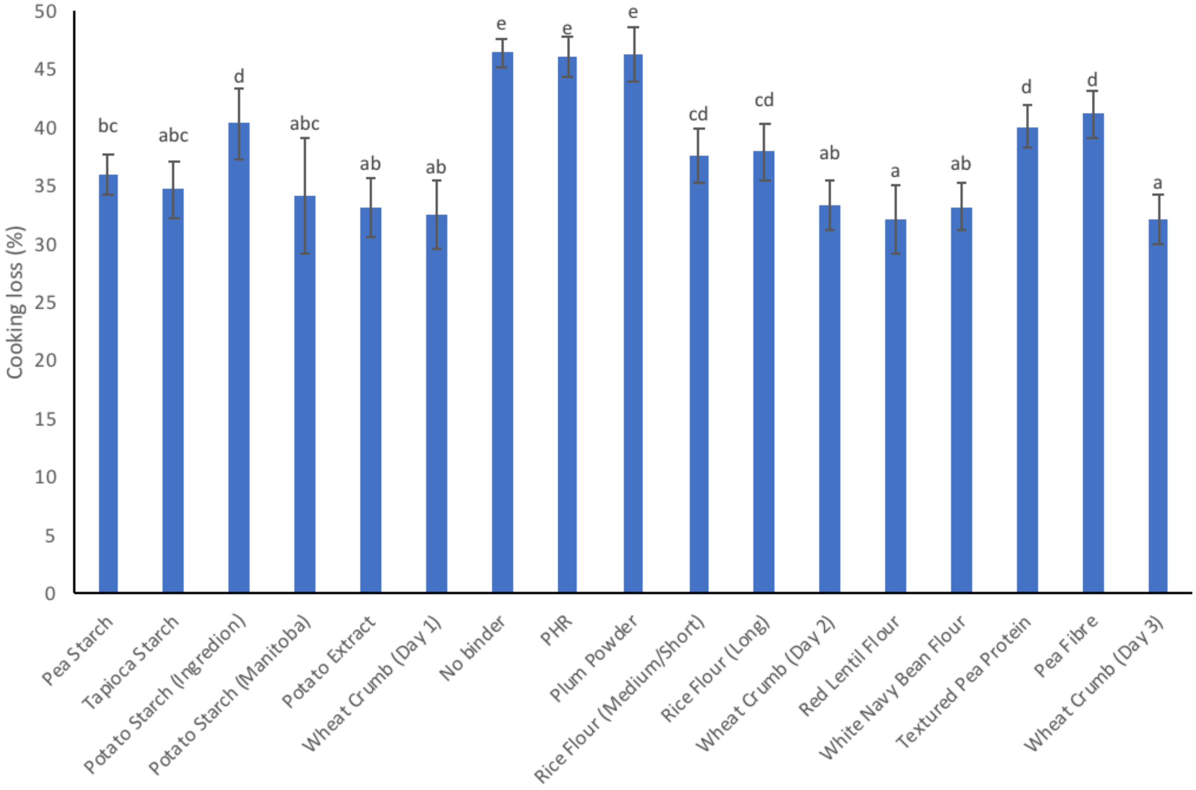


Figure 3.1. Cooking loss of burgers formulated with different binders. Abbreviation: PHR: a commercial clean label phosphate replacer. Vertical bars represent standard deviations. $^{a–e}$Means with different lowercase letters are significantly different (Tukey's HSD, $p < 0.05$).

3.1.2.3.2. Small consumer sensory evaluations of burgers

Although the panels were conducted on different days, there was no significant difference in terms of all the sensory features among wheat crumb controls evaluated on three days (Table 3.2). As for overall hedonic score, burgers processed without any binder or with pea fibre had the lowest overall scores. Pea starch, tapioca starch, potato starch (Ingredion) increased the overall acceptability compared to PHR, and were comparable to wheat crumb. There were no significant (p > 0.05) differences among burgers with potato-based ingredients, rice flours and pulse flours. Consumers also assigned the lowest scores to burgers manufactured without binder or those incorporated with PHR. Wheat crumb burgers had relatively higher appearance scores. Products without any binder or with PHR exhibited lower flavour scores compared to burgers with pea starch, tapioca starch, and potato starch (Manitoba), but were similar to the wheat crumb control. Consumers preferred the texture of burgers processed with wheat crumb, pea starch, potato starch, rice flour, and textured pea protein to burgers with PHR or no binder control. Consumers did not differentiate treatments in terms of aftertaste.

Table 3.2. Liking scores of cooked beef burgers formulated with different binders evaluated by the internal sensory panel.

Treatment	Overall acceptability	Appearance	Flavour	Texture	Aftertaste
Day 1					
Pea Starch	6.94±1.64^{c}	6.85±1.44^{bc}	7.03±1.36^{c}	6.73±1.66^{d}	6.18±1.83
Tapioca Starch	6.58±1.75^{c}	6.39±1.68^{abc}	6.85±1.58^{bc}	5.73±2.00^{bcd}	5.91±1.61
Potato Starch (Ingredion)	6.73±1.68^{c}	6.06±1.85^{abc}	6.52±1.80^{abc}	6.12±1.98^{cd}	5.48±1.64
Potato Starch (Manitoba)	6.45±1.66^{bc}	6.39±1.37^{abc}	6.76±1.39^{bc}	5.97±1.86^{cd}	5.82±1.49
Potato Extract	5.67±1.98^{abc}	6.39±1.58^{abc}	6.21±1.92^{abc}	5.52±2.17^{bcd}	5.52±1.84
Wheat Crumb	6.03±2.01^{abc}	6.76±1.71^{bc}	6.27±1.79^{abc}	6.06±2.22^{cd}	5.70±1.86
Day 2					
No Binder	5.06±1.90^{a}	5.50±1.83^{a}	5.34±1.81^{a}	4.06±1.95^{a}	5.16±1.48
PHR	5.25±1.90^{ab}	5.50±1.55^{a}	5.47±1.54^{a}	4.31±1.75^{ab}	5.19±1.40
Plum Powder	5.88±2.11^{abc}	6.16±1.78^{abc}	5.91±2.04^{abc}	5.00±2.27^{abc}	5.50±1.76
Rice Flour (Medium/Short)	6.47±1.83^{bc}	6.09±1.87^{abc}	6.25±1.83^{abc}	6.09±2.07^{cd}	6.03±1.89
Rice Flour (Long)	6.28±1.84^{abc}	6.41±1.46^{abc}	6.25±1.81^{abc}	5.88±1.84^{cd}	5.72±1.89
Wheat Crumb	6.38±2.18^{bc}	7.06±1.44^{c}	6.22±2.14^{abc}	5.75±2.36^{cd}	5.88±1.76
Day 3					
Red Lentil Flour	5.94±1.95^{abc}	6.30±1.76^{abc}	6.00±1.75^{abc}	5.58±1.97^{bcd}	5.82±1.53
White Navy Bean Flour	5.85±2.09^{abc}	5.94±1.80^{abc}	6.15±1.92^{abc}	5.61±2.06^{bcd}	5.64±1.98
Textured Pea Protein	6.55±1.77^{bc}	6.64±1.52^{abc}	6.52±1.70^{ac}	6.18±1.99^{cd}	6.15±1.87
Pea Fibre	5.06±2.00^{a}	5.88±1.85^{ab}	5.76±1.75^{ab}	4.94±2.15^{abc}	5.67±1.67
Wheat Crumb	6.58±1.64^{c}	6.61±1.58^{abc}	6.82±1.76^{bc}	5.91±2.20^{cd}	6.33±1.41

Results are presented as mean±standard deviation. $^{a–d}$Means with different lowercase letters in the same column are significantly different (Fisher's LSD, $p < 0.05$). Scored on 9-point hedonic scales where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like/dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely. Abbreviation: PHR: a commercial clean label phosphate replacer.

Percentage of frequency distribution for JAR scale of colour are illustrated in Appendix A (Figure A1). JAR was explored for selected binders to get further insight into what improvements may be required to increase consumer acceptability liking scores for burgers formulated with them. Burgers with textured pea protein, rice flour (long), and potato extract were rated most frequently as products with just-about-right colour. PHR and potato starch (Ingredion) treatment contributed to relatively lower just-about-right colour frequency.

Based on the JAR frequencies distribution, penalty table identified potential directions for improvement of the colour attribute. It was concluded that burgers formulated with PHR, pea fibre, potato starch, red lentil flour, and rice flour (medium/short) were penalized too pale (over 20% responses were received). None of the tested binders produced a significant penalty for burgers rated “too dark”.

The frequency figure in Appendix A (Figure A2) shows that grilled flavour, savoury, beefy, and salty were the most frequently checked flavour descriptors by consumers. The map of correspondence analysis (Figure 3.2) shows the differences between the products in terms of their flavour profiles. The first two dimensions explained 51.28% of total inertia. Burgers processed with potato starch, pea starch, potato extract, textured pea protein, plum powder, red lentil flour, rice flour (medium/short), and tapioca starch were close to beefy, salty, savoury, and grilled flavour attributes. However, burgers processed with white navy bean flour and rice flour (long) were relatively far from these attributes. Addition of rice flour (long) led to more off-flavour and tangy flavour. Burgers containing PHR and pea fibre were relatively too bland. Principal coordinate analysis including attributes and liking scores further indicated correlation coefficients and visualized in a two-dimensional map (Figure 3.3). The first two dimensions explain 31.91% of the variation. It was demonstrated that flavour liking score was associated with the attributes grilled flavour, savoury, beefy, and salty.

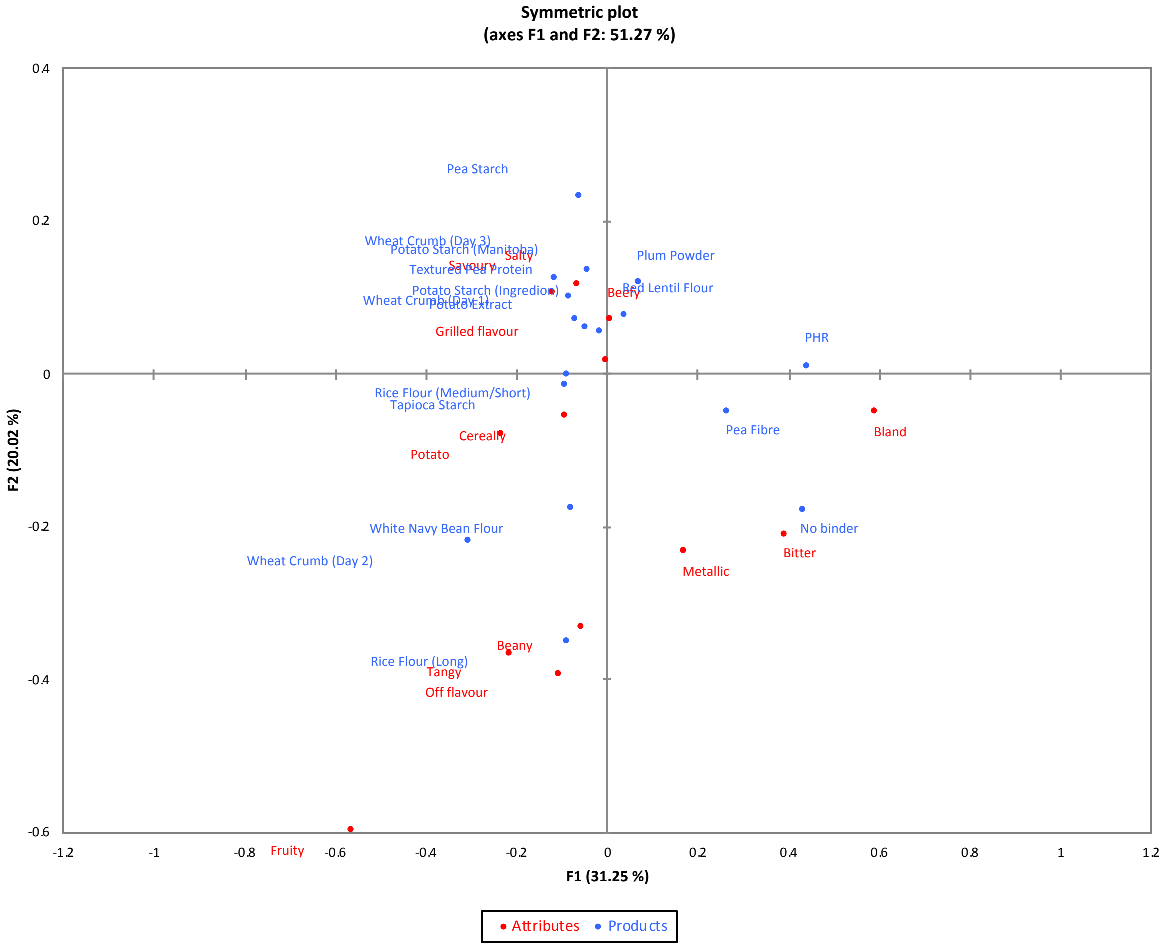


Figure 3.2. Correspondence analysis for Check-All-That-Apply scale of flavour for cooked burgers formulated with different binders evaluated by the internal sensory panel. F1 and F2 account for 31.25% and 20.02% of total variance respectively. Abbreviation: PHR: a commercial clean label phosphate replacer.

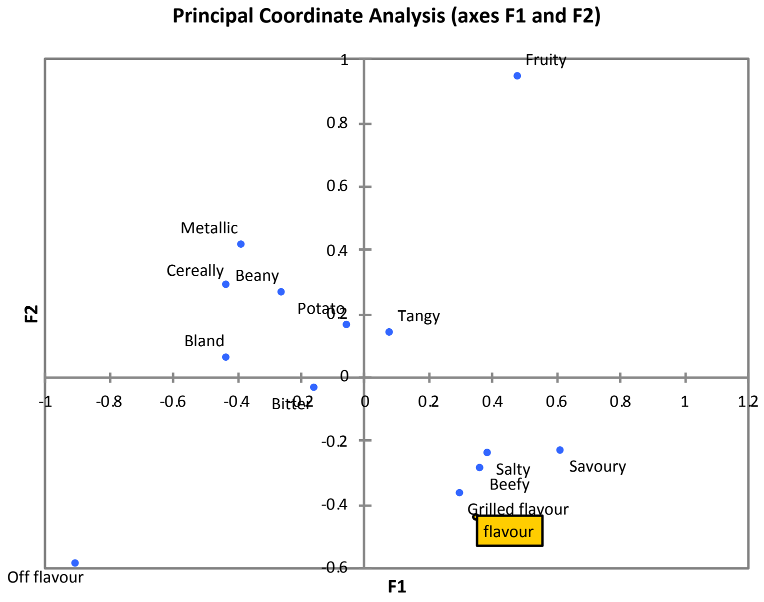


Figure 3.3. Principal coordinate analysis for Check-All-That-Apply scale of flavour for cooked burgers formulated with different binders evaluated by the internal sensory panel.

Percentage of frequency distribution for JAR scale of firmness and juiciness are also illustrated in Appendix A (Figure A3 & Figure A4). Over 60% of consumers evaluated the firmness of burgers with textured pea protein, red lentil flour, rice flour (long), and pea starch as just-about-right. In contrast, only 15.63% consumers believed that the firmness of no binder burgers was acceptable. More than 75% panellists thought textured pea protein, red lentil flour, and pea starch provided ideal juiciness to beef burgers. Based on the frequency, penalty table identified potential directions needed for improvement of texture attribute. Burgers incorporated with PHR, pea fibre, plum powder, potato starch (Ingredion), texture pea protein and burger without binder were significantly too firm. In addition, burgers with PHR, pea fibre, plum powder, potato starch (Ingredion), rice flour, wheat crumb and burger without binder were significantly ($p < 0.05$) too dry, and pea fibre addition resulted in the greatest penalty (mean drop was 3.309).

The frequency distribution figure in Appendix A (Figure A5) shows that PHR, plum powder, and pea fibre burgers were frequently perceived as chewy by consumers. No binder, PHR, plum powder, and pea fibre treatments contributed to rubbery and tough texture in burgers. Red lentil flour and white navy bean flour burgers were most checked as mushy/pasty texture. Texture of pea starch, potato starch (Manitoba), rice flour (long), textured pea protein and wheat crumb burgers were evaluated as nice bite more frequently than other burgers.

The map of correspondence analysis (Figure 3.4) shows the differences among the products in terms of their texture profiles. The first two dimensions explained 88.16% of total inertia. Burgers with white navy bean flour, red lentil flour, potato starch (Manitoba), tapioca starch, and rice flour (medium/short) were close to the attributes mushy/pasty, greasy, chalky, and crumbly. To the contrary, burgers added with PHR, plum powder, pea fibre, potato starch (Ingredion) or processed without binder featured rubbery, tough/leathery, and chewy texture. Burgers with rice flour (long) and wheat crumb (Day 1 & 2) was close to a mouth coating but nice bite. Textured pea protein and pea starch burgers were related to granular texture. Principal coordinate analysis including attributes and liking scores further indicated correlation coefficients and visualized in a two-dimensional map (Figure 3.5). The first two dimensions explained 40.98% of the variation. It is demonstrated that texture liking score was associated to the attributes nice bite, chewy, and granular texture.

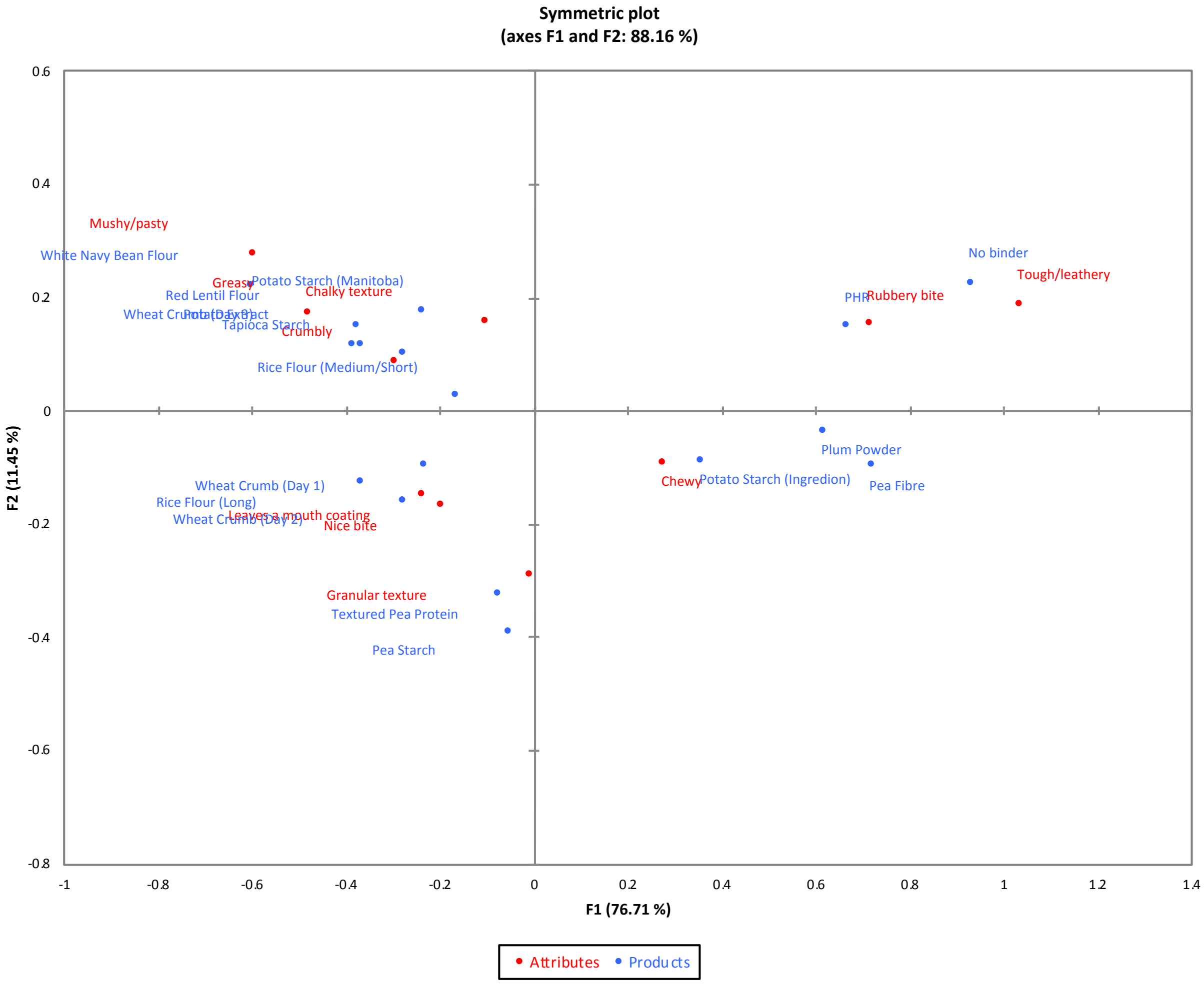


Figure 3.4. Correspondence analysis for Check-All-That-Apply scale of texture for cooked burgers formulated with different binders evaluated by the internal sensory panel. F1 and F2 account for 76.71% and 11.45% of total variance respectively. Abbreviation: PHR: a commercial clean label phosphate replacer.

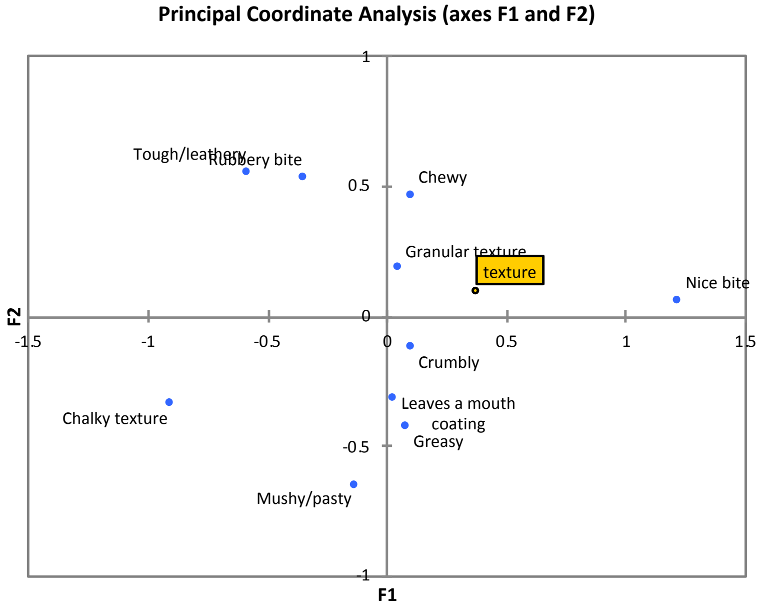


Figure 3.5. Principal coordinate analysis for Check-All-That-Apply scale of texture for cooked burgers formulated with different binders evaluated by the internal sensory panel.

Percentage of frequency distribution for linear intensity of lingering and pleasant aftertaste are illustrated in Figure 3.6 and Figure 3.7. Over 50% of consumers believed that burgers containing white navy bean flour, red lentil flour, wheat crumb, rice flour (long and medium/short), potato extract, potato starch (Manitoba and Ingredion), tapioca starch and pea starch produced lingering aftertaste. When it came to pleasantness among these treatments, wheat crumb, white navy bean flour, rice flour (medium/short), potato starch (Manitoba) and pea starch resulted in pleasant aftertaste in burgers rated by more than half of consumers.

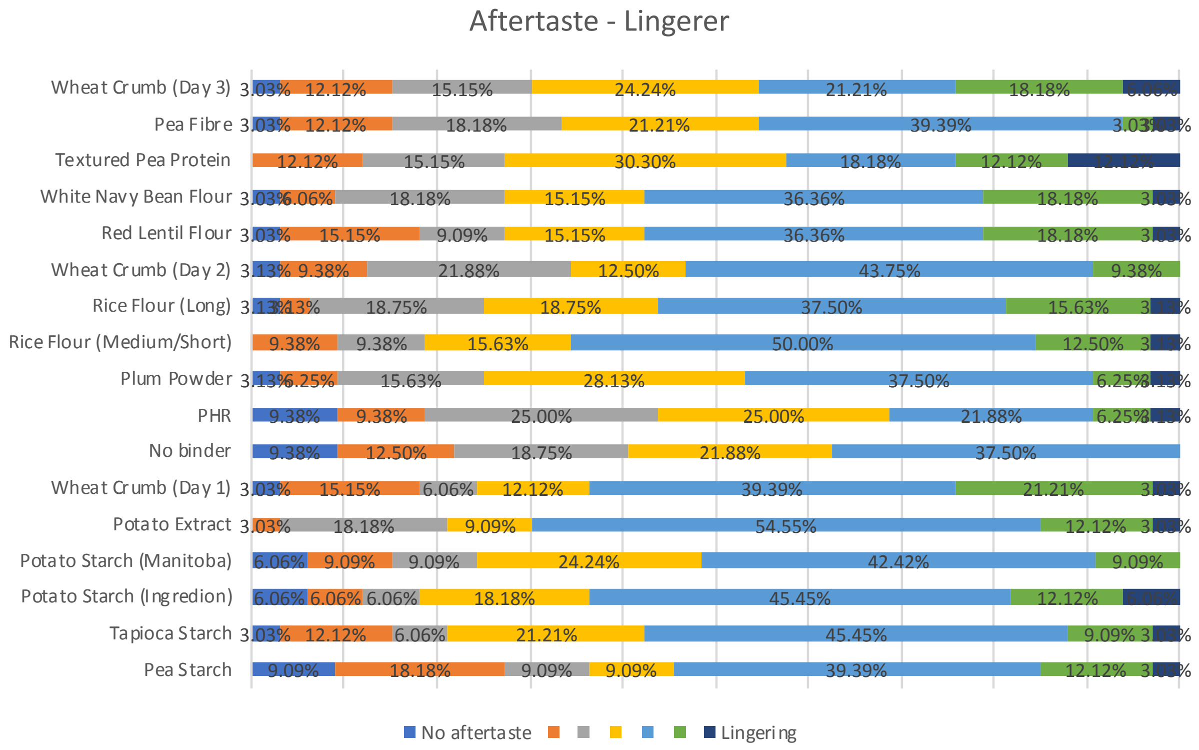


Figure 3.6. Percentage of frequency distribution for linear intensity of lingering aftertaste for cooked burgers formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = no aftertaste, 7 = lingering. Abbreviation: PHR: a commercial clean label phosphate replacer.

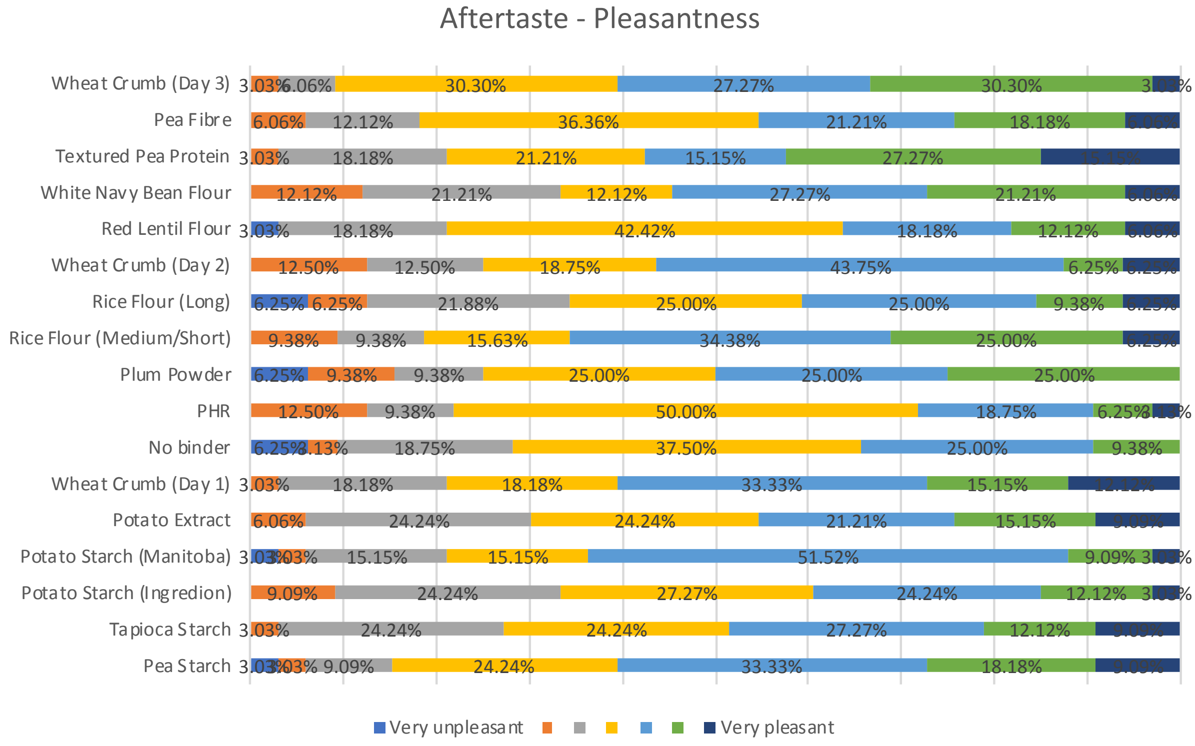


Figure 3.7. Percentage of frequency distribution for Just-about-right scale of pleasant aftertaste for cooked burgers formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = very unpleasant, 4 = just-about-right, 7 = lingering/very pleasant. Abbreviation: PHR: a commercial clean label phosphate replacer.

Percentage of purchase intent from consumers was calculated and demonstrated in Figure 3.8. More than half of panellists indicated they would likely or definitely purchase beef burgers containing wheat crumb, textured pea protein, rice flour (medium/short), and pea starch. Consumers showed relatively lower purchase intent for burgers with both potato starches than these four binders but still higher than other binders. No binder, PHR, pea fibre, and plum powder treatments were less popular (less than 30%) among all the treatments.

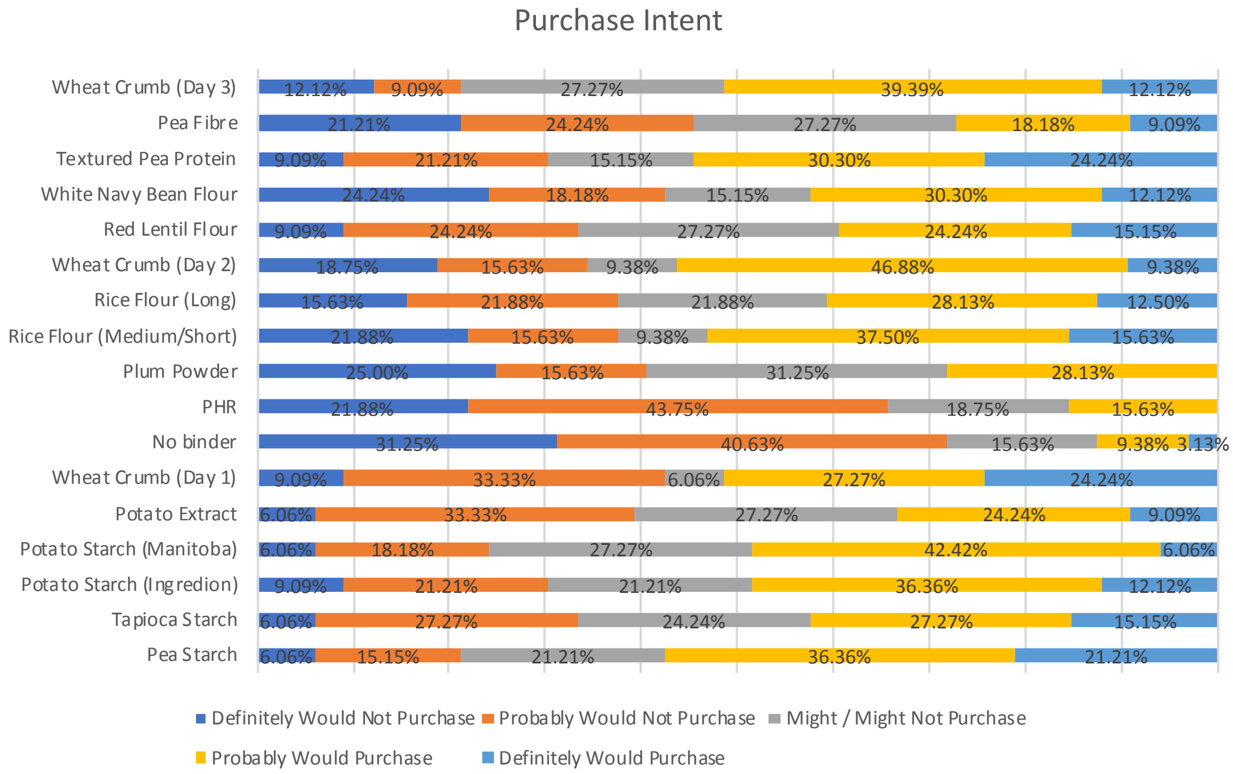


Figure 3.8. Percentage of frequency distribution of purchase intent for cooked burgers formulated with different binders evaluated by the internal sensory panel. Scored on 5-point scales where 1 = definitely would not purchase, 2 = probably would not purchase, 3 = might/might not purchase, 4 = probably would purchase, 5 = definitely would purchase. Abbreviation: PHR: a commercial clean label phosphate replacer.

Figure 3.9 shows the biplot of PCA. The first two principal components represent 89.00% of the initial variability of the data. The horizontal axis is linked with cooking loss, purchase intent, and sensory attributes more than the vertical axis because squared cosine values of the variables (attributes) to the first principal component were always larger than values to the second principal component calculated from PCA. Sensory attributes including appearance, aftertaste, texture, flavour and overall acceptability were positively correlated to each other. The observations (treatments) on the two-dimensional biplot were located according to the principal component scores (Table 3.3). On the horizontal axis, burgers added with potato extract, white navy bean flour, pea fibre, PHR, plum powder and no binder were on the opposite side of the centre, which indicated that they had higher cooking loss and lower sensory scores than products with other binders. Wheat crumb controls were located in the first quadrant, as well as red lentil flour treatment. Two kinds of rice flour were close to each other, but potato starch from Manitoba had higher principal component score on both axes. Location of potato starch (Manitoba) was close to tapioca starch, meaning similar characteristics. The pea starch and textured pea protein were located close to texture, flavour, aftertaste, and purchase intent attributes owing to higher hedonic scores presented early. The dendrogram of hierarchical clustering (Figure 3.10) also further shows the grouping results of treatment characterized by similar comprehensive attributes based on the two principal component scores.

Table 3.3. Principal component scores of biplot for different binders of cooked burgers in the first two dimensions.

Treatment	Principal Component 1	Principal Component 2
Pea Starch	3.47663	-0.78875
Tapioca Starch	1.51965	-0.22694
Potato Starch (Ingredion)	0.49666	-1.30169
Potato Starch (Manitoba)	1.49937	-0.04812
Potato Extract	-0.31587	1.16995
Wheat Crumb (Day 1)	1.13526	0.99638
No binder	-5.19073	-0.16559
PHR	-4.58723	-0.38185
Plum Powder	-2.1428	-0.95138
Rice Flour (Medium/Short)	0.77806	-0.55978
Rice Flour (Long)	0.31073	-0.19171
Wheat Crumb (Day 2)	1.41158	0.83666
Red Lentil Flour	0.21556	1.09912
White Navy Bean Flour	-0.54029	0.75

Textured Pea Protein	1.86782	-0.87809
Pea Fibre	-2.48417	0.42107
Wheat Crumb (Day 3)	2.54977	0.22073

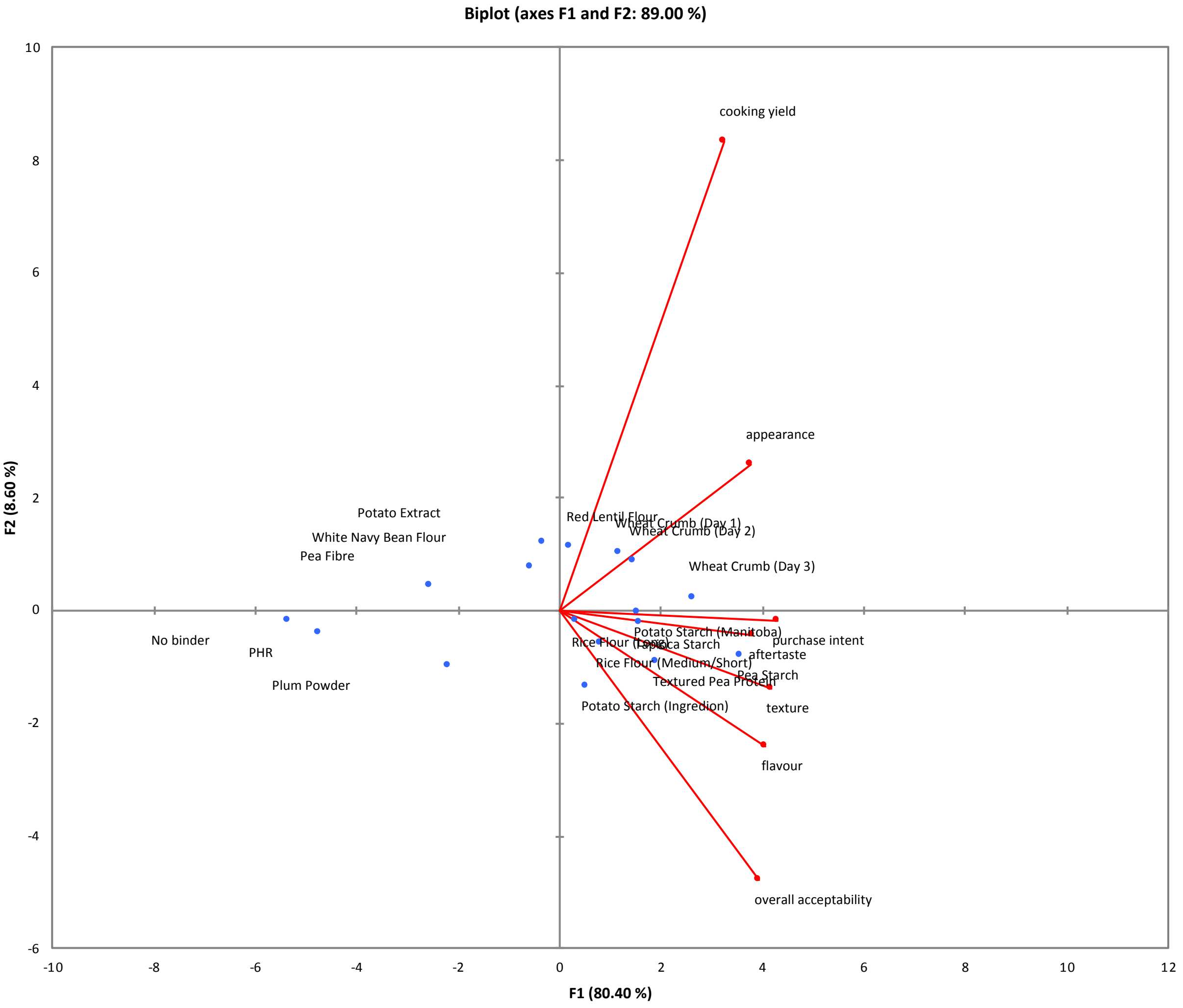


Figure 3.9. Biplot of principal component analysis for attributes of cooked burgers formulated with different binders evaluated by the internal sensory panel. F1 and F2 account for 80.40% and 8.60% of total variance respectively. Rays represent loadings of variables, and points represent treatments.

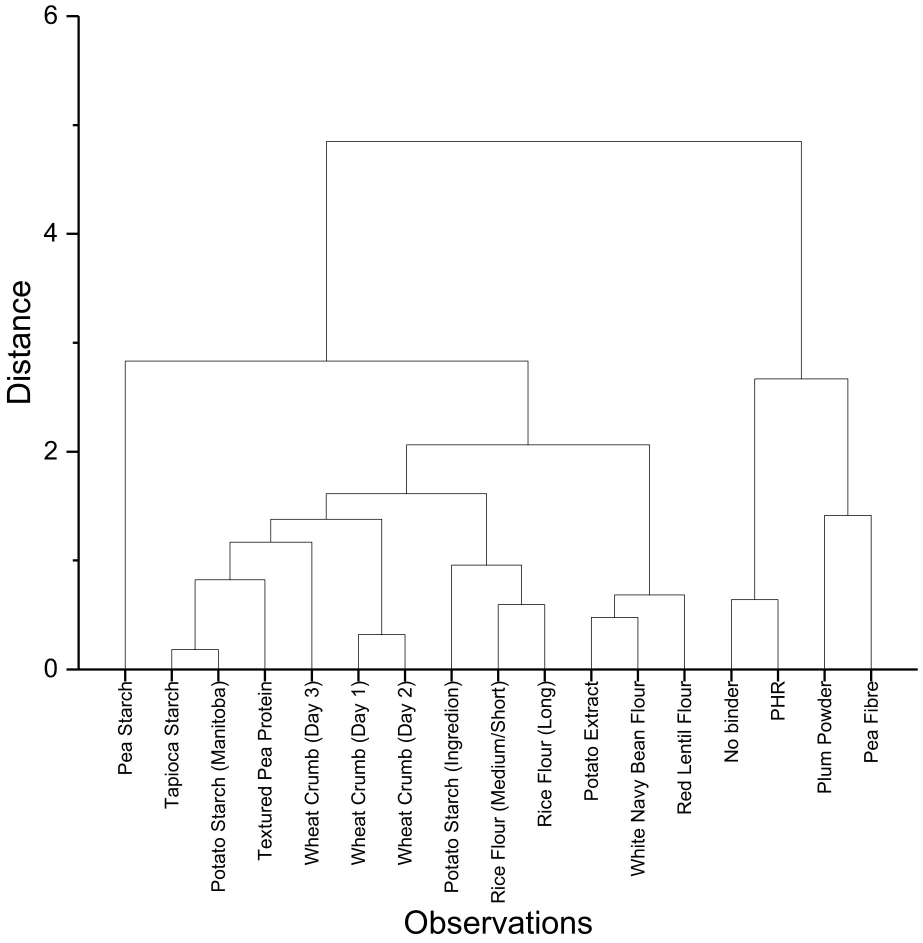


Figure 3.10. Dendogram of hierarchical cluster analysis for attributes of cooked burgers formulated with different binders evaluated by the internal sensory panel.

3.1.3. Conclusion

Overall, burgers with pea starch, tapioca starch, potato starch, rice flour, and textured pea protein had similar comprehensive hedonic scores to a wheat crumb control and relatively higher scores than other treatments. Therefore, they could be applied in the next stage of experiments.

PHR, pea fibre, plum powder, and no binder treatment resulted in firm and dry texture of burgers, which consumers disliked. Burgers containing potato extract, red lentil flour, and white navy bean flour, and tapioca starch burgers featured soft even mushy texture. Thus, these ingredients were not considered for further studies.

Burgers with potato starch (Ingredion) were tougher and drier than ones with potato starch (Manitoba). Rice flour (long) caused unpleasant aftertaste in burgers compared to rice flour (medium/short). Cluster analysis indicated that tapioca starch was similar to potato starch (Manitoba). Consequently, native potato starch and short rice flour would be selected as the representatives of potato starch and rice flour respectively.

Based on the penalty analysis, it was suggested that the texture of burgers with potential ingredients such as native pea starch, short rice flour was too soft or dry, while texture of burgers with other ingredients, like textured pea protein, was considered too firm. Incorporation levels of the potential ingredients mentioned above in this chapter were all 3%. These opposite results indicated that in order to explore their effect on functional and sensory attributes in a full-scale experiment more effectively, both higher and lower concentrations should be included in further research.

In conclusion, consumer sensory data demonstrated that textured pea protein, pea starch, potato starch (Manitoba), and rice flour (medium/short) had the best potential for wheat crumb replacement in beef burgers. To verify these initial findings, a full-scale experiment evaluating the effectiveness of these binders incorporated at two levels (2%, 4%) into burger patties was completed using a pilot plant equipment.

3.2. Identifying and selection of non-allergen binders for wheat flour replacement in emulsion type meat systems

3.2.1. Preliminary screening of non-allergen binders for pork bolognas

The objective of sensory evaluation conducted during this phase of the study was to provide feedback and guidelines about the acceptability of regular salt pork bolognas formulated with varying types and levels of non-allergen ingredients. The recipes of the products with the best performance were then refined as the project moved into the next stage of product development. Similar to screening the non-allergenic ingredients for burgers, the preliminary screening of binders for emulsion type product was performed informally by the project team members. Bench top emulsion type products were developed using a small-scale food processor. Each treatment was evaluated for flavour and textural properties. Texture and flavour characteristics were the major focus with each participant describing the flavour and textural attributes they were perceiving, and assigned a liking, disliking or neutral rating for each bologna treatments. Participants were also asked to identify any samples they would eliminate based on strong, objectionable pulse flavours or pasty/rubbery texture. Comments on flavour and texture were recorded and liking frequency was tallied.

Throughout the screening process, different pulse (deflavoured faba protein, deflavoured pea protein, deflavoured chickpea, red lentil flour, yellow lentil flour, white navy bean flour, pea starch, pea protein, two types of insoluble pea fibre, two types of rice flour, yellow pea flour, faba bean flour, precooked garbanzo flour) and non-pulse (potato extract, two types of potato starch, potato flour, tapioca starch, hydrolysed collagen, plum and citrus extracts) ingredients were tested.

Out of the tested ingredients, based on the total liking frequency of each treatment after several preliminary sensory screening rounds and cooking loss of the products, the best performing binders shown in Table 3.4 were selected for further evaluations.

The addition levels for each treatment in this experiment were selected according to a typical commercial usage to reflect the major chemistry component of the binder. Starch-based and flour-based ingredients were added at 3%, fibre-based ingredients were used at 2–3% and protein-based ingredients were added at 1–2%.

Table 3.4. Treatment information and percentage used in regular salt bologna formulations.

Treatment	Source	Content in the formulation
Citrus fibre	Newly Weds Foods Inc., Chicago, IL, United States	1%
Potato starch (Ingredion)	Ingredion Canada Inc., Mississauga, ON, Canada	3%
Native Potato starch	Manitoba Starch Products, MB, Canada	3%
White navy bean flour	Infra-Ready Foods, Saskatoon, SK, Canada	3%
Tapioca starch	Pacific Blends Ltd., Port Coquitlam, BC, Canada	3%
Hydrolysed collagen (Solugel 5000)	PB Leiner, Davenport, IA, United States	1%
Pea fibre (Centara 3)	Nutri-Pea Limited, Portage la Prairie, MB, Canada	2%
Pea fibre (Uptake 80)	Nutri-Pea Limited, Portage la Prairie, MB, Canada	3%
Native Pea starch (Accu-Gel)	Nutri-Pea Limited, Portage la Prairie, MB, Canada	3%
Medium/Short Rice flour	PGP International Inc., Woodland, CA, United States	3%
Sodium Tripolyphosphate	Newly Weds Foods Inc., Chicago, IL, United States	0.3%

3.2.1.1. Bench top development for preliminary screening

Bench top development was conducted in a culinary lab at FPDC. The frozen pork leg lean meat and back fat were obtained and kept in the freezer (-20 °C). Prior to processing, the meats were thawed at 4 °C for 24 h, then trimmed and ground separately through a 3 mm plate (K & G Wetter, Model AW114, Mississauga, ON, Canada). Samples were taken from each batch of ground meat and fat, and proximate composition was determined using a Foss FoodScan analyser

(FoodScan Lab, Type 78800, FOSS, Hilleroed, Denmark). For each of the treatments, protein (from lean meat and back fat) content was adjusted to a constant level of 14% and fat to 10% in all formulations by adding water and shredded ice. Lean pork, 1.52% NaCl, and 0.3% Prague Powder (containing 6.5% sodium nitrite and 93.5% sodium chloride) were added into a Vertical cutter (UMC 12 F, Stephan Machinery GmbH, Hameln, Germany) pre-cooled to lower than 8 °C using circulating ice water, with half amount of water for 90 s of processing at 3000 rpm. Each treatment (Table 3.4) or no binder and 1% spices (0.72% dextrose, 0.15% black pepper, 0.05% nutmeg, 0.05% garlic powder, 0.03% onion powder) were added, with half amount of water for 90 s of processing at 3000 rpm. Fat was added and mixed under vacuum for another 2 min at 6000 rpm. The final temperature of the batter never exceeded 15 °C. The total amount of each batch was 2 kg. The emulsion bologna batter was transferred into food-grade plastic containers and heat processed in a Combi-Steamer (SelfCookingCenter® SCC 62, Rational Canada Inc., Mississauga, ON, Canada) at 70 °C for 15 min and then at 80 °C to an endpoint temperature of 71 °C. When the central temperature reached 71 °C, the bolognas were cooled immediately in running water until they reached a core temperature of 30 °C and were stored at 4 °C until analysed.

3.2.1.2. Sensory reference to preliminary screening

The preliminary screening evaluations were used to assist with defining product specific attributes to be used for future ballot question generation. Preliminary screening was performed informally by the project team members (n = 6). Bolognas were sliced into 5 mm pieces. Each treatment was individually evaluated for appearance, flavour and textural properties. Flavour and textural attributes were considered as major screening criteria. Each participant described the flavour and textural properties they were perceiving, and assigned a liking, disliking or neutral rating for each burger. Participants identified which treatment they would eliminate based on strong unpleasant binder flavours or pasty/rubbery texture or objectionable colour. Comments on flavour and texture were collected and liking frequency was tallied. Preliminary screening was used to determine which binders would be evaluated in a small consumer sensory screening panel.

3.2.1.3. Preliminary screening results

After tasting sessions, the descriptors that were most frequently used by panellists to characterise flavour and texture of bolognas were tallied and summarized in Table 3.5. Citrus fibre led to unpleasant fruity flavour in the bologna. Potato starch (Ingredion) caused off flavour and loose structure. Although bolognas with sodium tripolyphosphate tasted flavourful and juicy, the tough texture was still a problem. Tapioca starch and rice flour (medium/short) both contributed to mushy texture in bolognas. Although treatments such as native pea starch, native potato starch, white navy bean flour, hydrolysed collagen, and pea fibre (Centara 3) also caused different flavour and texture of bologna, evaluation panel still believed the overall palatability of bolognas was acceptable and could be selected for a pilot plant trial. Based on these evaluations it was decided to select white navy bean flour, hydrolysed collagen, pea fibre (Centara 3), pea starch (Accu-Gel), and potato starch (Manitoba) as binders in scale up trials.

Table 3.5. Sensory reference to flavour and texture of pork bolognas formulated with different binders for preliminary screening.

Treatment	Flavour	Texture
citrus fibre	fruity, sweet	firm, fibrous
potato starch (Ingredion)	off flavour	soft, falls apart
no binder	bland, salty, peppery, metallic aftertaste	firm, grainy
sodium tripolyphosphate	flavourful, salty	rubbery, juicy, springy
white navy bean flour	okay flavour	rubbery, fibrous
tapioca starch	tangy, bland, metallic aftertaste	pasty, mushy, fibrous
hydrolysed collagen	salty	good bite, juicy, a bit rubbery
pea fibre (Centara 3)	savoury, meaty	rubbery, little soft
pea fibre (Uptake)	cereal off flavour	dry, rubbery
pea starch (Accu-Gel)	bland	harder but not rubbery, mealy but not soft
rice flour (medium/short)	bolo flavour, savoury	pasty, soft
potato starch (Manitoba)	salty, a bit tangy, meaty	firm, dry, fibrous

3.2.2. Scale-up evaluation of selected non-allergen binders for pork bolognas

3.2.2.1. Pilot plant production for scale-up evaluation

All manufacturing was carried out in a refrigerated pilot plant (<7 °C) at Food Processing Development Centre (FPDC) (Leduc, AB, Canada). The frozen pork leg lean meat and back fat were obtained from a local processor and kept in the freezer (-20 °C). Before processing, the meats were thawed at 2 °C for 48 h, trimmed and separately ground through a 4 mm plate (Model AW114, K & G Wetter, Mississauga, ON, Canada). Samples were taken from each batch of ground meat and fat, and proximate composition was determined using a Foss FoodScan analyser (Type 78800, FoodScan Lab, FOSS, Hilleroed, Denmark). Protein (from lean meat and back fat) content was adjusted to a constant level of 14% and fat to 10% in all formulations by adding water and shredded ice. Lean pork, 1.8% NaCl, and 0.02% sodium nitrite were added into a 30 L bowl silent cutter (Seydelmann, Stuttgart, Germany), with half amount of water and chopped for 90 s at low speed (3000 rpm knife speed). The respective binders (3% white navy bean flour, 3% potato starch, 3% pea starch, 2% pea fibre, 1% hydrolysed collagen) and spice mix (0.72% dextrose, 0.15% black pepper, 0.05% nutmeg, 0.05% garlic powder, 0.03% onion powder) were added with remaining amount of water and chopped for another 90 seconds. During chopping fat was added and the bowl chopper was stopped and the lid and sides were scrapped to evenly distribute ingredients. Finally, the meat batter was chopped (intermediate bowl and 6000 rpm knife speed) under vacuum (-0.8 bar) for 2 min. The final temperature of the batter never exceeded 8 °C. The total amount of each batch was 10 kg. The emulsion batter mixture was vacuum stuffed (Handtmann, Model VF80, Waterloo, ON, Canada) into moisture-proof casings (105 mm diameter) at full vacuum. Casings were tensioned and clipped, and the bologna sausages were thermally processed in a smokehouse (Fessmann GmbH u. Co., Winnenden, Germany) to a final internal temperature of 71 °C using a HH23 Microprocessor thermometer (Omega Engineering Inc., Stamford, CT) with copper constantan thermocouples inserted in the geometrical centre of the sausages. The product was cooled overnight before each chilled bologna was removed from its casing and weighed to determine cooking loss. Overall cooking loss was calculated as a percentage of raw stuffed weight before cooking. One chub per formulation was prepared as 2 mm slices that were vacuum packed (10 slices per package) in highbarrier, mylar/polyethylene pouches (Ulma TF-Supra packaging machine, CyE.S. Coop., Ltd., ONATI, Spain). The remainder was vacuum packaged and all samples were stored in cartons at 2 °C until sampling for sensory and instrumental evaluations.

3.2.2.2. Small consumer sensory evaluation of bologna

Participants were recruited from the FPDC, Agrivalue Processing Business Incubator (APBI) (Leduc, Alberta), or residents of Leduc who frequently consume bologna type sausages. The evaluation of the regular salt bolognas was conducted in the dedicated sensory evaluation lab at the FPDC. Three-digit blinding codes were used to label each treatment during panel set up, and all products were refrigerated (LHU-113, ESPEC Corp., Osaka, Japan) until prepared for serving. All sensory panel responses were collected using a computerized program specific for sensory evaluation (Compusense Cloud, Compusense Inc., Guelph, Ontario). A complete block design was used to conduct panel evaluations. Samples were presented one at a time. Each panellist evaluated six sliced bolognas. Forty-seven panellists over 18 years of age (20 males and 27 females) received verbal instructions upon arrival at the FPDC, and were seated at individual testing booths lit with white lighting where written instructions were integrated into electronic ballot presentation. Samples were placed on a 6-inch white coded styrofoam plate (Genpak) and passed through serving hutches to each panellist in a sequential, monadic manner. A forced 90 sec break was administered and room temperature water and unsalted crackers were provided for palate cleansing between samples. Panellists were asked to rate overall acceptability and the acceptability of the appearance, flavour, overall texture, juiciness, firmness, chewiness and aftertaste using a 9-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely. The Check-All-That-Apply (CATA) method was used to further define the flavour attribute with the supplied terms: bitter, metallic, cereally/starchy, bland, off flavour, salty, flavourful, typical bologna flavour, savoury, spicy. A 7-point Just-about-right (JAR) scale was used to further describe texture characteristics: juiciness, firmness and chewiness. The JAR scale was anchored with 1 = too dry/soft/crumbly, 2 = moderately dry/soft/crumbly, 3 = slightly dry/soft/crumbly, 4 = just-about-right, 5 = slightly juicy or moist/firm/rubbery, 6 = moderately juicy or moist/firm/rubbery, and 7 = too juicy or moist/firm/rubbery. A 5-point hedonic scale was also used to evaluate purchase intent of each sample, where 1 = definitely would not purchase, 2 = probably would not purchase, 3 = might/might not purchase, 4 = probably would purchase, 5 = definitely would purchase.

Consumer sensory data were analysed using two-way ANOVA with treatment as fixed effect and panellist as random effect using by Compusense Cloud, and Fisher's LSD was used to test for least squared mean differences ($p < 0.05$). Principle Component Analysis (PCA), Penalty Analysis, Cochran's Q Test, Correspondence Analysis, Principal Coordinate Analysis, and Cluster Analysis were generated by XLStat 2016 (Addinsoft, Paris, France) and Origin 2017 (OriginLab, Northampton, MA, USA).

3.2.2.3. Small scale sensory evaluation results of bolognas

Table 3.6 shows the liking scores of several sensory attributes for bolognas formulated with different binders evaluated by an internal panel. Bolognas processed with white navy bean flour and hydrolysed collagen had significantly ($p < 0.05$) higher overall acceptability than ones with pea fibre. There were no statistical differences among bolognas with potato starch, pea starch and no binder bolognas in terms of overall acceptability. However, bolognas with potato starch and pea fibre scored higher than those with pea starch and no binder in terms of appearance. Hydrolysed collagen resulted in higher flavour liking score than pea fibre in bolognas and no significant difference was found among other treatments. Although from the radar plot juiciness mean scores of selected samples seemed to be different, it could not be distinguished statistically. In terms of overall texture, firmness, chewiness, and aftertaste, bolognas formulated with hydrolysed collagen were the most preferred by the internal panel. Hydrolysed collagen bolognas had higher texture score than pea starch, pea fibre, and potato starch bolognas. Addition of pea fibre decreased the bologna hedonic scores for chewiness and aftertaste properties compared to hydrolysed collagen. Sausages added with white navy bean flour were similar to products without binder when it comes to overall texture, firmness, chewiness, and aftertaste characteristics.

Table 3.6. Liking scores of pork bolognas formulated with different binders evaluated by the internal sensory panel.

Treatment	Overall acceptability	Appearance	Flavour	Juiciness	Texture	Firmness	Chewiness	Aftertaste
Collagen	6.57±1.47^{a}	6.45±1.57^{abc}	6.64±1.42^{a}	6.51±1.57	6.60±1.53^{a}	6.53±1.52^{a}	6.55±1.38^{a}	6.38±1.69^{a}
Pea Starch	6.36±1.69^{ab}	6.28±1.57^{bc}	6.26±1.61^{ab}	6.19±1.70	5.94±1.65^{b}	5.94±1.70^{b}	5.91±1.67^{b}	6.06±1.65^{ab}
White Navy Bean Flour	6.68±1.48^{a}	6.62±1.33^{ab}	6.40±1.64^{ab}	6.36±1.65	6.38±1.71^{ab}	6.21±1.65^{ab}	6.28±1.48^{ab}	5.98±1.70^{ab}
Pea Fibre	6.04±1.69^{b}	6.72±1.56^{a}	6.09±1.87^{b}	6.23±1.45	6.06±1.67^{b}	6.26±1.58^{ab}	5.98±1.58^{b}	5.70±1.91^{b}
Potato Starch	6.53±1.92^{ab}	6.74±1.41^{a}	6.34±1.80^{ab}	6.40±1.66	6.02±1.91^{b}	6.43±1.78^{ab}	6.21±1.82^{ab}	6.09±1.67^{ab}
No Binder	6.36±1.76^{ab}	6.21±1.83^{c}	6.55±1.95^{ab}	6.30±1.71	6.21±1.47^{ab}	6.13±1.74^{ab}	6.17±1.56^{ab}	6.11±1.78^{ab}

Results are presented as mean±standard deviation. $^{a–c}$Means with different lowercase letters in the same column are significantly different (Fisher's LSD, $p < 0.05$). Scored on 9-point hedonic scales where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like/dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremel

The frequency figure in Appendix A (Figure A6) shows that salty, flavourful, typical bologna flavour, savoury, and spicy were frequently checked by the internal panel. Hydrolysed collagen bologna was most frequently checked as typical bologna flavour product, which is significantly higher than pea fibre bologna according to Cochran's Q test ($p < 0.05$), followed by pea starch and no binder bologna. No binder control was the most flavourful and savoury sample, followed by pea fibre bologna. Pea fibre bologna was selected as the most salty and spicy one. The map of correspondence analysis (Figure 3.11) shows the differences among the products in terms of their flavour profiles. The first two dimensions explained 72.25% of total inertia. Metallic, bitter, and off-flavour spot were relatively far from samples. Pea fibre bologna was close to salty, spicy, and savoury. White navy bean flour and no binder bolognas tended to be savoury, salty, and flavourful. Bolognas processed with potato starch and pea starch bologna were close to bland and hydrolysed collagen bolognas were close to typical bologna flavour. Principal coordinate analysis including attributes and liking scores further indicated correlation coefficients and visualized in a two-dimensional map (Figure 3.12). The first two dimensions explains 53.62% of the variation. The flavour liking score was associated to the attribute typical bologna flavour, savoury, flavourful, and salty.

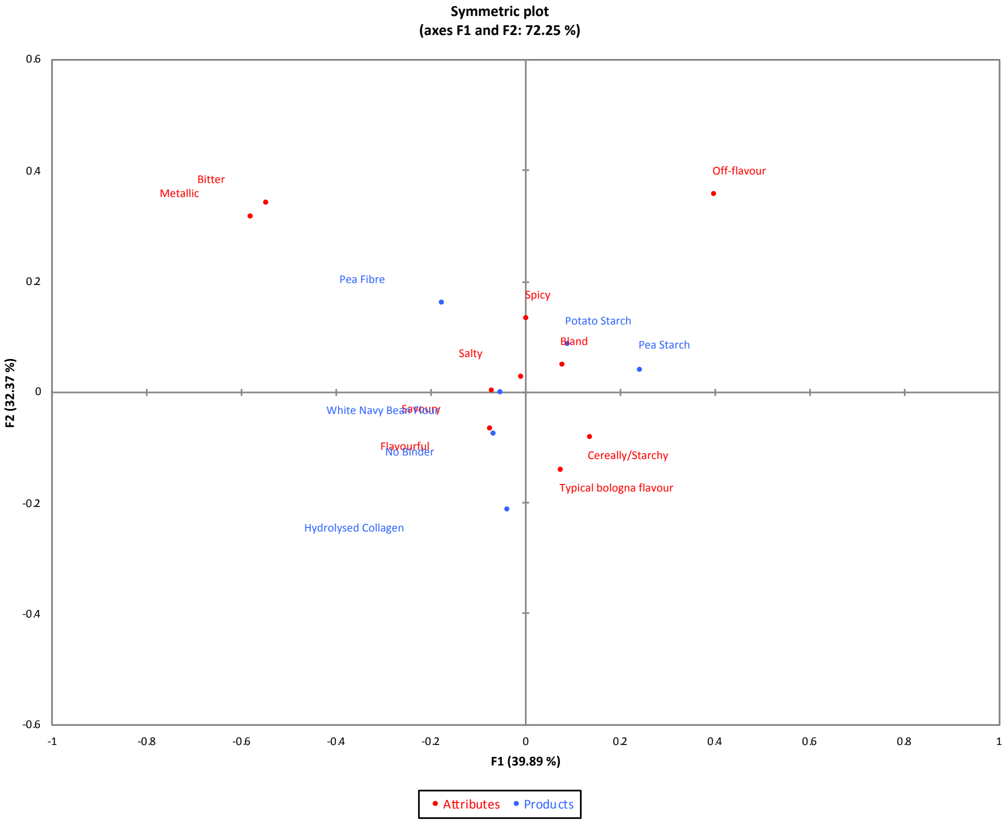


Figure 3.11. Correspondence analysis for Check-All-That-Apply scale of flavour for bolognas formulated with different binders evaluated by the internal sensory panel. F1 and F2 account for 39.89% and 32.37% of total variance respectively.

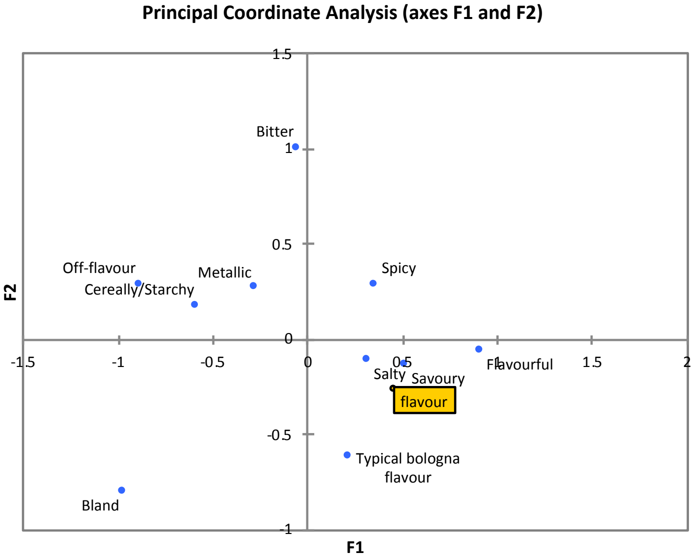


Figure 3.12. Principal coordinate analysis for Check-All-That-Apply scale of flavour for bolognas formulated with different binders evaluated by the internal sensory panel.

Percentage of frequency distribution for JAR scale of firmness is illustrated in Appendix A (Figure A7). More specifically, 61.70% panellists believed that the firmness of hydrolysed collagen bologna was just-about-right, followed by bolognas with white navy bean flour (57.45%), whereas only 40.43% people rated firmness of pea starch bolognas as just-about-right. Based on the frequency, the penalty table identified potential directions to the firmness attribute. Bologna made with white navy bean flour or without binder were significantly ($p < 0.01$) too soft. On the contrary, bologna containing pea fibre, pea starch, and potato starch were too firm for panellists.

Percentage of frequency distribution for JAR scale of juiciness is shown in Appendix A (Figure A8). Results suggested that no panellist found hydrolysed collagen bologna being too dry, and 57.45% people believed the juiciness was just-about-right, which was more than that for the white navy bean flour bologna (55.32%). No binder bologna was chosen by the least number of participants (46.81%) as right juicy product. Based on the frequency, penalty table identified potential directions to the juiciness attribute. Bolognas with hydrolysed collagen or without binder were significantly too juicy for panellists compared to ones with white navy bean flour. In contrast, addition of pea fibre, pea starch, and potato starch caused significantly ($p < 0.05$) too dry products.

Percentage of frequency distribution for JAR scale of chewiness is demonstrated in Appendix (Figure A9). It was indicated that 65.96% and 63.83% consumers believed hydrolysed collagen and white navy bean flour contributed to right chewy texture of bolognas, respectively. Chewiness of pea fibre bologna was scored the least as just-about-right. Based on the frequency, penalty table identified potential directions to the chewiness property. Bolognas processed with hydrolysed collagen, pea fibre, pea starch, and potato starch were considered significantly ($p < 0.05$) too rubbery and potato starch bolognas were panelised the most by 48.94% of panellists with

2.087 penalty score. No binder bolognas were significantly penalized for both too crumbly and too rubbery attributes.

Percentage of purchase intent from consumers was calculated and shown in Figure 3.13. Approximately 60% consumers were likely or definitely had the desire to purchase hydrolysed collagen bologna. The percentage of consumer intending to purchase pea fibre bologna was the least (less than 40%).

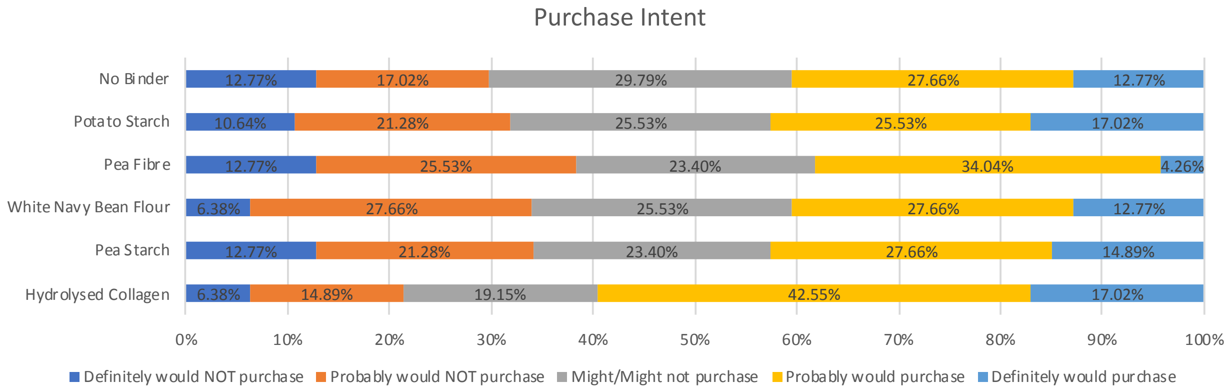


Figure 3.13. Percentage of frequency distribution of purchase intent for bolognas formulated with different binders evaluated by the internal sensory panel. Scored on 5-point scales where 1 = definitely would not purchase, 2 = probably would not purchase, 3 = might/might not purchase, 4 = probably would purchase, 5 = definitely would purchase.

Figure 3.14 shows the biplot of PCA for attributes of bolognas with different binders. The first two principal components represent 84.38% of the initial variability of the data. The horizontal axis is linked with attributes of overall acceptability, flavour, juiciness, texture, firmness, chewiness, aftertaste, and purchase intent, whereas the vertical axis is linked with appearance attribute and cooking yield. All sensory attributes, except for appearance, were significantly positively correlated to each other. The observations (treatments) on the two-dimensional biplot were located on the basis of the principal component scores (Table 3.6). On the horizontal axis, bolognas with pea fibre and pea starch were on the opposite side of the centre and far from others due to most of the lower liking scores. Pea fibre bologna represented higher appearance scores than pea starch bologna so that it was located in the second quadrant and close to appearance. Hydrolysed collagen bologna was positioned in close proximity to overall acceptability, overall texture, purchase intent, and juiciness. Potato starch and white navy bean flour bolognas were also located in the first quadrant, close to firmness and juiciness attributes. No binder bolognas were close to flavour and aftertaste attributes, which caused by flavourful and pleasant taste. Pea starch bologna on the horizontal axis was closer to major sensory characteristics than pea fibre bologna. The dendrogram of hierarchical clustering (Figure 3.15) also further shows the grouping results based on the two principal component scores. White navy bean flour and potato starch bolognas were in the same group, interpreting similar properties, and pea starch bologna and bologna without binder were grouped together.

Table 3.7. Principal component scores of biplot for different binders of bolognas in the first two dimensions.

Treatment	Principal Component 1	Principal Component 2
Hydrolysed Collagen	4.05255	0.13649
Pea Starch	-2.06749	-1.30512
White Navy Bean Flour	0.54689	0.88678
Pea Fibre	-3.0724	1.13726
Potato Starch	0.26732	1.50127
No Binder	0.27313	-2.35668

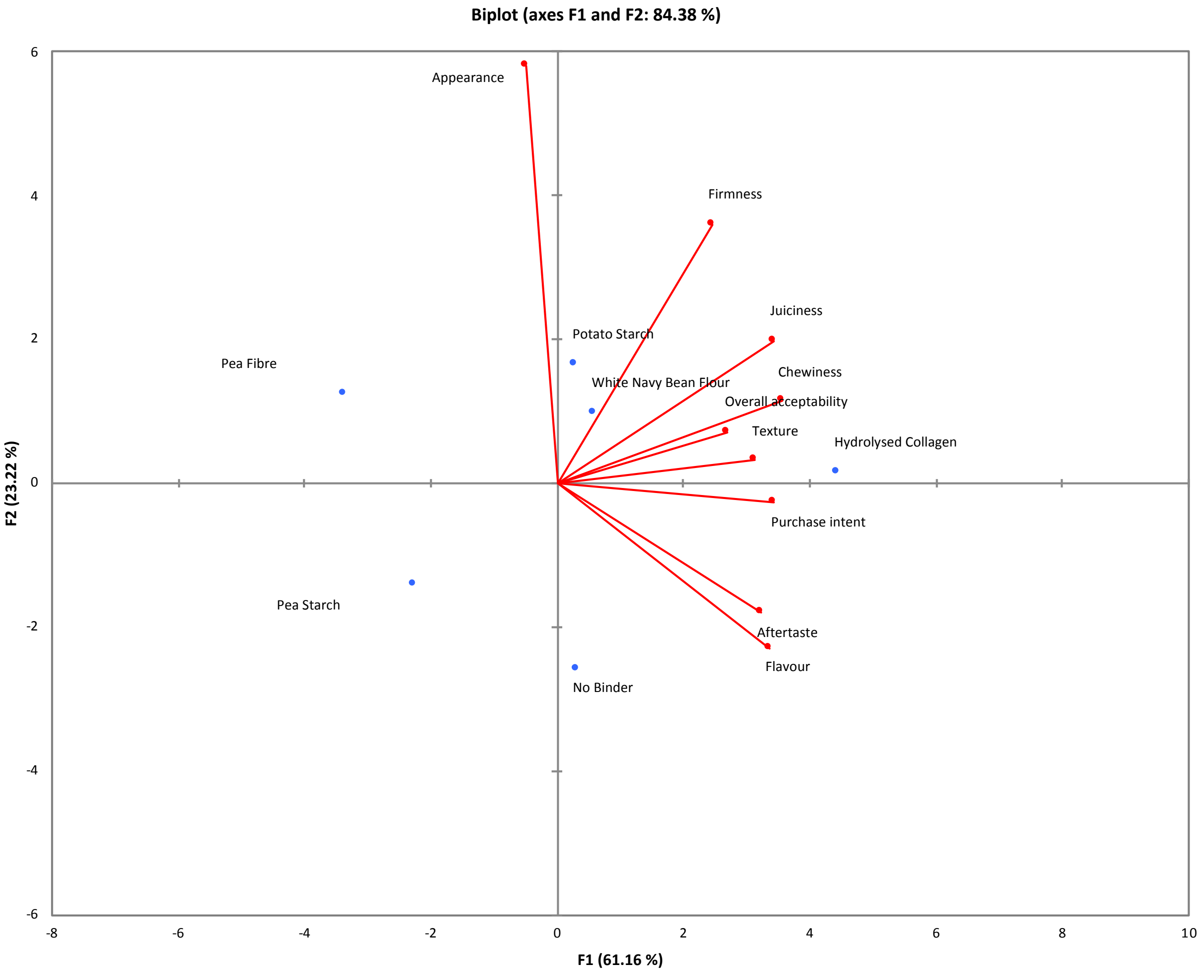


Figure 3.14. Biplot of principal component analysis for attributes of bolognas formulated with different binders evaluated by the internal sensory panel. F1 and F2 account for 61.16% and 23.22% of total variance respectively. Rays represent loadings of variables, and points represent treatments.

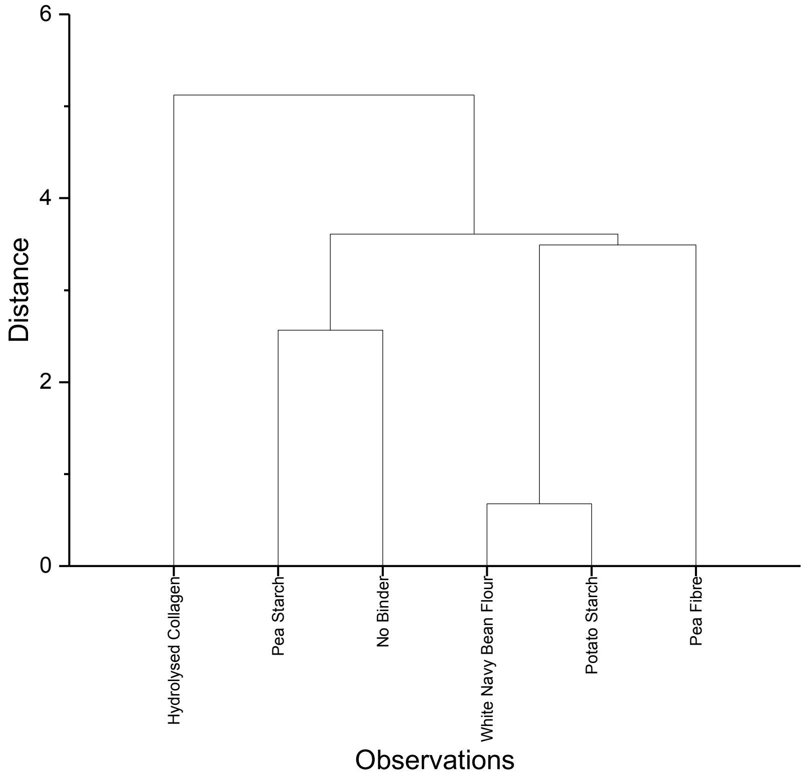


Figure 3.15. Dendogram of hierarchical cluster analysis for attributes of bolognas formulated with different binders evaluated by the internal sensory panel.

3.2.3. Conclusion

Preliminary screening results showed that citrus fibre, potato starch (Ingredion), sodium tripolyphosphate, tapioca starch, pea fibre (Uptake 80), and rice flour (medium/short) were characterized by unpleasant off-flavour or unacceptable pasty texture and thus were eliminated for further evaluation. In regular salt pork bologna model, hydrolysed collagen and white navy bean flour led to higher overall acceptability of bologna hedonic scores than pea fibre. Hydrolysed collagen bologna featured juicy texture and typical bologna flavour and white navy bean flour contributed to flavourful and savoury flavour. Although native pea starch, native potato starch, and pea fibre bolognas were relatively tough, dry, and rubbery, the overall palatability of bolognas with native pea starch, native potato starch was still more acceptable than that containing pea fibre. Pea fibre bologna had the lowest overall acceptability and purchase intent percentage on account of lacking bologna flavour and aftertaste. Therefore pea fibre was eliminated from the lists for the next full-scale experimental stage.

The results of these preliminary evaluations allowed for selection of best alternative ingredients which might be the replacement of wheat flour in emulsified sausages. Pea starch, potato starch, white navy bean flour and hydrolysed collagen were identified to have the best potential without compromising sensory acceptability, and they were selected for further evaluation in a reduced sodium system.

Chapter 4. Effect of non-allergen binders on functionalities and sensory characteristics of beef burgers

4.1. Introduction

Food allergy is one of the high-incidence diseases among the population in the world. It is estimated that globally, 220–250 million people may suffer from food allergy (Mills *et al*., 2007). Recent statistics also show that 2% to 3% of adults and 6% to 8% of children are affected by food allergies (Rachid & Keet, 2018). Approximately 2.5 million Canadians self-report having at least one food allergy. More than 40% of Canadians read food labels looking for allergen information (Allergy, Genes and Environment Network, 2015). From 2009 to 2010 in the U.S., 8% children (infant to 18) have a food allergy, 30.4% of food allergic children have multiple food allergies. In 2012, 5.6% or 4.1 million children reported food allergies in the past 12 months (Bloom, Cohen & Freeman, 2013). In the UK, about 2 million people were diagnosed with food allergy (Wearne, 2017).

According to Health Canada, priority food allergens have been identified, including eggs, milk, mustard, peanuts, crustaceans and molluscs, fish, sesame seeds, soy, sulphites, tree nuts, wheat and triticale (Health Canada, 2011). Among these, wheat, soy, and whey are widely used in meat products as binders or fillers to enhance the texture, flavour, or cooking characteristics. Rosli *et al*. (2011) produced chicken patties with oyster mushroom, potato starch and isolated soy protein as binders. Addition of 25% oyster mushroom instead of chicken breast increased the springiness of chicken patties and was suitable for commercial chicken patties. Cornsilk incorporated with potato starch and isolated soy protein were applied by Wanrosli *et al*. (2011) in beef patties. A gradual replacement of 6% potato starch with cornsilk fibre was effective in enhancing cooking yield, moisture and fat retention and improving texture of beef patties. Some binders are also used as a fat replacer. The blend of tapioca starch, oat fibre and whey protein in low-fat beef burgers could significantly increase cooking yield and water holding capacity and burgers were acceptable in terms of flavour and texture (Troy, Desmond & Buckley, 1999). Soncu *et al*. (2015) developed low-fat beef hamburger with bread crumbs and different levels of carrot and lemon fibre. The addition of carrot fibre resulted in more tender, gummy, springy, and smoother hamburgers and higher sensory scores compared to those processed with lemon fibre. However, lemon fibre demonstrated better cooking yield, shrinkage, moisture and fat retention.

Demand for low-fat meat products from consumers is growing due to the increasing awareness of health issues related to diet (Yang *et al*., 2015). Some alternative non-allergen binders have been researched in both regular and low-fat burgers over the last few years. Addition of thermally micronized chickpea and green lentil flours into low-fat beef burgers was studied by Shariati-Ievari *et al*. (2016). Low-fat beef burgers containing 6% lentil and chickpea flour had better physicochemical properties and consumer acceptability compared to those without added pulse flour. Cooking losses from burgers processed with nonmicronized and micronized flours at both 130 °C and 150 °C were significantly lower than those with no binders. Turhan, Sagir & Sule (2005) reported that hazelnut pellicle reduced redness, lightness and yellowness but improved cooking yield and resulted in a lower dimensional shrinkage of burgers. It was also concluded that 1–2% pellicle addition could be utilized as a dietary fibre filler in low-fat beef burger production. In addition, oatmeal flour, flour of green banana pulp, flour of green banana peel, flour of apple peel and pulp of green banana (Bastos *et al*., 2014), gari (Akwetey & Knipe, 2012), albedo-fibre powder (López-Vargas *et al*., 2014), *Aloe vera* (Soltanizadeh & Ghiasi-Esfahani, 2015), tiger nut fibre (Sánchez-Zapata *et al*., 2010), and destoned olive cake (Hawashin *et al*., 2016) were successfully added to beef or pork burgers as fat substitutes and/or functional binders.

Pulse flours or fractions were formulated in different types of meat products due to not only nutritional and health benefits from protein and fibre, but also the improvement of functional properties (Boye, Zare & Pletch, 2010). Canada has a plentiful source of pea, and accounts for 25% of total world pea production (Ratnayake, Hoover & Warkentin, 2002). Non-pulse ingredients such as potato and rice have also been successfully applied in meat products (Malav *et al*., 2015, García-García & Totosaus, 2008, Malekian *et al*., 2014). These ingredients have potential to act as allergen binder substitutes. In this study, based on preliminary tests of more than 20 types of commercial plant ingredients, several allergen-free binders including textured pea protein, pea starch, rice flour and potato starch were evaluated in a regular fat burger system and their performance was compared to the wheat crumb control.

4.2. Materials and methods

4.2.1. Ingredients

Fresh beef shoulder clod and fat trimmings (85:15 and 50:50, w/w) were purchased from local processor and stored at 2 °C. Spices were purchased from local grocery stores. Native potato starch was purchased from Manitoba Starch Products (MB, Canada). Native pea starch was purchased from Nutri-Pea Limited (Accu-Gel^{TM}, Portage la Prairie, MB, Canada). Textured pea protein was produced by Sotexpro (textured pea protein 24/30, La Croix Forzy, Bermericourt, France). Rice flour was shipped from PGP International Inc. (medium/short rice flour, Woodland, CA, United States). Wheat crumb was obtained from Newly Weds Foods (Breader B34216 White #50, Edmonton, AB, Canada).

4.2.2. Burger manufacture

All manufacturing was carried out in a refrigerated pilot plant (<7 °C) at Food Processing Development Centre (FPDC) (Leduc, AB, Canada). Beef trim and fat (85:15 and 50:50, w/w) were separately ground through a 4 mm plate (Model AW114, K & G Wetter, Mississauga, ON, Canada). Samples were taken from each batch of ground meat and proximate composition was determined using a Foss FoodScan analyzer (Type 78800, FoodScan Lab, FOSS, Hilleroed, Denmark). The lean meat to fat ratio was adjusted to 75:25. The required quantities of ground beef were combined with 0.8% salt, 0.1% black pepper, 0.15% onion powder, 12% ice water, and respective binders (9 treatments including 5% wheat crumb, 2% and 4% of potato starch, pea starch, textured pea protein and rice flour), and mixed at a low mixing speed using a Hobart mixer (A-200T, Hobart, Trot, OH, US) with a paddle attachment for 45 s. The total weight of individual batch was 8 kg. The mixture was then formed into 140 g patties (120 mm diameter patties with 13.5 mm thickness) using a forming machine (Super 54 Patty machine, Hollymatic, Countryside, IL). Three batches were produced following the same protocol. Four patties from each treatment were packaged individually on Styrofoam® trays over-wrapped with an O_2-permeable film (8,000 $cm^3/m^2/24$ h, Vitafilm, Huntsman Film Products of Canada, Toronto, ON, Canada). The patties were held in a simulated retail display deck cabinet under 24 h fluorescent lighting with an average intensity of 1630 lx at 4 °C for 4 days (Pietrasik, Gaudette & Klassen, 2016). All the remaining burgers were packed with hamburger patty paper in cardboard boxes with a plastic liner and kept frozen at -20 °C until evaluation.

4.2.3. Qualities of raw patties during simulated retail display

4.2.3.1 Surface colour

The instrumental colour was measured using a Minolta CM-2500C handheld spectrophotometer (Konica-Minolta, Osaka, Japan), with 10° observer angle and illuminant A and calibrated against a white tile. The surface colour measurements of fresh burgers were taken at day 0, and then daily up to 4 d of retail display. The CIE L^* (lightness), a^*(redness), b^* (yellowness) values for each sample were presented as the average of three readings. Hue and Chroma were calculated for each sample using the following formulas. The spectral reflectance ratio of 630/580 nm was also measured.

$$\text{hue} = \tan^{-1} {}^{b^*}\!/_{a^*}$$

$$\text{chroma} = (a^{*2} + b^{*2})^{\frac{1}{2}}$$

4.2.3.2 2-Thiobarbituric acid reactive substance (TBARS) value

TBARS tests were performed on fresh burgers (stored at 4 °C) on Day 1 and Day 3, and on frozen burgers (stored at -20 °C) after 3 months, following the method of Pietrasik, Gaudette & Klassen (2016). Briefly, 3 g sample was placed in a 50 mL centrifuge tube, homogenized with 15 mL deionized distilled water and 50 μL butylated hydroxytoluene (7.2%) for 15 s. Homogenate (1 mL) was transferred to a test tube (15 mL), mixed with 2 mL thiobarbituric acid/trichloroacetic acid (20 mM TBA and 15% [w/v] TCA) solution. The mixture was vortexed for 10 s and incubated in a 90 °C water bath for 15 min. After cooling for 10 min in cold water, the tubes were vortexed and centrifuged at 3000 rpm (Rotor #11457, MPWMed Instruments, Warsaw, Poland) for 15 min. The absorbance of the resulting upper layer was read at 531 nm against a blank prepared with 1 mL deionized water and 2 mL TBA/TCA solution using a UV–vis spectrophotometer. TBARS values were calculated from a 1,1,3,3-tetraethoxypropane standard curve and expressed as mg malondialdehyde per kg raw meat.

4.2.4. Qualities of cooked burgers

4.2.4.1. Measurement of cooking characteristics

4.2.4.1.1. Cooking loss

Frozen burgers were placed on a preheated (about 200 °C) electric grill (Garland ED-42B electric broiler, Russell Food Equipment Ltd, Edmonton, AB, Canada). Burgers were cooked and flipped every 2 min, until the internal temperature reached 71 °C. Cooked patties were placed in trays and allowed to cool for about 5 min. The weights of both frozen and cooked samples were used for calculating the cooking loss.

$$\text{cooking loss} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100\%$$

4.2.4.1.2. Dimensional shrinkage

The diameter and thickness were determined on both raw and cooked burgers using an electronic calliper. Two diameter readings were taken in two orthogonal directions and four thickness readings were taken 90° apart from each other from the bottom to the top. Means for each parameter of burger were used to calculate the dimensional shrinkage.

$$\begin{aligned}&\text{dimensional shrinkage}\\&\quad = \frac{\text{raw thickness} - \text{cooked thickness} + \text{raw diameter} - \text{cooked diameter}}{\text{raw thickness} + \text{raw diameter}}\\&\quad \times 100\%\end{aligned}$$

4.2.4.1.3. Moisture and fat retention

The moisture and fat percentage of both raw and cooked burgers were determined by a Foss FoodScan analyser (Type 78800, FoodScan Lab, FOSS, Hilleroed, Denmark), and moisture and fat retentions were calculated using the following formulas.

$$\text{moisture retention} = \frac{\text{cooked weight } \times \text{ cooked moisture content}}{\text{raw weight } \times \text{raw moisture content}} \times 100\%$$

$$\text{fat retention} = \frac{\text{cooked weight } \times \text{ cooked fat content}}{\text{raw weight } \times \text{raw fat content}} \times 100\%$$

4.2.4.2. Physicochemical analyses

4.2.4.2.1. Interior colour

Cooked burgers were cut horizontally into two pieces with a knife and the interior colour was measured using a Minolta CM-2500C handheld spectrophotometer (Konica-Minolta, Osaka, Japan), with 10° observer angle and illuminant D65 and calibrated against a white tile. The CIE L^* (lightness), a^*(redness), b^* (yellowness) values for each sample were presented as the average of three readings. Hue and Chroma were calculated for each sample using the formulas shown in the section ***4.2.3.1***.

4.2.4.2.2. pH

PH of both raw and cooked burgers was measured by an Orion 5 Star pH meter (Thermo Fisher Scientific, Ottawa, ON, Canada) with an electrode (pHoenix Electrode Co., Houston, TX) after 5 g of each sample was homogenized with 50 mL deionized distilled water for 30 s.

4.2.4.2.3. Expressible moisture

A cork borer with 1.7 cm diameter was used for sampling. Cooked burger samples (each sample was around 3 g) were wrapped in Whatman No. 3 filter paper and placed in 50 mL centrifuge tubes. The samples were centrifuged at 963 × g (MPWMed Instruments, Warsaw, Poland) for 10 min. The sample weights before and after centrifugation were used for calculation of expressible moisture using the following formula.

$$\text{expressible moisture} = \frac{\text{weight before centrifuge} - \text{weight after centrifuge}}{\text{weight before centrifuge}} \times 100\%$$

4.2.4.2.4. Texture profile analysis (TPA)

Core samples were cut from the centre of each patty using a cork borer with 2 cm diameter, and put in an Instron Universal Testing System (Model 5565, Instron Corporation, Burlington, ON, Canada). All the samples were compressed twice to 30% of original height with a 9 cm flat ended steel plunger at a constant cross-head speed of 60 mm/min. The following parameters were obtained: hardness (N), the peak force of the first bite; cohesiveness, the ratio of the active work under the second force–displacement curve to that under the first compression curve; springiness (mm), the distance the sample recovers after the first bite and chewiness (N mm), hardness × cohesiveness × springiness (Pietrasik, Pierce & Janz, 2012).

4.2.4.2.5. Shear force

A 2.5 cm width strip was cut from each burger. Each prepared sample was sheared at three evenly distributed sample locations by a straight-edge blade fixture using a crosshead speed of 200 mm/min. The peak shear force (N) was recorded.

4.2.5. Sensory evaluation

Participants were recruited who frequently consume beef burgers and the evaluation of the burgers was conducted in the dedicated sensory evaluation lab at Consumer Product Testing Centre (CPTC, Edmonton, AB, Canada) over 4 days. All burgers were cooked following the method described in the section ***4.2.4.1.1***. Three-digit blinding codes were used to label each treatment during panel set up, and all burgers were cut into thirds immediately after being removed from the grill, individually wrapped in aluminium foil, and placed into a 60 °C chamber (LHU-113, ESPEC Corp., Osaka, Japan) until prepared for serving. All sensory panel responses were collected using a computerized program specific for sensory evaluation (Compusense Cloud, Compusense Inc., Guelph, Ontario). An incomplete block design was used for each panel to conduct sensory evaluations. Each panellist evaluated six treatments of burgers. Panellists (167) over 18 years of age (53 males and 114 females) received verbal instructions upon arrival at the CPTC, and were seated at individual testing booths lit with white lighting where written instructions were integrated into electronic ballot presentation. Samples were placed on a 6-inch white coded styrofoam plate (Genpak) and passed through serving hutches to each panellist in a sequential, monadic manner. A forced 90 sec break was administered and room temperature water and unsalted crackers were provided for palate cleansing between samples. Panellists were asked to rate overall acceptability and the acceptability of the appearance, flavour, texture, and aftertaste using a 9-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely. The Check-All-That-Apply (CATA) method was used to further define the flavour attribute with the supplied terms: grilled flavour, cereally/grainy, beany, bitter, metallic, off flavour, savoury, tangy/sour, bland, beefy, salty, seasoned, potato, fatty/oily flavour; and textural attribute: rubbery bite, tough/leathery, crumbly, mushy/pasty, chewy, granular texture, greasy, leaves a mouth coating, nice bite, chalky texture. A 7-point Just-about-right (JAR) scale was used to further describe appearance (colour), texture (firmness and juiciness), and aftertaste (lingerer and pleasantness) characteristics. The JAR scale was anchored with 1 = too pale/soft/dry or no aftertaste/very unpleasant, 2 = moderately pale/soft/dry, 3 = slightly pale/soft/dry, 4 = just-about-right, 5 = slightly dark/firm/juicy, 6 = moderately dark/firm/juicy, and 7 = too dark/firm/juicy or lingering/very pleasant. A 5-point hedonic scale was also used to evaluate purchase intent of each sample, where 1 = definitely would not purchase, 2 = probably would not purchase, 3 = might/might not purchase, 4 = probably would purchase, 5 = definitely would purchase.

4.2.6. Statistical analysis

Data from instrumental analyses were evaluated by SPSS 23 software (IBM Corp., Armonk, NY, United States) using linear mixed model with treatment as a fixed effect and trial as a random effect, except for shelf-life data in which retail display days and treatment × days interaction were also included as fixed effects. Tukey's HSD test was used to determine the differences between the least squared means ($p < 0.05$). Sensory data were analysed by SPSS 23 software, using two-way ANOVA with treatment as a fixed effect and panellist as a random effect. Tukey's HSD test was used to determine the differences between the least squared means ($p < 0.05$). Principal component analysis, penalty analysis and cluster analysis were run by XLSTST 2016 software (Addinsoft, Paris, France) and Origin 2017 software (OriginLab, Northampton, MA, United States).

4.3. Results and discussion

4.3.1. Colour of fresh burgers

Table 4.1 and Table 4.2 show colour changes of fresh burgers during 4 days of retail display. The interaction between storage time and treatment was significant ($p < 0.05$) for a*, C*, h, and $A_{630nm/580nm}$ (Table 4.1), indicating that the discolouration rate of these fresh burgers was treatment dependent. In contrast, the interaction between storage time and treatment for L* and b* was not significant ($p > 0.05$) (Table 4.2). Lightness decreased on the first day, followed by an increase over the next three days. Yellowness of all treatments declined within 4 days. Other researchers reported similar changing trends of colour parameters (a*, b*, C*) of raw meat during aerobic display (Pietrasik, Gaudette & Klassen, 2016, Garner *et al.*, 2014).

Table 4.1. Least squares means for interactions of treatment combination with storage time for redness (a*), chroma (C*), hue angle (h), and $A_{(630nm/580nm)}$ of raw burgers formulated with selected binders.

Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Standard error	*p* value
a*(A)							
5% Wheat Crumb	30.35^{A}	22.73^{abcB}	21.01^{abcC}	17.90^{abD}	15.05^{aE}	0.37	<0.001
2% Potato Starch	29.72^{A}	21.00^{dB}	19.10^{dC}	14.05^{dD}	11.28^{dE}	0.31	<0.001
4% Potato Starch	29.63^{A}	21.51^{cdB}	18.88^{dC}	15.95^{cD}	12.76^{cdE}	0.28	<0.001
2% Pea Starch	30.34^{A}	21.93^{bcdB}	18.49^{dC}	15.70^{cD}	12.76^{cdE}	0.28	<0.001
4% Pea Starch	30.59^{A}	22.90^{abcB}	19.88^{bcdC}	17.18^{bcD}	13.74^{abcE}	0.29	<0.001
2% Short Rice Flour	30.12^{A}	22.07^{abcdB}	19.75^{cdC}	16.71^{bcD}	13.31^{bcE}	0.28	<0.001
4% Short Rice Flour	31.24^{A}	23.62^{aB}	21.24^{bcC}	19.34^{aD}	15.39^{aE}	0.31	<0.001
2% Textured Pea Protein	29.98^{A}	22.90^{abcB}	21.48^{aC}	17.16^{bcD}	13.76^{abcE}	0.32	<0.001
4% Textured Pea Protein	29.71^{A}	23.21^{abB}	22.22^{aB}	18.99^{aC}	14.70^{abD}	0.43	<0.001
Standard error	0.41	0.36	0.34	0.36	0.39		
p value	0.134	<0.001	<0.001	<0.001	<0.001		
C*(A)							
5% Wheat Crumb	39.64^{abA}	30.75^{abB}	28.92^{abcC}	25.95^{abcD}	24.16^{aD}	0.45	<0.001
2% Potato Starch	38.84^{abA}	28.97^{bB}	27.24^{cdC}	22.41^{dD}	21.01^{cD}	0.38	<0.001
4% Potato Starch	38.43^{bA}	29.90^{abB}	26.85^{dC}	24.10^{cdD}	22.15^{bcE}	0.34	<0.001
2% Pea Starch	39.55^{abA}	30.09^{abB}	26.54^{dC}	24.20^{cdD}	22.52^{abcE}	0.37	<0.001
4% Pea Starch	40.18^{abA}	31.48^{aB}	28.25^{bcdC}	25.45^{bcD}	22.69^{abcE}	0.37	<0.001
2% Short Rice Flour	39.09^{abA}	30.22^{abB}	27.69^{bcdC}	24.97^{bcD}	21.99^{cE}	0.38	<0.001
4% Short Rice Flour	40.63^{aA}	31.92^{aB}	29.47^{abC}	27.69^{aD}	23.84^{abE}	0.39	<0.001
2% Textured Pea Protein	39.31^{abA}	31.01^{aB}	29.38^{abC}	25.21^{bcD}	22.79^{abcE}	0.39	<0.001
4% Textured Pea Protein	38.94^{abA}	31.41^{aB}	30.27^{aB}	26.73^{abC}	23.83^{abD}	0.46	<0.001
Standard error	0.49	0.46	0.41	0.42	0.41		
p value	0.058	<0.001	<0.001	<0.001	<0.001		
h(A)							
5% Wheat Crumb	40.06^{D}	42.40^{bCD}	43.45^{cdC}	46.54^{defB}	51.70^{deA}	0.59	<0.001
2% Potato Starch	40.05^{E}	43.62^{abD}	45.59^{abC}	51.23^{aB}	57.56^{aA}	0.45	<0.001
4% Potato Starch	39.55^{D}	44.12^{aC}	45.45^{abC}	48.66^{bcB}	54.98^{abcA}	0.43	<0.001
2% Pea Starch	39.92^{E}	43.23^{abD}	45.88^{aC}	49.59^{abB}	55.48^{abA}	0.43	<0.001
4% Pea Starch	40.48^{E}	43.58^{abD}	45.60^{abC}	47.82^{bcdeB}	52.88^{bcdA}	0.47	<0.001
2% Short Rice Flour	39.60^{D}	43.14^{abC}	44.58^{abcC}	48.06^{bcdB}	52.68^{cdeA}	0.51	<0.001
4% Short Rice Flour	39.76^{D}	42.43^{bC}	44.09^{bcdC}	45.88^{efB}	49.89^{eA}	0.43	<0.001
2% Textured Pea Protein	40.33^{D}	42.40^{bCD}	43.11^{cdC}	47.26^{cdeB}	52.91^{bcdA}	0.62	<0.001
4% Textured Pea Protein	40.28^{D}	42.54^{bCD}	42.99^{dBC}	45.20^{fB}	52.57^{cdeA}	0.63	<0.001
Standard error	0.29	0.33	0.34	0.46	0.63		
p value	0.266	<0.001	<0.001	<0.001	<0.001		

$A_{630nm/580nm}$							
5% Wheat Crumb	5.33^{A}	3.29^{abcB}	2.82^{bcdC}	2.20^{bcD}	1.70^{aE}	0.08	<0.001
2% Potato Starch	5.17^{A}	2.87^{dB}	2.39^{efC}	1.58^{fD}	1.17^{cE}	0.08	<0.001
4% Potato Starch	4.98^{A}	2.96^{cdB}	2.35^{efC}	1.84^{defD}	1.33^{bcE}	0.07	<0.001
2% Pea Starch	5.48^{A}	3.08^{bcdB}	2.24^{fC}	1.76^{efD}	1.33^{bcE}	0.08	<0.001
4% Pea Starch	5.26^{A}	3.32^{abcB}	2.52^{defC}	2.01^{cdeD}	1.49^{abE}	0.09	<0.001
2% Short Rice Flour	5.34^{A}	3.16^{abcdB}	2.56^{cdeC}	1.95^{cdeD}	1.47^{abE}	0.07	<0.001
4% Short Rice Flour	5.54^{A}	3.48^{aB}	2.83^{bcC}	2.39^{abD}	1.70^{aE}	0.09	<0.001
2% Textured Pea Protein	5.20^{A}	3.38^{abB}	2.96^{abC}	2.11^{cdD}	1.51^{abE}	0.07	<0.001
4% Textured Pea Protein	5.10^{A}	3.45^{abB}	3.16^{aB}	2.56^{aC}	1.71^{aD}	0.10	<0.001
Standard error	0.19	0.09	0.07	0.06	0.06		
p value	0.548	<0.001	<0.001	<0.001	<0.001		

$^{a–f}$Means with different lowercase letters in the same column of the same day are significantly different (Tukey's HSD, $p < 0.05$).

$^{A–E}$Means with different uppercase letters for the same treatment during 4 days are significantly different (Tukey's HSD, $p < 0.05$).

Colour was measured with illuminant A. Abbreviation: $A_{(630nm/580nm)}$: ratio of absorbance.

Table 4.2. Least squares means for main effects of treatment combination with storage time for lightness (L*) and yellowness (b*) of raw burgers formulated with selected binders.

Treatment	Day	L*(A)	b*(A)
5% Wheat Crumb		50.43^{ab}	20.64^{abc}
2% Potato Starch		50.49^{ab}	19.85^{d}
4% Potato Starch		50.43^{ab}	20.02^{cd}
2% Pea Starch		49.93^{b}	20.34^{bcd}
4% Pea Starch		51.27^{a}	20.80^{ab}
2% Short Rice Flour		49.89^{b}	20.11^{cd}
4% Short Rice Flour		51.41^{a}	21.10^{a}
2% Textured Pea Protein		49.44^{b}	20.50^{abc}
4% Textured Pea Protein		50.43^{ab}	20.76^{ab}
Standard error		0.26	0.15
p value		<0.001	<0.001
	0	51.25^{a}	25.20^{a}
	1	49.06^{c}	20.85^{b}
	2	49.79^{bc}	19.71^{c}
	3	50.13^{b}	18.47^{d}
	4	51.83^{a}	18.05^{d}
	Standard error	0.18	0.10
	p value	<0.001	<0.001

$^{a–d}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$). Colour was measured with illuminant A.

In terms of redness, hue angle, and $A_{630nm/580nm}$, no differences were observed among the treatments on Day 0 after processing (Table 4.1), indicating that binders added at different levels did not affect these parameters of raw burgers. After one day of storage, redness of all burgers started to decrease significantly ($p < 0.05$). Only burgers incorporated with 4% textured pea protein had less discolouration on Day 2. However, on Day 4, burgers processed with 2% pea starch, 2% rice flour and both 2% and 4% potato starch had significantly ($p < 0.05$) lower redness compared to the wheat control. The ratio 630nm/580nm represents the redness owing to either oxymyoglobin or deoxymyoglobin and is used to follow discolouration during display. It showed similar changes to a* value. Four percent pea starch and both levels of rice flour and textured pea protein helped maintain similar redness of burgers compared to 5% wheat crumb at the end of storage. On day 4, only the hue angles of patties containing 2% pea starch or 2% and 4% potato starch were significantly ($p < 0.05$) higher than that of patties with wheat crumb and other treatments. Within each kind of binder, level of addition of binders did not affect hue of fresh burgers. Chroma could be described as colour saturation or intensity of burgers (Serratosa *et al*., 2008). On day 0, chroma of patties with non-allergen binders was comparable to that of control patties, but burgers with 4% rice flour had statistically higher chroma than those with 4% potato starch burgers. Burgers processed with 4% rice flour, 4% textured pea protein and 5% wheat crumb had less chroma decrease than patties with 2% rice flour. At the end of the storage, burgers added with 4% rice flour and any level of pea starch and textured pea protein showed significantly ($p < 0.05$) higher chroma values than burgers with other non-allergen binders and were similar to control burgers, indicating higher vividness and colour intensity of burgers were achieved.

Increasing the incorporation level of pea starch and rice flour from 2% to 4% resulted in significantly ($p < 0.05$) increased lightness of raw burgers. None of the selected non-allergen binders added at either 2% or 4% contributed to significant ($p < 0.05$) difference from wheat crumb in lightness. Two percent of potato starch led to significant ($p < 0.05$) lower yellowness value in comparison of 5% wheat crumb, but no differences were observed from wheat control among other allergen-free treatments. Kilincceker (2018) reported lightness increase of raw patties only when the amount of potato starch reached 5%.

According to the American Meat Science Association, Illuminant A is recommended for measuring meat colour because it places more emphasis on the proportion of red wavelengths and is recommended for samples where detection of differences in redness is the priority (Hunt & King, 2012). Therefore, in this research, Illuminant A was used to measure colour of raw burgers. Oxidation of ferrous deoxymyoglobin to ferric metmyoglobin causes brown discolouration in meat (Hunt & King, 2012). Lynch, Kastner & Kropf (1986) reported that 74% of consumers indicated that colour of fresh red meat was important in product purchase decisions, and consumers usually associated bright cherry red colour with meat freshness. Zhu & Brewer (1999) concluded that instrumental colour parameters (a*, hue angle, and ΔR (630-580)) could be used to predict visual redness for raw beef model system. Based on the results obtained from Table 4.1 and Table 4.2, 4% short rice flour and textured pea protein could be ideal replacement of wheat crumb in terms of colour.

Researchers investigated colour stability of coarse meat products added with other pulses and potato ingredients. Colle *et al*. (2019) investigated the effect of selected binders on retail colour of beef patties. Discolouration scores (based on redness) were not differentiated on day 0 among treatments. On day 4, beef patties with 2% potato extracts were more discoloured than patties with 2% soy flour (textured vegetable protein). The results were similar to those of the present study. Ergezer, Akcan & Serdaroğlu (2014) reported similar lightness changing trend of meatballs with

10% potato puree during 2°C storage for 6 days rather than meatballs containing 20% potato puree or 10% bread crumb, while storage period had no impact on redness and yellowness as for all treatments. Der (2010) processed fresh burgers with toasted wheat crumb, green lentil and red lentil flours. It was suggested that on day 0, increasing lentil flour amount from 6% to 12% had no effect on redness and lightness of burgers, but increased yellowness. Addition of 6% wheat crumb resulted in significantly ($p < 0.05$) lower redness and yellowness of burgers, but no difference in lightness compared to 6% and 12% of green lentil and red lentil flour. After 7 days, no significant ($p > 0.05$) differences were observed in redness and lightness, while only 12% red lentil flour burgers had significantly ($p < 0.05$) greater yellowness among these five treatments. The different results in our study might be due to different types and contents of protein and carbohydrates in binders, different storage conditions, and different meat processing systems.

4.3.2. TBARS of fresh and frozen burgers

TBA method was used as a quantitative assessment to measure the content of lipid-derived carbonyls, especially malonaldehyde (MDA) (Rodríguez-Carpena, Morcuende & Estévez, 2012). TBARS values of fresh and frozen burgers are shown in Table 4.3 and Table 4.4, respectively. Mixed model indicated that the interaction between time and treatment for fresh burgers was not significant ($p > 0.05$) (Table 4.3). There were no effects of binder incorporation levels on TBARS values of burgers (Table 4.3). However, addition of textured pea protein at both 2% and 4% significantly ($p < 0.05$) reduced TBARS values of burgers compared to rice flour when added at either 2% or 4%, indicating that burgers made with rice flour tended to have more lipid oxidation than those made with textured pea protein. Only burgers with 4% textured pea protein had significantly ($p < 0.05$) reduced burger TBARS values compared to the wheat crumb control. Additionally, effect of binders on TBARS of frozen burgers stored for three months exhibited similar results to that of fresh ones. Burgers with either 2% or 4% textured pea protein had significantly ($p < 0.05$) lower lipid oxidation compared to rice flour. Non-allergen treatments did not lead to significant ($p > 0.05$) alteration in TBARS compared to the wheat control. Shariati-Ievari *et al*. (2016) reported that infrared micronization at high temperature could significantly ($p < 0.05$) reduce the lipoxygenase activity leading to generation of lower amounts of volatile organic compounds and thus inhibiting oxidation capacity of pulses. Due to the fact that textured pea protein used in our study was subjected to an extrusion process, the high pressure and temperature might have led to the denaturation of lipoxygenase and in turn resulted in a decreased production of TBARS in burgers.

Table 4.3. Least squares means for main effects of treatment combination with storage time for 2-thiobarbituric acid reactive substance (TBARS) of fresh raw burgers formulated with selected binders.

Treatment	Day	TBARS (mg MDA/kg)
5% Wheat Crumb		1.07^{ab}
2% Potato Starch		1.06^{abc}
4% Potato Starch		1.04^{abc}
2% Pea Starch		1.03^{abc}
4% Pea Starch		1.02^{abc}
2% Short Rice Flour		1.22^{a}
4% Short Rice Flour		1.18^{a}
2% Textured Pea Protein		0.87^{bc}
4% Textured Pea Protein		0.85^{c}
Standard error		0.05
p value		<0.001
	Day 1	0.94^{B}
	Day 3	1.14^{A}
	Standard error	0.02
	p value	<0.001

$^{a\text{–}c}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).
$^{A\text{–}B}$Means with different uppercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).
Abbreviation: MDA: malonaldehyde.

Table 4.4. Least squares means of treatment for 2-thiobarbituric acid reactive substance (TBARS) of frozen raw burgers formulated with selected binders.

Treatment	TBARS (mg MDA/kg)
5% Wheat Crumb	0.82^{ab}
2% Potato Starch	0.79^{ab}
4% Potato Starch	0.80^{ab}
2% Pea Starch	0.80^{ab}
4% Pea Starch	0.82^{ab}
2% Short Rice Flour	1.00^{a}
4% Short Rice Flour	1.02^{a}
2% Textured Pea Protein	0.66^{b}
4% Textured Pea Protein	0.60^{b}
Standard error	0.06
p value	<0.001

$^{a–b}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).
Abbreviation: MDA: malonaldehyde.

For raw burgers, the longer they were displayed under light, the more lipid oxidation occurred. Other research also indicated similar results (McKenna *et al*., 2005, Garner *et al*., 2014, Pietrasik, Gaudette & Klassen, 2016). However, freezing treatment could delay the reaction after three months compared to storage at 4 °C for one day (Table 4.3 and Table 4.4). Kanner (1994) mentioned that oxidation was slowed by freezing but not completely prevented. Some lipid-free radicals were more stable at low temperature and further continued the reaction, which still caused the slower increase of TBARS values. Moreover, Pietrasik, Gaudette & Klassen (2016) mentioned that lipid oxidation could also affect colour and colour stability. However, even though 4% rice flour contributed to higher TBARS, the redness of fresh burgers was not negatively affected by rice flour compared to control as much as potato starch (Table 4.2).

For meat products, consumers could accept oxidized beef with about 2 mg MDA/kg as threshold. Additionally, rancid flavour could be tasted with TBARS value higher than 0.6 mg MDA/kg (Forell *et al*., 2010). All treatments had TBARS value lower than 2 in this study. Lipid oxidation is usually not considered a limiting factor for shelf-life, which is attributed to faster process of discoloration and growth of microorganisms than lipid oxidation during aerobically storage (Zhao, Wells & Mcmillin, 2010). Furthermore, in this study, the TBARS values were higher than some other studies (Kilincceker, 2018) and it may be attributed to higher amount of fat (25%) in this formula.

Hawashin *et al*. (2016) tested the lipid oxidation of beef patties formulated with 0%, 2%, 4%, and 6% olive cake powder during 14 days storage. TBARS values of patties containing olive cake powder were consistently lower than those of untreated controls. Meanwhile, increasing the level of olive cake powder to 6% concentration resulted in a gradual reduction in TBARS values. However, the fat content was 9% in each treatment. This might explain why linear changes were not observed in this study due to higher (more than twice) amount of fat and higher possibility of oxidation. Colle *et al*. (2019) reported that lipid oxidation of beef patties under retail display increased from day 0 to day 4 in terms of all treatments with textured soy protein flour or three dry potato extracts. In addition, on day 0, fresh patties incorporated with 2% potato extracts had comparable TBARS values to those with 2% textured soy protein flour. However, significantly ($p < 0.05$) higher TBARS values of potato extract burgers were observed on day 4. Ergezer, Akcan & Serdaroğlu (2014) found that incorporation of 10% or 20% potato puree did not significantly ($p > 0.05$) influence TBARS as compared to 10% bread crumb during 6 days storage period.

Colle *et al*. (2019) also compared TBARS results of patties during 3 weeks of frozen storage. Although lipid oxidation of cooked patties increased for all treatments on day 21 compared to day 0, TBARS of patties processed with potato extracts or textured soy protein flour did not differ from each other.

4.3.3. Cooking characteristics

Burgers tend to lose water and fat with shrinkage during cooking on account of the denaturation of the meat proteins (Serdaroğlu & Değırmencioğlu, 2004). Increasing incorporation level of binders significantly ($p < 0.05$) improved cooking properties (Table 4.5). In specific, all non-allergen binders added at 4% contributed to lower cooking loss, dimensional shrinkage, and higher moisture retention than those incorporated at 2% level. All burger treatments processed with binders at 4% had shrinkage and water retention similar to that of the wheat crumb control. Treatments with 4% potato starch, pea starch and rice flour did not result in significant ($p > 0.05$) differences in cooking loss of burgers compared to wheat crumb control. Addition of 4% potato starch and pea starch provided lower expressible water than 2% addition, which meant higher amount of these binders enhanced the water holding capacity. However, the increased amount of rice flour and textured pea protein in burgers did not obviously improve the water holding capacity compared to 2%. Fat retention was not variable among different treatments.

Table 4.5. Least squares means of treatment for cooking characteristics and expressible moisture of cooked burgers formulated with selected binders.

Treatment	Cooking Loss (%)	Dimensional Shrinkage (%)	Fat Retention (%)	Moisture Retention (%)	Expressible Moisture (%)
5% Wheat Crumb	28.78^{e}	14.47^{c}	64.12	64.98^{ab}	8.93^{c}
2% Potato Starch	33.71^{bc}	16.66^{a}	60.54	59.85^{cd}	11.52^{ab}
4% Potato Starch	28.47^{e}	14.95^{bc}	59.88	66.67^{ab}	8.94^{c}
2% Pea Starch	32.79^{c}	15.94^{ab}	65.02	59.75^{cd}	12.71^{a}
4% Pea Starch	28.58^{e}	14.62^{c}	60.12	68.15^{a}	10.78^{b}
2% Short Rice Flour	35.46^{ab}	16.87^{a}	62.66	55.17^{de}	9.92^{bc}
4% Short Rice Flour	30.11^{de}	14.95^{bc}	62.68	62.98^{bc}	10.06^{bc}
2% Textured Pea Protein	36.67^{a}	16.93^{a}	61.41	54.81^{e}	10.98^{ab}
4% Textured Pea Protein	31.70^{cd}	15.26^{bc}	61.41	63.98^{abc}	10.00^{bc}
Standard error	0.47	0.23	1.85	0.97	0.40
p value	<0.001	<0.001	0.524	<0.001	<0.001

$^{a–e}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).

These results could be attributed to the water and fat holding capacity of binders during cooking (Traynham *et al.*, 2007). Adding more flours or starches could favour absorbing more water and fat loss during cooking. During the cooking processes, gel matrix formation of added starch could favour retaining moisture in patties (Kilincceker, 2018), and the moisture loss altered the size and shape of patties (Alakali, Irtwange & Mzer, 2010). Kilincceker (2018) reported similar results that the cooking yield of meat patties with potato starch or pea starch improved by increasing the percentage of added starch. In their study, except for 3% pea starch, which led to higher cooking yield than 3% potato starch, 1% and 5% of both starches resulted in similar yield. It was in agreement with the results reported by Anderson & Berry (2001). Moreover, Anderson & Berry (2001) found that pea starch had better fat absorbing ability than water absorption compared to potato starch. However in this study, fat retention of each treatment was not significantly ($p > 0.05$) different. This might be caused by different cooking process, usage amount of binder, and the original fat content in formulation. Kurt & Kilincceker (2012) evaluated burgers added with 5% wheat, barley, oat, rye, rice, corn, soy, chickpea and yellow lentil flours as binders. They also found that fat retention of burgers was not significantly ($p > 0.05$) affected by the binder type. Cooking loss, moisture and expressible moisture of burgers with 5% wheat flour and rice flour were not statistically different as well, which was similar to the results of burgers containing 5% wheat crumb and 4% rice flour in our research. It was concluded that those cereals and legumes except for lentil flour could improve physical and chemical properties of beef patties. Serdaroğlu, Yıldız-Turp & Abrodímov (2005) reported that addition of 10% blackeye bean flour or lentil flour as extenders resulted in higher cooking yield and moisture retention, and lower diameter reduction than rusk and chickpea flour in low fat meatballs. Meatballs included with 10% rusk had higher water holding capacity than ones with other legume flours. Anderson & Berry (2001) investigated the use of inner pea fibre in high fat ground beef and concluded that pea fibre could retain fat during high temperature heating and increase cooking yield. Hawashin *et al*. (2016) reported that incorporation of olive cake powder in beef patties could significantly ($p < 0.05$) increase cooking yield, moisture retention and fat retention compared to control and decrease dimensional shrinkage when the concentration of olive cake powder was more than 2% ($p \leq 0.05$). Other research revealed that addition level of moringa seed powder and bambara groundnut seed flour were related to the positive effect on cooking yield, moisture retention, and fat retention of beef patties (Al-Juhaimi *et al*., 2016, Alakali, Irtwange & Mzer, 2010). Ergezer, Akcan & Serdaroğlu (2014) reported that increasing amount of potato puree from 10% to 20% in meatballs could significantly ($p < 0.05$) enhance cooking yield, moisture and fat retention. Meatballs containing 10% bread crumb had higher cooking yield than ones with 20% potato puree, while moisture and fat retention between these two treatments did not statistically differ.

The concept of "swelling power (SP)" is defined as the wet weight of the starch sedimented gel divided by its dry weight (Wang & Seib, 1996) and it is related to the water holding capacity of swelled starch. Gujska, Reinhard & Khan (1994) reported that the swelling power of field pea starch was ~6 g/g at 71 °C. Lai & Varriano-Marston (1979) measured swelling power of wheat starch and suggested that SP was 6 g/g at 70 °C. Vandeputte *et al*. (2003) indicated that swelling powers of 3 normal rice starches were all less than 10 g/g at around 71 °C. Similar result of unmodified normal rice starch was presented by Liu, Ramsden & Corke (1999). This might explain similar cooking loss and moisture retention of burgers when adding similar amount of binders. However, swelling power of potato starch at 71 °C was much higher than other starches according to researches (Srichuwong *et al*., 2005, Kim *et al*., 1996), while cooking loss and moisture retention of burgers with 4% potato starch did not differ from ones with similar amount of binders.

This might be caused by the limited available moisture in beef patties affecting the gelatinization of starch at low water content (Vainionpää, Forssell & Virtanen, 1993).

4.3.4. Colour of cooked burgers

During cooking, the colour of burgers changed due to Maillard reaction, protein denaturation, and fat and water loss (Sánchez-Zapata *et al*., 2010) compared to raw burgers. Redness of burgers was significantly ($p < 0.05$) reduced after cooking and differences among treatments were shown (Table 4.6). Pea starch was the only binder affecting redness with increased addition amount, and 4% pea starch contributed to less red colour of burgers than 2% pea starch. This trend was not determined in the research of Kilincceker (2018). In comparison to wheat crumb control, 2% pea starch and rice flour treatments resulted in redder colour in beef burgers. No significant ($p > 0.05$) differences in lightness were observed among treatments compared to wheat crumb. However, burgers with 2% pea starch and both 2% and 4% rice flour had higher lightness value than those processed with 4% potato starch. Incorporation of 4% textured pea protein increased yellowness and chroma of burgers compared to wheat crumb. This might be caused by the own yellow colour of pea protein. Hue angle of burgers with 4% pea starch and rice flour and 2% textured pea protein was similar to that with wheat crumb control. Wan Rosli *et al*. (2011) found adding up to 50% oyster mushroom in chicken patties reduced lightness and yellowness of products without affecting redness. Thus, colour changes with addition of binders may differ among various types of binders and incorporation percentage.

Table 4.6. Least squares means of treatment for colour (L*, a*, b*, C*, h) of cooked burgers formulated with selected binders.

Treatment	L*(D65)	a*(D65)	b*(D65)	C*(D65)	h(D65)
5% Wheat Crumb	48.08^{ab}	5.91^{cd}	15.16^{bc}	16.28^{bc}	68.71^{bc}
2% Potato Starch	47.88^{ab}	6.33^{abc}	14.79^{c}	16.09^{bc}	66.81^{de}
4% Potato Starch	47.05^{b}	6.30^{abc}	14.61^{c}	15.93^{c}	66.67^{de}
2% Pea Starch	48.67^{a}	6.66^{a}	15.26^{bc}	16.67^{ab}	66.44^{e}
4% Pea Starch	47.90^{ab}	6.14^{bcd}	14.91^{bc}	16.14^{bc}	67.53^{cde}
2% Short Rice Flour	48.65^{a}	6.37^{ab}	14.99^{bc}	16.30^{bc}	66.95^{de}
4% Short Rice Flour	48.77^{a}	6.10^{bcd}	15.10^{bc}	16.30^{bc}	67.99^{bcd}
2% Textured Pea Protein	48.39^{ab}	5.85^{d}	15.53^{ab}	16.60^{abc}	69.32^{ab}
4% Textured Pea Protein	48.19^{ab}	5.72^{d}	16.18^{a}	17.17^{a}	70.49^{a}
Standard error	0.31	0.10	0.16	0.17	0.33
p value	0.003	<0.001	<0.001	<0.001	<0.001

$^{a–e}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).
Colour was measured with illuminant D65.
Abbreviations: L*: lightness; a*: redness; b*: yellowness; C*: chroma; h: hue angle.

4.3.5. pH of raw and cooked burgers

There were no significant ($p > 0.05$) differences among tested binders in the pH of both raw and cooked burgers compared to wheat control (Table 4.7), suggesting that replacing wheat crumb with selected non-allergen binders did not affect pH of beef burgers. Similarly, Kurt & Kilincceker (2012) concluded that no significant ($p > 0.05$) difference of pH for both raw and cooked patties was observed between wheat flour treatment and rice flour treatment. Kilincceker (2018) also compared different percentage of potato starch and pea starch in burgers and concluded that binders did not affect the pH of raw patties. Gök *et al*. (2011) reported that pH range of meat burgers was from 5.83 to 6.08. It was noticeable that the pH of burgers after cooking decreased compared to raw patties in this study, which was opposite to other research results.

Table 4.7. Least squares means of treatment for pH of raw and cooked burgers formulated with selected binders.

Treatment	Raw	Cooked
5% Wheat Crumb	6.01	5.88
2% Potato Starch	6.02	5.89
4% Potato Starch	6.05	5.84
2% Pea Starch	5.94	5.92
4% Pea Starch	5.95	5.94
2% Short Rice Flour	5.96	5.81
4% Short Rice Flour	5.96	5.79
2% Textured Pea Protein	6.07	5.90
4% Textured Pea Protein	5.98	5.94
Standard error	0.04	0.05
p value	0.26	0.24

4.3.6. Textural properties and shear force

Texture can be assessed via instrumental device which has both shearing and compression resistance types (De Huidobro *et al.*, 2005). The TPA and shear force results are demonstrated in Table 4.8. With the increase of amount of four non-allergen binders, textural property values of burgers including hardness, cohesiveness, springiness, and chewiness significantly ($p < 0.05$) declined. Whereas, unlike hardness values, shear force values did not differentiate the texture of burgers produced with 2% and 4% of pea starch and potato starch. Addition of two percent of pea starch, rice flour, and textured pea protein led to a tougher burger texture compared to 5% wheat crumb treatment, while inclusion of 2% potato starch resulted in hardness similar to that of wheat crumb burgers. With increasing the incorporation level from 2 to 4%, the hardness of burgers containing pea starch, rice flour, and textured pea protein tended to be insignificantly ($p > 0.05$) different from the control. In contrast, addition of 4% potato starch resulted in significantly ($p < 0.05$) softer burgers compared to wheat crumb control. TPA hardness was more sensitive than shear force for differentiating burger treatments. Compared to control, only 2% textured pea protein burgers exhibited significantly ($p < 0.05$) higher shear force. Also, there was no significant ($p > 0.05$) force difference between treatments with 2% and 4% of potato starch or pea starch burgers. Both levels of pea starch and textured pea protein increased textural values of cohesiveness, springiness, and chewiness of beef burgers in comparison to 5% wheat crumb. Adding 2% potato starch and rice flour in beef burgers formulations led to higher cohesiveness and springiness values compared to 5% wheat crumb controls. When increasing their addition level from 2% to 4%, lower or similar cohesiveness and springiness of burgers did occur compared to control. No significant ($p > 0.05$) difference was observed in terms of chewiness of burgers containing 2% potato starch and 4% rice flour compared to wheat control.

Table 4.8. Least squares means of treatment for textural properties of cooked burgers formulated with selected binders.

Treatment	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (N-mm)	Maximum Load (N)
5% Wheat Crumb	94.65^{cd}	0.31^{c}	4.94^{c}	143.46^{de}	20.89^{bc}
2% Potato Starch	95.49^{cd}	0.33^{b}	5.23^{b}	167.36^{cd}	22.11^{abc}
4% Potato Starch	73.83^{e}	0.28^{d}	4.93^{c}	103.00^{f}	19.57^{c}
2% Pea Starch	133.38^{a}	0.41^{a}	5.54^{a}	303.58^{a}	25.00^{ab}
4% Pea Starch	106.36^{bc}	0.34^{b}	5.31^{b}	191.38^{bc}	21.54^{bc}
2% Short Rice Flour	112.50^{b}	0.35^{b}	5.26^{b}	208.51^{b}	24.85^{ab}
4% Short Rice Flour	89.41^{d}	0.29^{cd}	4.85^{c}	128.14^{ef}	19.15^{c}
2% Textured Pea Protein	135.66^{a}	0.41^{a}	5.64^{a}	316.24^{a}	25.94^{a}
4% Textured Pea Protein	106.92^{bc}	0.35^{b}	5.27^{b}	198.76^{bc}	21.63^{bc}
Standard error	2.98	0.01	0.05	7.60	0.95
p value	<0.001	<0.001	<0.001	<0.001	<0.001

$^{a–f}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).

Desmond, Troy & Buckley (1998) evaluated effect of tapioca starch, oat fibre and whey protein on quality of low-fat beef burgers and indicated tapioca starch resulted in succulent and tender products. Soltanizadeh & Ghiasi-Esfahani (2015) utilized different concentrations (0%, 1%, 3%, 5%) of *Aloe vera* to improve quality of beef burgers and the significant ($p < 0.05$) influence on texture was reported. Compression force of raw burgers increased with the formulated level up to 3% and remained unchanged with further increase to 5%. However, the shear force of cooked burgers decreased with the increase of *Aloe vera* levels. Wan Rosli *et al*. (2011) reported similar trend of hardness changes with addition of oyster mushroom in chicken patties. The toughness of chicken patties decreased proportionally as the level of oyster mushroom increased. The relationship between binder addition level and hardness of cooked products was opposite from that reported in this research. For example, Der (2010) found that increase of green lentil flour incorporation level from 6% to 12% did not cause significant ($p > 0.05$) texture change in beef burgers in terms of shear force, hardness, cohesiveness, and springiness, while increase of red lentil flour addition contributed to significantly ($p < 0.05$) greater hardness values but stable cohesiveness and springiness. The differences in texture might be attributed to different addition level, or content and types of proteins and polysaccharides in different binders.

Although AMSA recommended both shear force and compression measurements for ground beef patties (Belk *et al*., 2015), De Huidobro *et al*. (2005) suggested that TPA compression results had highly significant ($p < 0.05$) correlations with sensory hardness and could be performed as a better predictor of this sensory attribute than Warner–Bratzler shear test. In this study, it was found that TPA was more efficient to differentiate hardness among treatments than shear test. It was likely due to the formation of crust on the surface of patties during grilling that might have affected the results. Consequently, shear force results might not be an ideal indication of the interior texture.

4.3.7. Consumer acceptability

The results of hedonic liking scores are presented as a radar plot in Figure 4.1 and Table 4.9. In general, increased addition level within the same non-allergen binder had limited effect on burger liking scores of sensory attributes. The effect of increased incorporation level from 2 to 4% was significant only with addition of textured pea protein for appearance of burgers. There were no significant ($p > 0.05$) changes of flavour, texture, and aftertaste attribute with increased addition level of non-allergen binders in burgers. Among all treatments, the overall acceptability score of burgers processed with 4% pea starch was significantly ($p < 0.05$) higher than that of burgers with 5% wheat crumb. Incorporation of all other non-allergen binders in beef burgers did not significantly ($p > 0.05$) impact sensorial hedonic scores in terms of appearance, flavour, texture, and aftertaste.

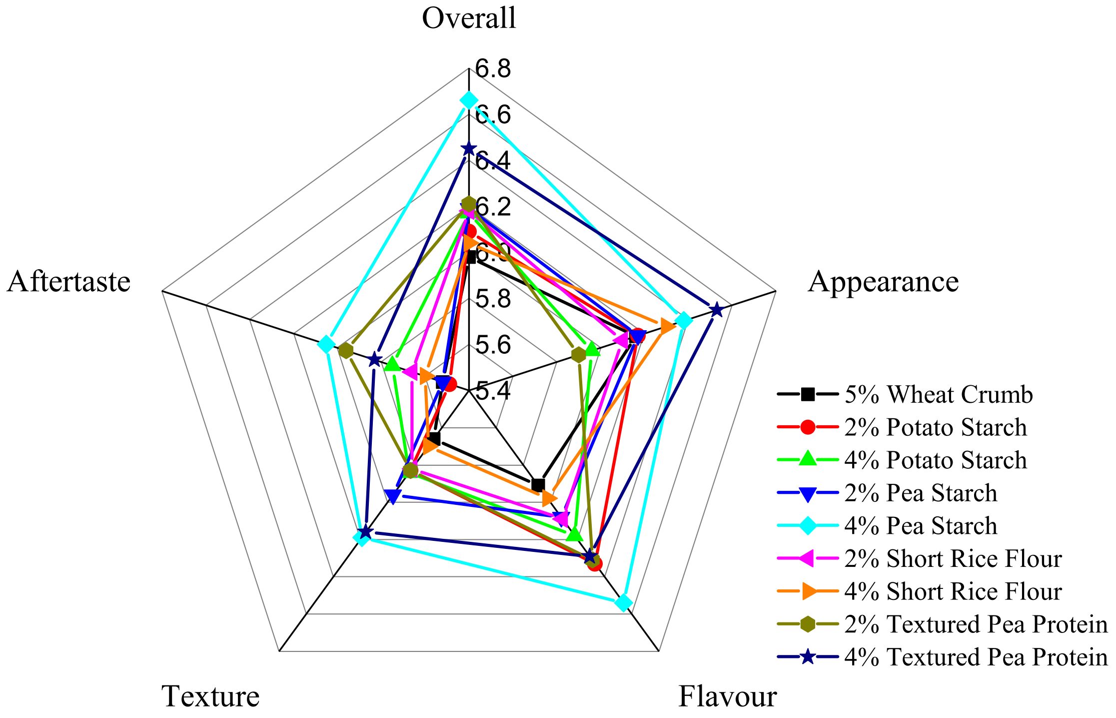


Figure 4.1. Radar plot of hedonic scores of cooked burgers formulated with selected binders evaluated by the consumer sensory panel. Scored on 9-point hedonic scales where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like/dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.

Table 4.9. Least squares means of treatment for hedonic scores of cooked burgers formulated with selected binders evaluated by the consumer sensory panel.

Treatment	Overall	Appearance	Flavour	Texture	Aftertaste
5% Wheat Crumb	5.98^{b}	6.16^{ab}	5.91	5.66	5.52
2% Potato Starch	6.09^{ab}	6.17^{ab}	6.33	5.83	5.49
4% Potato Starch	6.17^{ab}	5.96^{ab}	6.18	5.84	5.75
2% Pea Starch	6.19^{ab}	6.17^{ab}	6.08	5.96	5.52
4% Pea Starch	6.66^{a}	6.38^{ab}	6.54	6.19	6.05
2% Short Rice Flour	6.18^{ab}	6.10^{ab}	6.09	5.82	5.66
4% Short Rice Flour	6.04^{ab}	6.30^{ab}	5.98	5.70	5.60
2% Textured Pea Protein	6.21^{ab}	5.90^{b}	6.31	5.83	5.96
4% Textured Pea Protein	6.45^{ab}	6.53^{a}	6.29	6.16	5.83
Standard error	0.14	0.14	0.15	0.15	0.14
p value	0.034	0.024	0.223	0.254	0.095

[a–b]Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$). Scored on 9-point hedonic scales where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like/dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.

Although means of liking scores especially for flavour, texture, and aftertaste attributes did not significantly differentiate burger treatments, and most of differences between burgers in overall acceptability and appearance were not significant probably due to the more conservative multiple comparisons that Tukey's HSD brought about, some significant differential details still could be perceived by consumers through Just-about-right and Check-all-that-apply methods (Appendix B). In terms of appearance, 72.5% consumers thought interior colour of 2% pea starch burger was just-about-right. Burgers with 4% pea starch had the lowest just-about-right frequency of interior colour. Cooked burgers containing 4% potato starch and 2% textured pea protein were perceived as too dark ($p < 0.01$) as for interior colour, causing lower scores on appearance. On the contrary, 4% rice flour led to significant ($p < 0.01$) too light colour of burgers. Burgers with 4% pea starch had highest checking frequency when it comes to "grilled flavour", "savoury", and "seasoned". Wheat crumb control had highest checking frequency of "beefy" and "fatty/oily" flavour. Textured pea protein burgers at 4% were mostly considered not only "salty", "seasoned", and "savoury", but also “beany” and “off-flavour”. Burgers with 4% rice flour were checked by more consumers as “beany”, “off-flavour”, and “fatty/oily”, which might be the reason of lower flavour liking scores. As for texture, 59.5% and 58.3% panellists believed that the firmness of burgers with 4% textured pea protein and 4% pea starch was just-about-right respectively, while 2% potato starch products were checked the least for just-about-right firmness. On the other hand, burgers with 2% and 4% pea starch accounted for the highest just-about-right juiciness portion. Burgers with 2% potato starch were perceived as products with the least just-about-right juiciness, and they were checked by consumers as the products with the most “rubbery bite” and “tough/leathery” texture. Burgers containing 4% rice flour were checked under the term “leaves a mouth coating” and “chalky texture” the most. Products with 2% textured pea protein obtained the highest check of “chewy”. Four percent of pea starch and textured pea protein burgers received the higher number of counts under the term “nice bite”. Penalty analysis indicated that 5% wheat crumb burgers were significantly ($p < 0.01$) too juicy, and 4% rice flour and textured pea protein caused significantly ($p < 0.01$) too soft texture. Due to the fact that each treatment had different textural profile, the comprehensive texture liking score did not differ too much (in Table 4.9, p value of texture was higher than other properties). Additionally, most consumers thought the aftertaste of burgers with 4% pea starch was pleasantly lingering. At last, 55.6% panellists and 50% panellists would have the intent to purchase burgers with 4% pea starch and textured pea protein respectively. When comparing sensory texture to instrumental data, the two sets of data were not totally in alignment with each other. Unlike TPA results, liking scores did not show any significant difference in texture. The liking scores of textures were not strongly related to instrumental data.

Kilincceker (2018) reported that no significant ($p > 0.05$) differences were observed in sensory hedonic scores of meat patties processed with potato starch, corn starch, and pea starch added at 1%, 3%, and 5% level when it came to appearance, colour, odour, flavour, and texture. Selani *et al*. (2015) concluded that pineapple, passion fruit or mango by-products added at 4 concentrations (1%, 1.5%, 2%, 2.5%) did not affect sensory attributes in terms of colour, odour and overall acceptance of the burgers. Some other studies, however, indicated that the utilization of different binders at specific concentration could improve sensory properties. Modi *et al*. (2004) concluded that the burgers with 8% black gram dhal flour had better sensory quality attributes compared to other legumes such as soya bean, bengal gram, and green gram. Soncu *et al*. (2015) suggested that low-fat hamburgers with 2% carrot fibre presented comparable overall sensory properties to regular hamburgers, but utilization amount over 2% led to deficient results. Colle et al. (2019) found that beef patties processed with 2% potato extracts exhibited more desirable juiciness than ones with 2% textured soy protein flour, while consumers did not perceive significant ($p > 0.05$) difference in terms of texture, flavour, and overall acceptability.

Based on the liking scores, PCA was selected as a statistical method to visually describe the relationship between hedonic scores of 5 sensory attributes and 9 burger treatments. Two principal components were extracted which represented 91.11% of the initial variability of the data. The loading plot and the score plot (Figure 4.2) shows that texture and flavour affected the overall hedonic score, but the appearance is not related to the aftertaste. Based on PCA results, the principal component scores from the first two principal components for each treatment were utilized to run hierarchical cluster analysis. Four percent of pea starch and textured pea protein treatment were firstly grouped together other than other treatments (Figure 4.3). This was a further indication that 4% pea starch and 4% textured pea protein contributed similarly to sensory characteristics of regular fat beef burgers.

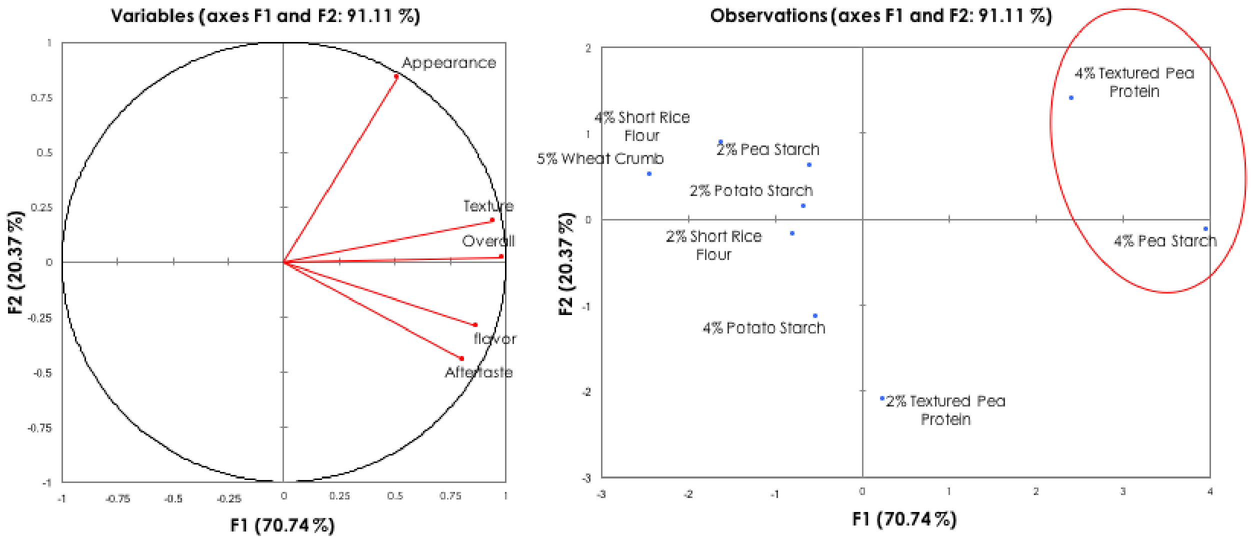


Figure 4.2. Loading plot and score plot of principal component analysis for attributes of cooked burgers formulated with selected binders evaluated by the consumer sensory panel. F1 and F2 account for 70.74% and 20.37% of total variance respectively. Rays represent loadings of variables, and points represent treatments.

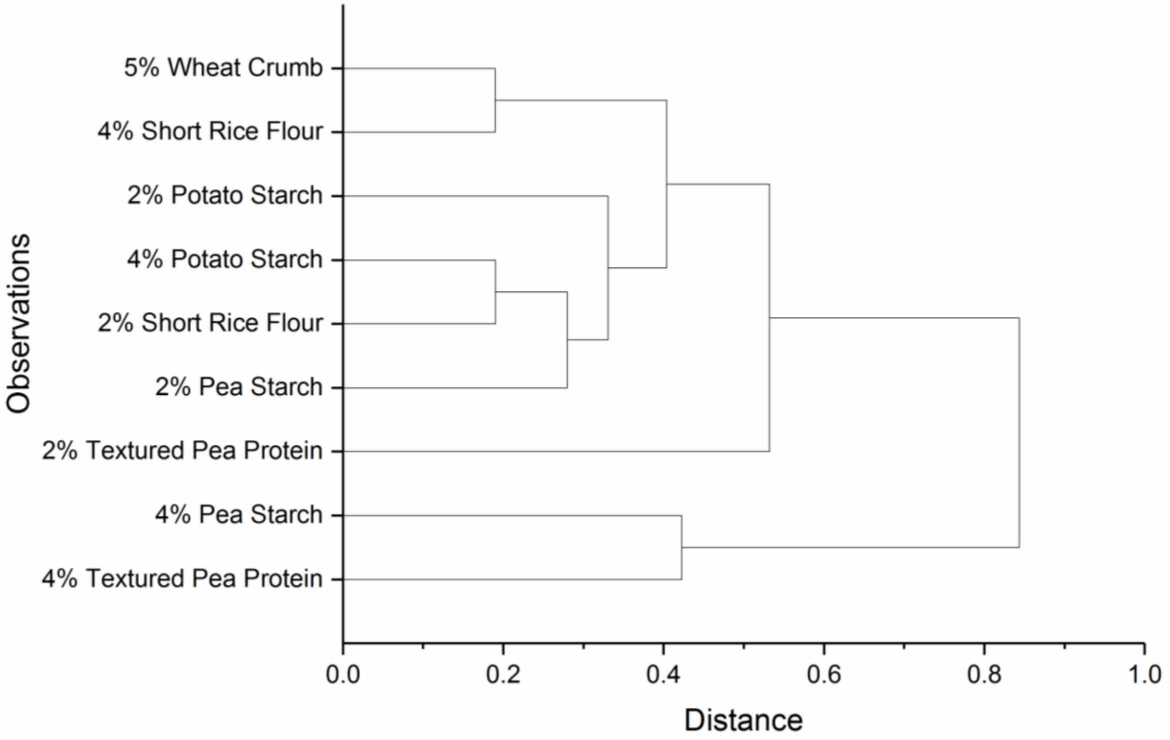


Figure 4.3. Dendogram of hierarchical cluster analysis for attributes of cooked burgers formulated with selected binders evaluated by the consumer sensory panel.

4.4. Conclusions

In this study, potato starch, pea starch, short rice flour, and textured pea protein were selected as non-allergen binder alternatives to wheat crumb, and the effects of addition level on qualities of both raw and cooked beef burgers were evaluated. During the 4 days storage under simulated retail display, burgers processed with 4% rice flour and 4% textured pea protein had better effect on maintaining colour stability of redness, similar to 5% wheat crumb. Addition of 4% textured pea protein effectively delayed lipid oxidation of raw burgers. All allergen-free binders added at 4% in beef burgers contributed to similar cooking properties to 5% wheat crumb control and better cooking properties than 2%. Sensory evaluation indicated that consumer preferred burgers incorporated with 4% pea starch and 4% textured pea protein for appearance and overall acceptability. Overall, based on the shelf-life results of raw burgers, and functionalities and sensory properties of cooked burgers, pea starch and textured pea protein have potential to be exploited as gluten-free substitutes for wheat crumb as binders to improve or maintain functional and sensory properties of coarse meat products in practical applications. Future study could focus on the optimization of combination of different binders, or the application of non-allergen binders on low-fat ground meat, restructured, and comminuted products.

Chapter 5. Effect of non-allergen binders on functionalities and sensory characteristics of low sodium pork bolognas

5.1. Introduction

Food allergy is one of the chronic diseases without a cure at this time (Shroba, Rath & Barnes, 2018). In Canada, approximately 2.5 million people self-report having at least one food allergy and more than 40% of Canadians read food labels looking for allergen information (Allergy, Genes and Environment Network, 2015). Food allergy is also an increasing concern for children, adolescents and their families all over the world, with most recent Australian reports showing that food allergy occurs in 1-in-12 children (Fong, Katelaris & Wainstein, 2018). In 2012, 5.6% or 4.1 million children in the U.S. reported food allergies in the past 12 months (Bloom, Cohen & Freeman, 2013). Eggs, milk, mustard, peanuts, crustaceans and molluscs, fish, sesame seeds, soy, sulphites, tree nuts, wheat and triticale have been identified as priority food allergens by Health Canada (Health Canada, 2011). Among these, wheat flour, soy protein, and whey protein are widely used in meat products as binders or fillers to enhance the texture, flavour, or cooking characteristics (Lauck, 1975, Bejosano & Corke, 1998).

Meanwhile, populations around the world are consuming much more sodium than is physiologically necessary according to the World Health Organization, which is relative to a number of noncommunicable diseases (NCDs) including hypertension, cardiovascular disease and stroke. Decreasing sodium intake may reduce blood pressure and the risk of associated NCDs (WHO, 2012). Consumption of processed meats has been criticized because they contain high levels of sodium (Horita *et al*., 2011). In meat products, salt plays an important role in processing, and affects their physicochemical characteristics, shelf life and palatability. (Choi *et al*., 2014). NaCl contributes to the emulsion stability of emulsified meat products resulting in the formation of a desirable gel texture upon cooking (Terrell, 1983). Approaches to salt reduction in meat products include: 1) gradual reduction of salt employed in formulations to acceptable levels; 2) the use of salt substitutes, in particular, potassium chloride (KCl), associated with masking agents; 3) the use of flavour enhancers, which enhance the saltiness of meat products when used in combination with salt instead of providing a salty taste; 4) the optimisation of the physical form of salt so that it becomes more taste bioavailable and therefore less salt is needed (Desmond, 2006).

A lot of research on utilization of different binders in comminuted or emulsion type meat products such as frankfurter, mortadella, and bologna sausage has been published in the past several decades (Barbut, 2007, Bhat & Bhat, 2011, Zayas, 2012, Petracci *et al*., 2013). Sanjeewa *et al*. (2010) screened six high-yielding chickpea varieties and two kinds of chickpea flours were reported to improve the low-fat pork bologna's instrumental and sensory texture properties as extended at 2.5% and 5.0%. Omana, Pietrasik & Betti (2012) found that soy protein isolate could be replaced by poultry protein isolate without negatively affecting low-fat turkey bologna characteristics as evident from cooking yield and purge loss data. Connective tissue gels from pork skin, poultry skin and desinewed beef were also investigated in reduced-fat bolognas and results indicated that they could act as water-binders and texture-modifying agents in low-fat comminuted meat products (Osburn, Mandigo & Eskridge, 1997, Osburn & Mandigo, 1998, Osburn, Mandigo & Calkins, 1999). Chickpea flour (Verma, Ledward & Lawrie, 1984) and black gram flour (Chaudhray & Ledward, 1988) were incorporated in British fresh skinless sausages to reduce the cost of products without compromising acceptability. In addition, pork skin and wheat fibre mixture in frankfurter-type sausages (Choe *et al*., 2013), barley flour and pea flour in low-sodium fish balls (Ganie, Kumar & Tanwar, 2017), pigeon-pea flour, corn flour, walnut paste and sesame paste in beef emulsion sausages (Tahmasebi *et al*., 2016), navy bean paste in gnocchi-type beef emulsion (Liu *et al*., 2016), potato flour in buffalo sausage (Ponsingh *et al*., 2010), modified corn and potato starch in bologna-type sausages (Aktaş & Genccelep, 2006), were successfully added to enhance meat emulsion stability.

On the basis of previous research, allergen binders were successfully exploited in low-sodium restructured meat products (Tsao *et al*., 2002). The objective of this study was to evaluate the functional effectiveness and consumer acceptance of selected alternative non-allergen ingredients (hydrolysed collagen, white navy bean flour, potato starch, pea starch) as binders in low fat reduced sodium pork bolognas.

5.2. Materials and methods

5.2.1. Ingredients

The frozen pork leg lean meat and back fat were obtained from a local processor and kept in the freezer (-20 °C) until used for processing. Spices were purchased from local grocery stores. Saltwell® sea salt (Salinity AB, Göteborg, Sweden) was purchased from a local distributor. Saltwell® sea salt (containing 65% sodium chloride and 30% potassium chloride, the rest contents including moisture, anti-caking agent and iodine) was utilized in this experiment as a commercial salt replacer. Native potato starch was donated from Manitoba Starch Products (MB, Canada). Native pea starch was purchased from Nutri-Pea Limited (Accu-Gel™, Portage la Prairie, MB, Canada). White navy bean flour was produced by Infra-Ready Foods (Saskatoon, SK, Canada). Hydrolysed collagen was shipped from PB Leiner (Solugel 5000, Davenport, IA, United States). Wheat flour was obtained from a local grocery store (Robin Hood Mills, Saskatoon, SK, Canada). Wheat flour treatment was included to serve as allergen control currently used in meat industry.

5.2.2. Bologna manufacture

All manufacturing was carried out in a refrigerated pilot plant (<7 °C) at Food Processing Development Centre (FPDC) (Leduc, AB, Canada). For each of three replications, seven bologna treatments were processed: two no binder (NB) controls with NaCl regular salt (RS) or Saltwell low sodium salt (LS) where NaCl was fully substituted with salt replacer; and five binder treatments (wheat flour: WF, hydrolysed collagen: HC, white navy bean flour: WNBF, potato starch: PoS, pea starch: PeS) formulated with Saltwell salt replacer. The amount of non-meat ingredient usage is shown in Table 5.1.

Table 5.1. Percentage of non-meat ingredients used for raw bologna batter manufacture.

Treatments[1]	NB-RS	NB-LS	WF-LS	HC-LS	WNBF-LS	PoS-LS	PeS-LS
Wheat flour			3				
Hydrolysed collagen				1			
White navy bean flour					3		
Potato starch						3	
Pea starch							3
Regular salt (RS)	1.8						
Low sodium salt (LS)		1.8	1.8	1.8	1.8	1.8	1.8
Spices	1	1	1	1	1	1	1
Sodium nitrite	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Formulation treatments[1]: NB-RS: no binder-regular salt; NB-LS: no binder-low sodium; WF-LS: wheat flour-low sodium; HC-LS: hydrolysed collagen-low sodium; WNBF-LS: white navy bean flour-low sodium; PoS-LS: potato starch-low sodium; PeS-LS: pea starch-low sodium; RS: treatments processed with NaCl; LS: treatments processed with Saltwell® sea salt.

Before processing, the meats were thawed in the 2 °C cooler, then ground separately through a 3 mm plate (Model AW114, K & G Wetter, Mississauga, ON, Canada). Samples were taken from each batch of ground meat and fat, and proximate composition was determined using a Foss FoodScan analyser (Type 78800, FoodScan Lab, FOSS, Hilleroed, Denmark). Protein (from lean meat and back fat) content was adjusted to a constant level of 14% and fat to 10% in all formulations by adding water and shredded ice. Lean pork, NaCl or Saltwell, and sodium nitrite were added into a 30 L bowl silent cutter (Seydelmann, Stuttgart, Germany), with half amount of water and chopped for 90 seconds at low speed (3000 rpm knives speed). Then the respective binders and spice mix (0.72% dextrose, 0.15% black pepper, 0.05% nutmeg, 0.05% garlic powder, 0.03% onion powder) were added with remaining amount of water and chopped for another 90 seconds. During chopping fat was added and the bowl chopper was stopped and the lid and sides were scrapped to evenly distribute ingredients. Finally, the meat batter was chopped (intermediate bowl and 6000 rpm knife speeds) under vacuum (-0.8 bar) for another 2 minutes. The final temperature of the batter never exceeded 8 °C. The total amount of each batch was 10 kg. Batter samples were taken from each treatment for back extrusion and rheological tests. The remaining emulsion batter mixture was vacuum stuffed (Handtmann, Model VF80, Waterloo, ON, Canada) into moisture-proof casings (105 mm diameter) at full vacuum. Casings were tensioned and clipped, and the bologna sausages were thermally processed in a smokehouse (Fessmann GmbH u. Co., Winnenden, Germany) to a final internal temperature of 71 °C monitored using a HH23 Microprocessor thermometer (Omega Engineering Inc., Stamford, CT) with copper constantan thermocouples inserted in the geometrical centre of the sausages. The product was initially cooled with cold running water until 40 °C was reached, then cooled overnight before each chilled bologna was removed from its casing and weighed to determine cooking loss. One chub per formulation was prepared as 2 mm slices that were vacuum packed (10 slices per package) in high barrier, mylar/polyethylene pouches (Ulma TF-Supra packaging machine, CyE.S. Coop., Ltd., ONATI, Spain). Three packages of sliced bolognas for each treatment were placed in a simulated retail display deck cabinet under 24 h fluorescent lighting with an average intensity of 1630 lx at 4 °C and displayed for 8 weeks. The remainder chubs were vacuum packaged and all samples were stored in cardboard boxes at 2 °C until sampling for sensory and instrumental evaluations.

5.2.3. Measurement on bologna batter

5.2.3.1. Back extrusion

Approximately 30 g batter was stuffed into 50 mL beakers. A 12.5 mm-diameter metal plunger attachment of the Instron Universal Testing System (model 5565, Instron Corporation, Burlington, ON, Canada) was utilized to back extrude the batter at a speed of 20 mm/min. The batter resistance to flow was represented as the peak force (N) to push the plunger into the sample.

5.2.3.2. Rheology

The dynamic viscoelastic properties of bologna batter were determined by a Physica MCR Rheometer (Anton Paar GmbH, Ashland, VA) according to the method described by Omana, Pietrasik & Betti (2012) with some modifications. Samples were loaded between a 2.5 cm diameter measuring plate and the bottom plate with 1 mm gap. The rheological measurements were performed under oscillatory mode with a controlled strain of 5% at a fixed frequency of 1.0 Hz. The storage modulus (G') and the loss modulus (G") were recorded during heating the raw batter sample from 4 to 71 °C at a heating rate of 2 °C/min.

5.2.4. Cooking loss

After cooking and overnight storage, chilled meat chubs were removed from casings and weighed to determine cooking loss. Overall cooking loss was calculated by raw stuffed weight and cooked bologna weight.

$$\text{cooking loss} = \frac{\text{raw weight} - \text{cooked weight}}{\text{raw weight}} \times 100\%$$

5.2.5. Measurement on cooked bolognas

5.2.5.1. Interior colour and colour stability

The instrumental colour was measured by a Minolta CM-2500C handheld spectrophotometer (Konica-Minolta, Osaka, Japan) with a 10° observer angle and illuminant D65, calibrated against a white tile. The internal colour of cooked bolognas was measured on freshly cut surface from sliced pieces after manufacturing. In addition, the surface colour of vacuum-packed bologna stored in a simulated retail display deck cabinet under 24 h fluorescent lighting at

4 °C with an average intensity of 1630 lx was measured on Day 0 and after 4 weeks and 8 weeks. The CIE L^* (lightness), a^*(redness), b^* (yellowness) values for each sample were presented as the average of three readings. Hue and Chroma were calculated for each sample using the following formulas:

$$\text{hue} = \tan^{-1} {b^*}/{a^*}$$

$$\text{chroma} = ({a^*}^2 + {b^*}^2)^{\frac{1}{2}}$$

5.2.5.2. pH and sodium content

pH of cooked bolognas was measured using an Orion 5 Star pH meter (Thermo Fisher Scientific, Ottawa, ON, Canada) after 5 g of each sample was homogenized with 50 mL deionized distilled water for 30 s. The pH of the homogenate was adjusted to pH 9 with a sodium ionic strength adjuster (4 M NH_4Cl & 4 M NH_4OH, Fisher Scientific, Edmonton, AB) and sodium content was measured with an ion-selective combination sodium electrode (pHoenix Electrode Co., Houston, TX) connected to an ion meter (Thermo Fisher Scientific Orion 5-Star pH/ISE/Cond/DO, Beverly, MA), as described by Pietrasik, Gaudette & Klassen (2016). Sodium ion concentration was converted into mg/g.

5.2.5.3. Expressible moisture

A cork borer with 1.7 cm diameter and a pair of parallel knives with 1.5 cm gap was used for sampling. Each sample (around 3 g) was placed in 50 mL centrifuge tube fitted with a thimble consisting of Whatman No. 3 filter paper and centrifuged at 963 × g (MPWMed Instruments, Warsaw, Poland) for 10 minutes. Expressible moisture was expressed as the ratio of weight lost after centrifugation to the initial sample weight.

$$\text{expressible moisture} = \frac{\text{weight before centrifuge} - \text{weight after centrifuge}}{\text{weight before centrifuge}} \times 100\%$$

5.2.5.4. Texture profile analysis (TPA)

The texture profile analysis tests were performed using an Instron Universal Testing System (Model 5565, Instron Corporation, Burlington, ON, Canada). Six core samples for each treatment and batch were cut with a cork borer with 2 cm diameter and a pair of parallel knives with 1.5 cm distance, from the centre of each bologna chub and compressed twice to 30% of their original height with a 9 cm flat ended steel plunger at a constant cross-head speed of 60 mm/min. The following parameters were obtained: hardness (N), the peak force of the first bite; cohesiveness, the ratio of the active work under the second force–displacement curve to that under the first compression curve; springiness (mm), the distance the sample recovers after the first bite and chewiness (N mm), hardness × cohesiveness × springiness (Pietrasik, Pierce & Janz, 2012).

5.2.5.5 Purge loss

Purge accumulation from pre-weighed, cooked sliced product was determined on three vacuum-packaged bags for each treatment and batch. After packaging, the bags were stored at refrigerated temperature (4 °C) for either 4 or 8 weeks. Purge loss was measured by reweighing blotted slices from the packages stored under refrigerated temperature following each storage interval, and was expressed as a percentage of the initial slice weight.

5.2.5.6 Scanning electron microscopy

The microstructure of cooked bologna samples was visualized by scanning electron microscopy (SEM), according to Felisberto *et al*. (2015) with some modifications. The bologna sausage cubes (1 × 1 × 0.5 cm) were fixed with glutaraldehyde (3 g/100 g) in 0.1 mol/L phosphate buffer (pH 7.2–7.4), post-fixed with osmium tetroxide (1% with 0.2 mol/L phosphate buffer), washed, dehydrated in alcohol and hexamethyldisilazane solutions with increasing concentrations, air-dried overnight, sputter-coated with gold (Denton Desk II Sputter Coater, Denton Vacuum Inc., Moorestown, NJ) and scanned by SEM (Zeiss EVO MA10, Carl Zeiss Promenade, Jena, Germany) at 10 kV. A large number of micrographs were acquired to select the most representative ones (500× magnification).

5.2.6. Sensory evaluation

Evaluation of the bolognas was conducted in the dedicated sensory evaluation lab at Consumer Product Testing Centre (CPTC, Edmonton, AB, Canada). Participants (n = 150) over 18 years of age (77 males and 73 females) who frequently (at least once a month) consume bologna type sausages were recruited. Three-digit blinding codes were used to label each treatment during panel set up, and all products were refrigerated (LHU-113, ESPEC Corp., Osaka, Japan) until prepared for serving. All sensory panel responses were collected using a computerized program specific for sensory evaluation (Compusense Cloud, Compusense Inc., Guelph, Ontario). An incomplete block design was used to conduct panel evaluations. Each panellist evaluated five treatments of sliced bolognas. The panellists received verbal instructions upon arrival at the CPTC, and were then seated at individual testing booths lit with white lighting where written instructions were integrated into electronic ballot presentation. Samples were placed on a 6-inch white coded styrofoam plate (Genpak) and passed through serving hutches to each panellist in a sequential, monadic manner. A forced 90 sec break was administered and room temperature water and unsalted crackers were provided for palate cleansing between samples. Panellists were asked to rate overall acceptability and the acceptability of appearance, flavour, overall texture, juiciness, firmness, chewiness and aftertaste using a 9-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely. The Check-All-That-Apply (CATA) method was used to further define the flavour attribute with the supplied terms: bitter, metallic, cereally/starchy, bland, off flavour, salty, flavourful, savoury, spicy/peppery, sweet, chalky, sour/tangy, mouth-coating. A 7-point Just-about-right (JAR) scale was used to further describe appearance (colour and surface moisture), flavour (saltiness and bologna flavour intensity) and texture (juiciness, firmness and chewiness) characteristics. The JAR scale was anchored with 1 = too grey and dull/dry surface/blend/dry/soft/crumbly, 2 = moderately grey and dull/dry surface/blend/dry/soft/crumbly, 3 = slightly grey and dull/dry surface/blend/dry/soft/crumbly, 4 = just-about-right, 5 = slightly pink and bright/moist surface/strong/juicy or moist/firm/rubbery, 6 = moderately pink and bright/moist surface/strong/juicy or moist/firm/rubbery, and 7 = too pink and bright/moist surface/strong/juicy or moist/firm/rubbery. A 5-point linear intensity was used to evaluate purchase intent of each sample, where 1 = definitely would not purchase, 2 = probably would not purchase, 3 = might/might not purchase, 4 = probably would purchase, 5 = definitely would purchase. A 7-point linear intensity was also used to evaluate the aftertaste (lingerer and pleasantness) of each sample, where 1 = no aftertaste/unpleasant, 7 = lingering/pleasant.

5.2.7. Statistical analysis

Instrumental data were analysed using the Linear Mixed Model of SPSS 23 (IBM Corp., Armonk, NY, USA) including a fixed effect of treatment and a random effect of replication. Tukey's HSD was used to determine the differences between treatment means ($p < 0.05$). Consumer data were analysed using a two-way ANOVA of XLSTAT 2016 (Addinsoft, Paris, France) including a fixed effect of treatment and a random effect of consumer. Tukey's HSD test was used to determine the differences between the least squared means ($p < 0.05$). Penalty analysis was run by XLSTAT 2016.

5.3. Results and discussion

5.3.1. Back extrusion

Since the batter had to be pumped through pipelines when stuffed, back extrusion force was a useful parameter for designing equipment in the meat processing industry (Gujral *et al.*, 2002). Back extrusion results of different treatments are shown in Table 5.2. Back extrusion force of LS bologna batter without binder was significantly ($p < 0.05$) lower than that of RS bologna batter. Although the batter samples were not analysed for protein extractability in this study, it is perceived that the differences in back extrusion may be attributed to the amount of extracted proteins in LS and RS samples. During samples handling and preparation of specimens for testing, it was observed that batter with regular salt was stickier to the beaker wall than that with low sodium salt in the experiment. This could be explained that a slightly better meat protein extraction was achieved by NaCl compared with KCl (Trius *et al.*, 1994).

Table 5.2. Least squares means of treatment for peak values of back extrusion of raw batters formulated with selected salts and binders.

Treatment	Maximum force (N)
No binder RS	22.76^{cd}
No binder LS	20.36^{e}
Wheat flour LS	24.39^{bc}
Hydrolysed collagen LS	20.96^{de}
White navy bean flour LS	27.62^{a}
Potato starch LS	25.65^{ab}
Pea starch LS	25.25^{b}
Standard error	0.53
p value	<0.001

$^{a–e}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).

Except for hydrolysed collagen, incorporation of all binders significantly increased extrusion forces of LS batter. Of these binders, white navy bean flour, potato starch, and pea starch in LS batter even resulted in batter with a higher resistance to flow as compared to RS. Consequently, amount of extracted meat protein and application of binders were both responsible for the increase of viscosity. Addition of white navy bean flour in LS batter resulted in the highest back extrusion force. Compared to wheat flour, potato starch and pea starch caused similar bologna batter viscosity. The batters containing hydrolysed collagen exhibited smaller force values which were comparable to LS treatment processed without any binders. This may be due to the hydrolyzation of collagen and smaller molecular weight of peptides.

5.3.2. Rheological properties of different bologna batter

The storage and loss moduli were used to measure the viscoelastic characteristics of the emulsified meat product during the cooking process, and showed in Figure 5.1. At the initial stage, a slight decrease in storage modulus with an increase in temperature was presented from data although the figure did not significantly demonstrate this trend, and it could be related to the breaking of hydrogen bonds as temperature increased (Savadkoohi *et al*., 2013).

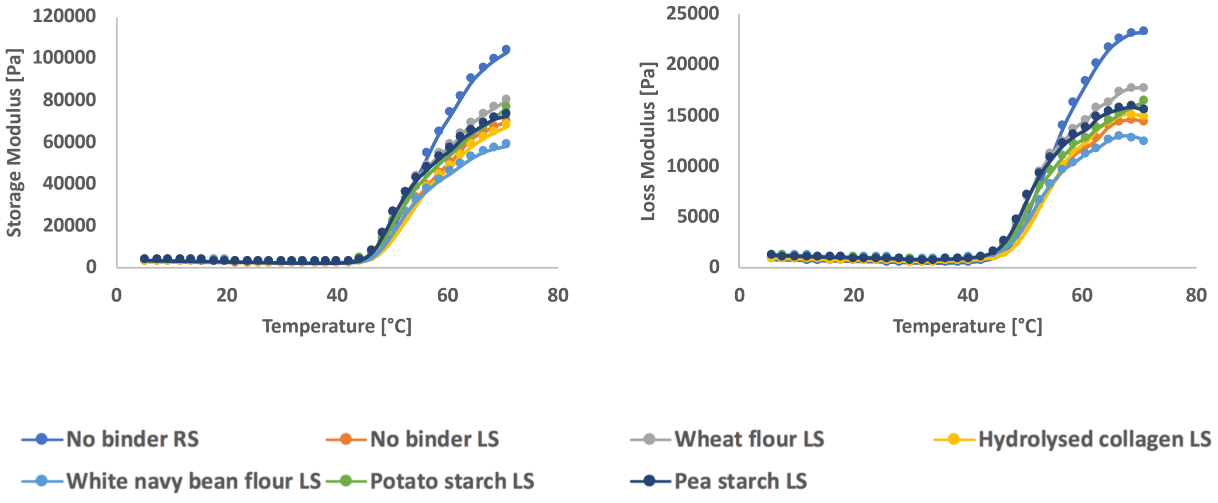


Figure 5.1. Changes of storage modulus and loss modulus of bologna batters formulated with selected salts and binders during heating process.

Both storage and loss modulus values increased significantly at around 45 °C, which had been related to aggregation of globular portion of myosin head and structural transformation of the batter protein from a loose network to an ordered cross-linked gel matrix; but the storage modulus values were higher than loss modulus after cooking, which means the batters were essentially elastic in nature (Khiari *et al*., 2014, Felisberto *et al*., 2015). It was noticeable that adding binders to LS batter did not retard myosin gelation (Figure 5.1). Storage modulus of regular salt batter observed at 71 °C was significantly higher than the one of low sodium batter with or without non-allergen binders. As pointed out earlier, these differences might be due to better extractability of myofibrillar proteins with addition of NaCl as compared to low sodium salt (Trius *et al*., 1994), which favoured formation of stronger protein gels. No statistical differences (p > 0.05) were observed among all the binder treatments at the end of heating. Loss modulus of each treatment showed the similar trend as storage modulus except for a slight decrease at the end of heating process of LS batter without binder or with pea starch, hydrolysed collagen, and white navy bean flour.

Orford *et al*. (1987) compared development of shear modulus with time for 30% (w/w) gelatinised starch gels of pea, potato, and wheat. It was observed that granule rigidity increased in the order potato<wheat<pea, and a similar trend was found for the rate of shear modulus as time increased. However, in the present study, after including these ingredients in meat batter, the storage modulus of each treatment showed similar trend in terms of potato starch and pea starch in most of the heating progress. This might indicate that addition amount of these binders did not significantly affect rheological properties of meat batters, or the interactions between potato starch and myofibrillar protein might occurred, which increased modulus of potato-meat batter so that significant differences between potato starch and pea starch did not exist.

5.3.3. Colour of sliced pork bolognas at different storage time

The colour of bologna is influenced by not only meat pigments but also fat, water and non-meat binders. Table 5.3 shows interior colour of bologna sausages. None of the colour parameters was impacted by replacing regular salt with low sodium salt. Horita *et al*. (2011) also concluded that replacement of NaCl by KCl did not alter colour of cooked mortadella. Pietrasik & Gaudette (2015) also published that OF45 and OF60 (sea salts with 45% and 60% less sodium than sodium chloride respectively, replaced by potassium, sulphate, and magnesium, etc.) increased yellowness and hue angle of homogeneous turkey sausages compared to NaCl control.

Table 5.3. Least squares means of treatment for interior colour of bolognas formulated with selected salts and binders on Day 0.

Treatment	L*(D65)	a*(D65)	b*(D65)	C*(D65)	h(D65)
No binder RS	67.73^{b}	7.99^{a}	9.74^{d}	12.60^{b}	50.65^{c}
No binder LS	67.92^{ab}	8.13^{a}	10.09^{cd}	12.96^{ab}	51.12^{c}
Wheat flour LS	67.48^{b}	8.01^{a}	10.24^{bc}	13.01^{ab}	52.00^{bc}
Hydrolysed collagen LS	67.41^{b}	8.25^{a}	10.18^{cd}	13.11^{a}	50.99^{c}
White navy bean flour LS	68.06^{ab}	7.89^{a}	10.65^{ab}	13.26^{a}	53.48^{b}
Potato starch LS	67.57^{b}	8.04^{a}	10.10^{cd}	12.91^{ab}	51.48^{bc}
Pea starch LS	68.75^{a}	7.40^{b}	10.83^{a}	13.12^{a}	55.69^{a}
Standard error	0.20	0.11	0.10	0.11	0.47
p value	<0.001	<0.001	<0.001	0.004	<0.001

$^{a–d}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).
Colour was measured with illuminant D65.
Abbreviations: L*: lightness; a*: redness; b*: yellowness; C*: chroma; h: hue angle.

Addition of wheat flour in low sodium bolognas led to similar colour parameters to no binder LS treatment. Bolognas incorporated with pea starch had the highest lightness and yellowness values, which were significantly ($p < 0.05$) higher than those of bolognas processed with wheat flour, hydrolysed collagen, and potato starch. Pea starch also contributed to significantly ($p < 0.05$) lower redness as compared to any other treatments and it was the only treatment affecting redness. No significant differences in lightness, redness and yellowness among bolognas with processed with wheat flour, hydrolysed collagen, white navy bean flour and potato starch were observed. Additionally, pea starch addition increased hue angle of cooked bologna compared to other binders, while chroma remained similar to that of other binder treatments.

In agreement with our results, Shand (2000) reported that the colour of low-fat pork bolognas with 4% potato starch and wheat flour had no significant ($p > 0.05$) change compared to no binder control. However, Pietrasik & Janz (2010) reported an opposite conclusion that 4% pea starch increased the redness of beef bolognas but had no effect on yellowness compared to no binder treatment, and there was no significant alteration of colour between potato starch and wheat flour bolognas. This difference from our research might be caused by different types of meat used for bologna processing due to higher myoglobin content in beef muscle than pork (Ginger, Wilson & Schweigert, 1954). Nevertheless, it was also mentioned that the magnitude of difference was small and considered to be of no practical importance. Devatkal *et al*. (2011) measured the interior colour of emulsified and cured chicken nuggets with binders, and reported that replacing 5% wheat flour in nuggets by 5% gluten-free sorghum flour significantly decreased redness and increase hue angle ($p < 0.05$).

The surface colour changes of vacuum-packed bologna exposed to light during 8-week storage period were shown in Table 5.4. There was no interaction between treatment and storage time, indicating that the types of binders or salts did not compromise colour stability of products. Within the first 4 weeks of storage, lightness of bolognas remained unchanged ($p > 0.05$), but significantly increased after next 4 weeks of exposure to light in a retail display. Redness, yellowness and chroma of vacuum packaged bolognas decreased significantly ($p < 0.05$) after 4 weeks and remained unchanged until the end of storage period. Hue angle maintained similar values during the whole experimental period. The effect of formulation treatments on surface colour of vacuum packaged bolognas over 8 weeks of simulated retail display was similar to that observed for interior colour, and the small variations among treatments might be attributed to available oxygen level in vacuum packages.

Table 5.4. Least squares means for main effects of treatment combination with storage time for surface colour of vacuum packaged bolognas formulated with selected salts and binders.

Treatment	Time	L*(D65)	a*(D65)	b*(D65)	C*(D65)	h(D65)
No binder RS		66.03^{ab}	7.51^{cd}	9.91^{c}	12.44^{d}	52.85^{b}
No binder LS		66.22^{ab}	7.70^{bc}	9.86^{c}	12.51^{d}	52.00^{bc}
Wheat flour LS		65.85^{ab}	7.90^{ab}	10.41^{b}	13.08^{b}	52.78^{b}
Hydrolysed collagen LS		66.20^{ab}	7.84^{abc}	9.93^{c}	12.66^{cd}	51.71^{bc}
White navy bean flour LS		66.27^{ab}	7.40^{bc}	10.97^{a}	13.44^{a}	54.77^{a}
Potato starch LS		65.72^{b}	8.08^{a}	9.99^{c}	12.86^{bc}	51.04^{c}
Pea starch LS		66.47^{a}	7.20^{d}	10.46^{b}	12.71^{cd}	55.48^{a}
Standard error		0.15	0.08	0.07	0.07	0.34
p value		0.007	<0.001	<0.001	<0.001	<0.001
	Day 0	65.83^{B}	7.84^{A}	10.37^{A}	13.01^{A}	52.91
	4 weeks	65.97^{B}	7.62^{B}	10.12^{B}	12.68^{B}	52.97
	8 weeks	66.53^{A}	7.66^{B}	10.17^{B}	12.74^{B}	52.96
	Standard error	0.10	0.05	0.05	0.05	0.22
	p value	<0.001	0.010	0.001	<0.001	0.979

$^{a–d}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).
$^{A–B}$Means with different uppercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).
Colour was measured with illuminant D65.
L*: lightness; a*: redness; b*: yellowness; C*: chroma; h: hue angle.

The rate of colour change depends on the total amount of oxygen available for reaction and the temperature of product storage (Omana, Pietrasik & Betti, 2012). Horita *et al.* (2011) reported that KCl salt did not change colour stability in mortadella after 60 days. In contrast, Pietrasik & Gaudette (2015) reported a significant decrease in yellowness and hue angle of turkey sausages containing sea salt replacers during 8 week exposure to light in a retail display case. In the present paper, the decrease of yellowness within 8 weeks' storage was in agreement with these results.

5.3.4. pH and sodium content

The pH of bolognas was around 6.3–6.5 (Table 5.5) and no significant differences were produced by adding different salts or binders. Pietrasik, Gaudette & Johnston (2017) reported similar conclusion that pH of naturally cured wieners with regular salt was similar to those with low salt where 50% of NaCl was substituted by modified potassium chloride salt replacer. Pietrasik & Gaudette (2015) also found OF45 and OF60 salt replacer had no effect on pH of turkey sausage.

Table 5.5. Least squares means of treatment for pH and sodium content of bolognas formulated with selected salts and binders.

Treatment	pH	Sodium (mg/g)
No binder RS	6.50	8.19[a]
No binder LS	6.36	4.38[c]
Wheat flour LS	6.39	4.42[c]
Hydrolysed collagen LS	6.48	4.58[c]
White navy bean flour LS	6.31	5.25[b]
Potato starch LS	6.41	4.08[c]
Pea starch LS	6.45	4.46[c]
Standard error	0.04	0.16
p value	0.051	<0.001

[a–c]Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).

As expected, sodium content of RS control bolognas was the highest. Replacing NaCl with low sodium salt substitute successfully decreased sodium content and resulted in approximately 46% lower sodium content in the final bologna sausage products. There were no significant differences in sodium content among LS products except for white navy been flour treatment, which resulted in higher sodium content after cooking. According to the nutritional profile of white navy bean flour, sodium content was 5 mg/100 g, which was even less than that of potato starch (8 mg/100 g) or other binders. Thus, higher sodium content in white navy bean bolognas might not be caused by the higher sodium content originally from navy bean flour. It was also not likely that the less liquid loss resulted in more remaining sodium based on its hydration properties shown in Table 5.5. The higher sodium content might be explained by some specific interactions between proteins or carbohydrates from flour helping in trapping more sodium ion in protein matrix.

5.3.5. Hydration properties of bolognas

Hydration properties such as cooking loss, expressible moisture, and purge loss were determined. Regular salt bologna processed with NaCl had significantly lower cooking loss as compared to products manufactured with low sodium salt (Table 5.6). Wheat flour and non-allergen binders effectively reduced cooking losses compared to no binder LS bologna. Furthermore, except for hydrolysed collagen, the cooking losses of all non-allergen binders were even lower than that of no binder RS control. Contrary to cooking loss results, replacing NaCl with low sodium KCl-based salt replacer had no significant effect on expressible moisture and purge accumulation during up to 8 weeks of storage. It was indicated that the moisture loss of no binder products occurred mainly during cooking, but after cooking, the moisture retention was stable within 8 weeks. In addition, the interaction between treatment and storage time for purge loss was insignificant. Wheat flour, hydrolysed collagen, and potato starch significantly ($p < 0.05$) reduced purge loss in LS bolognas compared to both control treatments processed without any binders. The amount of purge for potato starch bolognas was equivalent to that of wheat flour bolognas and significantly ($p < 0.05$) lower than that of white navy bean bolognas and pea starch bolognas during 8 weeks of storage. Moreover, wheat flour treatment showed the equally effective water holding capacity after cooking to non-allergen treatments indicated by the similar expressible moisture.

Table 5.6. Least squares means for main effects of treatment combination with storage time for hydration properties of bolognas formulated with selected salts and binders.

Treatment	Time	Cooking loss (%)	Expressible moisture (%)	Purge loss (%)
No binder RS		4.33^{b}	13.69^{a}	2.69^{a}
No binder LS		6.28^{a}	14.13^{a}	2.68^{a}
Wheat flour LS		3.10^{cd}	10.14^{b}	2.06^{c}
Hydrolysed collagen LS		3.96^{bc}	12.74^{ab}	2.24^{bc}
White navy bean flour LS		3.06^{cd}	13.52^{ab}	2.44^{ab}
Potato starch LS		2.33^{d}	10.80^{ab}	2.07^{c}
Pea starch LS		3.04^{cd}	13.38^{ab}	2.45^{ab}
Standard error		0.25	0.83	0.09
p value		<0.001	0.006	<0.001
	4 weeks			2.35
	8 weeks			2.41
	Standard error			0.05
	p value			0.364

$^{a\text{–}d}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).

The function of sodium chloride in formulation of processed meat products is well known and documented (Terrell, 1983, Ruusunen & Puolanne, 2005, Inguglia *et al*., 2017). The salt plays a critical role in maintaining adequate water and fat binding properties leading to the formation of a desirable gel texture and increase of cooking yield and juiciness upon cooking. This is attributed to solubilizing meat proteins and increasing protein hydration by added salt, which further increases the incorporation of fat to stabilize meat batter in processed meat products. Reducing added salt level while formulating meat products has an adverse influence on hydration properties (Desmond, 2006). However, reduction of NaCl level and utilization of KCl-based replacer to reach the same total salt amount in meat system still failed to alleviate cooking loss in our study. These results might testify some theories about the difference between sodium and potassium salts. Hamm (1961) reported a magnitude order of water holding capacity for chloride salt at pH 6.4: Li > Na > K > Mg > Ba > Ca > Zn. Also, Na^+ was considered as structure-making ion, causing a positive hydration effect, which means enforcing a hydrogen bound network of neighbouring water molecules to make water molecules less mobile and more structured; and K^+ (structure-breaking ion) was classified oppositely (Puolanne & Halonen, 2010). The differences between these two ions and their varying effect on thermal stability of batters were the likely reason why bolognas processed with NaCl had lower cooking loss than ones with Saltwell salt replacer containing 30% of KCl.

However, salt type did not differentiate the water holding capacity of cooked bologna sausages (expressible moisture data in Table 5.6). Salt could disrupt the myofibrillar structure, especially the chloride ion bounding to meat proteins more strongly than the sodium ion. This association results in an increase in the negative charges of proteins which promotes activation and swelling of myofilaments and leads to increasing of hydration and water-binding capacity in processed meats (Hamm, 1972, Puolanne & Halonen, 2010, Desmond, 2006). Due to the similar ionic strength of anions in RS and LS formulations, the cooked meat with either salt would have similar water holding capacity. It has been suggested that the ionic strength of the salts rather than types of ion is responsible for protein extraction and moisture retention capacity (Pietrasik & Gaudette, 2015). Pietrasik & Gaudette (2015) applied OF45 and OF60 salt replacer (as introduced previously, they were sea salts with 45% and 60% less sodium and mainly replaced by potassium) into turkey sausages and concluded that OF45 increased expressible moisture compared to OF60, which meant the sodium to potassium ratio affected water holding capacity of cooked sausage. It was clear that potassium could to some extend improve water holding ability at specific levels.

Binders from plant or animal sources were extensively researched and applied into comminuted meat products. Zayas & Lin (1988) reported that addition of 3% defatted corn germ protein derived from both supercritical-CO_2 and hexane extraction methods could significantly ($p < 0.05$) increase yield of frankfurters, while water holding capacities (expressible moisture) between control and experimental frankfurters with corn germ protein were not different. These results were similar to data presented in our study. Aktaş & Genccelep (2006) indicated that effect of starch type (potato starch and corn starch) on water holding capacity and emulsion stability (total expressible fluid) of bologna-type sausages was insignificant ($p > 0.05$). Shan *et al*. (2015) investigated hydration properties of frankfurters processed with shaddock albedo at six different levels (0, 2.5, 5, 7.5, 10, 12.5%). They found that all sausage treatments containing shaddock albedo had significantly ($p < 0.05$) lower cooking loss compared to control. Meanwhile, the presence of shaddock albedo decreased ($p < 0.05$) the total expressible fluid of frankfurters except for 12.5% addition treatment (probably due to the low pH negatively influencing meat batter stability). Jang, Lee & Chin (2016) incorporated 1% red bean protein isolate (RBPI) in pork myofibrillar protein gel and concluded that cooking yield was elevated by approximately 9% compared to non-RBPI control. Wang *et al*. (2018) applied collagen superfine powders without preheating processing as fillers at 1%, 3%, and 5% concentration in processing of Harbin red sausages. All collagen powder treatments significantly ($p < 0.05$) decreased cooking loss and it was explained by formation of collagen cross-links to meat protein and gel network during chopping and boiling, which increased the water-holding capacity. Dzudie, Scher & Hardy (2002) produced beef sausages with different amount of common bean flour. The significant ($p < 0.05$) reduction of cooking loss appeared when flour level was more than 5.0%. Additionally, water holding capacity was increased at 2.5% addition and no significant difference ($p > 0.05$) was found between treatments from 2.5 up to 10.0%.

Studies on substituting wheat with other plant sources have also been conducted by many researchers. Onweluzo *et al*. (2003) produced emulsified buffalo loaves containing seed flour of *Detarium microcarpum* (Dm) high in water-soluble non-starch polysaccharides and reported that loaves with 0.5% or 1.0% Dm seed flour presented similar ($p > 0.05$) cooking yield, consumer shrink, and water holding ability to control products where 3% wheat semolina was added as binder. Pietrasik & Janz (2010) reported that cooking yields of low-fat bolognas with 4% pea starch and wheat flour were comparable, and significantly ($p < 0.05$) higher than that of no binder control. These conclusions were similar to the results in the present research, in which legume flour like white navy bean flour was used at 3% as the same as wheat flour, and hydration properties like cooking loss and expressible moisture showed insignificant differences. Rosero-Chasoy & Serna-Cock (2017) evaluated plantain peel flour as a replacement of 6.45% wheat flour in frankfurter-type sausages. Water retention capacity of frankfurters, in which up to 50% wheat flour was substituted by plantain peel flour, was significantly ($p < 0.05$) higher than that of products with only wheat flour as binder. However, a decrease in water retention capacity of frankfurters was observed after further increasing the substitution level and with 100% replacement the water retention percentage was even lower than control. The ratio of different binders included in emulsion systems influenced water holding capacity variously.

High purge loss would leave unappealing impression on consumers, and the liquid accumulation within the package could shorten shelf-life of the product due to higher microbial growth in purge where nutrients and metabolites could easily disseminate (Omana, Pietrasik, and Betti, 2012, Yotsuyanagi *et al.*, 2016). Pietrasik, Gaudette & Johnston (2017) recognized that purge of both regular or low sodium wieners stored under vacuum stabilized after 4 weeks of refrigerated storage and there was no further significant increase. In our study, the purge loss tended to be stable after 4th week of storage (Table 5.6). However, Shand (2000) found the purge loss of low-fat bolognas with potato starch and wheat flour increased from 2nd week to 4th week. This may reveal that the purge loss mainly happened within first 4 weeks. Pietrasik & Janz (2010) reported that 4% pea starch led to significantly lower expressible moisture and purge loss of low-fat bolognas than no binder control, which was not observed in this research. The differences of hydration properties between their research and this study may due to a higher incorporation level of pea starch or different salt types used in formulations.

5.3.6. Textural properties of bolognas

TPA results indicated that no significant ($p > 0.05$) differences in textural properties of bolognas between RS and LS were observed (Table 5.7). This finding was in agreement with the results published by dos Santos Alves *et al*. (2017) that all textural parameters (hardness, springiness, cohesiveness, chewiness) of bologna-type sausages with 2.5% NaCl were statistically equal to those of products with 1.25% KCl and 1.25% NaCl. Horita *et al*. (2011) also reported similar results that in reduced-fat mortadella, hardness, cohesiveness, and chewiness of sausage with 2% NaCl or 1% NaCl associated with 1% KCl were not significantly different even after 60 days storage. Choi *et al*. (2014) produced low-sodium frankfurter sausage with low sodium salt (60% NaCl and 40% KCl) and found coincident results that low sodium salt did not affect hardness, cohesiveness, and chewiness, but increased springiness. Some other studies, however, suggested that salt replacers affected product texture. Pietrasik & Gaudette (2015) compared salt replacer OF45 and OF60 (commercial sea salts with 45% and 60% less sodium and mainly replaced by potassium) and reported that OF45 rather than OF60 decreased hardness of turkey sausages. Pietrasik, Gaudette & Johnston (2017) compared regular salt with salt replacer containing 50% of modified potassium chloride (single crystal that significantly reduces the bitter/metallic note) in naturally cured wieners, and found that salt replacer significantly reduced hardness, springiness, and chewiness.

Table 5.7. Least squares means of treatment for textural properties of bolognas formulated with selected salts and binders.

Treatment	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (N-mm)
No binder RS	36.06^{ab}	0.16^{ab}	3.42^{b}	20.09^{bcd}
No binder LS	33.46^{b}	0.16^{ab}	3.39^{b}	18.37^{cd}
Wheat flour LS	41.15^{ab}	0.16^{ab}	3.81^{ab}	25.02^{abc}
Hydrolysed collagen LS	36.30^{ab}	0.15^{b}	3.40^{b}	18.17^{d}
White navy bean flour LS	36.30^{ab}	0.16^{ab}	3.66^{b}	21.54^{bcd}
Potato starch LS	43.70^{a}	0.18^{a}	4.16^{a}	31.73^{a}
Pea starch LS	39.68^{ab}	0.17^{a}	3.63^{b}	25.13^{ab}
Standard error	1.85	0.01	0.11	1.57
p value	0.003	0.026	<0.001	<0.001

$^{a\text{–}d}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$).

Texture of processed meats depends on structure and integrity of the protein matrix formed during cooking (Pietrasik & Gaudette, 2014). Salts plays an essential role of extracting functional myofibrillar proteins that assist in binding meat particles together (Pietrasik & Gaudette, 2015). The efficiency of protein extraction in salt solutions mainly depends on the pH, ionic strength, and type of the salt. Some studies pointed out that with the increase of salt level formulated in meat products, the concentration of extracted myofibrillar protein increased until a threshold is reached (Munasinghe & Sakai, 2004). Both NaCl and KCl could increase the hydration and solubility of myofibrillar protein. At pH 7.0, NaCl had higher protein extractability than KCl from 0.4 to 1.8 molarity (Munasinghe & Sakai, 2004). However, the contents, types, and physical status of NaCl and KCl, and other residues may also contribute to different protein extraction efficiency. Utilization of Saltwell salts replacer in this research exhibited no significant difference in hardness and other textural properties of regular or low sodium sausages, indicating that extracted protein contents in RS and LS bologna systems were comparable and not affected by the types of salts. The pH and salt content in our bolognas, or other components in Saltwell might not result in protein extractability difference that Munasinghe & Sakai (2004) mentioned and were different from studies of Pietrasik & Gaudette (2015) and Pietrasik, Gaudette & Johnston (2017) due to types of commercial salts.

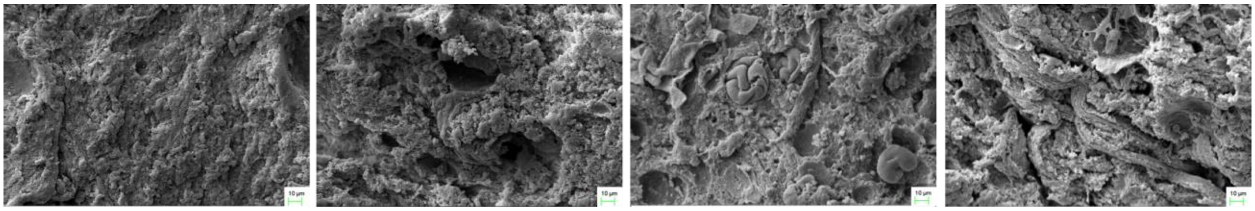
Addition of wheat flour resulted in no significant ($p > 0.05$) changes in hardness, springiness and chewiness of final products. However, potato starch treatment produced a harder texture of bolognas compared to LS treatment formulated without binder and exhibited greater springiness than other treatments except for wheat flour. Potato and pea starch addition increased cohesiveness and chewiness of LS bologna compared to hydrolysed collagen. Bolognas processed with potato starch also featured chewier texture than ones with white navy bean flour. Only hydrolysed collagen reduced the chewiness of bolognas among all binder treatments.

In the research from Pietrasik & Janz (2010), incorporation of 4% wheat flour or pea starch in low fat bolognas had the trend to increase the hardness, chewiness, springiness, and cohesiveness compared to no binder control. However, in the present study, only chewiness of bolognas processed with 3% pea starch instead of wheat flour was higher than that of no binder LS control. Shand (2000) reported that no difference existed in cohesiveness and springiness between potato starch bolognas and wheat flour bolognas, which was in agreement with our results. Although some research indicated that connective tissue protein had an equal to or larger detrimental effect upon firmness than muscle proteins in an emulsion system (Randall & Voisey, 1977, Comer, 1979), the addition amount of hydrolysed collagen (1%) might not be enough to significantly change bologna hardness, or the hydrolysis of collagen caused loss of its original function. Wang *et al*. (2018) revealed that increasing incorporation level of pork collagen superfine powder in sausages from 1% to 5% insignificantly ($p > 0.05$) changed product cohesiveness, while hardness, springiness, and chewiness of sausages tended to increase.

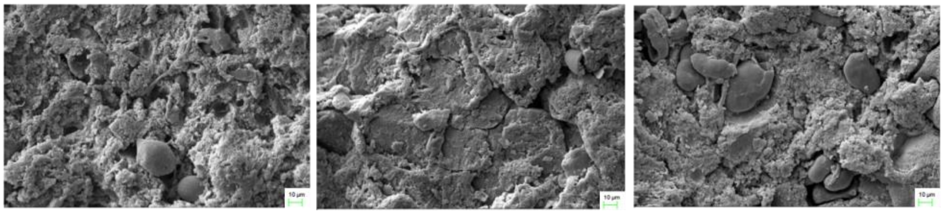
Other research on application of plant-based binders in comminuted meat or myofibril model were presented as follows. Onweluzo *et al*. (2003) concluded that when fat level in emulsified buffalo loaves reached 10%, Dm seed flour products at 0.5% addition level did not differ in shear force from controls incorporating 3% wheat semolina, while increasing Dm seed flour amount to 1.0% affected texture by reducing the shear force value. Zayas & Lin (1988) reported that 3% supercritical-CO_2 extracted corn germ protein treatment did not statistically affect Instron hardness of frankfurters, while sausages containing 3% hexane extracted corn germ protein had significantly ($p < 0.05$) lower hardness than control. Jang, Lee & Chin (2016) suggested that addition of 1% red bean protein isolate influenced texture of pork myofibrillar protein gel by significantly ($p < 0.05$) decreasing the gel strength. Dzudie, Scher & Hardy (2002) found that hardness, cohesiveness, and shear force of beef sausages processed with 2.5% common bean flour were similar to control. However, higher flour content (from 5% to 10%) was accompanied by a significant increase in cohesiveness, but a significant reduction in hardness and shear force of products, which was explained by dilution of meat proteins. Shan *et al*. (2015) found incorporation of shaddock albedo at 2.5% concentration increased hardness but did not significantly ($p > 0.05$) affect springiness, cohesiveness, and chewiness of frankfurters. Effects of these binders mentioned above on textural properties of different comminuted meat products might vary due to the factors such as the degree of extraction of myofibrillar protein, stromal protein content, degree of comminution and type and level of non-meat proteins (Dzudie, Scher & Hardy, 2002).

5.3.7. Microstructure of bolognas

Figure 5.2 shows the microstructure of bolognas processed with different salts or in presence of binders. White and grey areas represent meat protein aggregates or binders, while black areas represent pores of bologna network. In comparison to no binder LS bologna, RS bologna had more compact and denser topography and less porous structure, which helped trap more water and fat in the protein network. After adding binders, some of the granules swelled, collapsed and filled in the cavities or maybe even interacted with the myofibrillar proteins or fat granules. However, some of them cannot completely form gel with meat proteins during heating to 71 °C and act as fillers. Potato starch and wheat flour demonstrated relatively less whole binder granules or fibrils from 30 images of each treatment compared to hydrolysed collagen, white navy bean flour and pea starch.



No binder RS   No binder LS   Wheat flour LS   Hydrolysed collagen LS



White navy bean flour LS   Potato starch LS   Pea starch LS

Figure 5.2. Scanning electron microscopy images of bolognas formulated with selected salts and binders. Light areas represent meat proteins or binders, while dark areas represent pores.

Horita *et al*. (2014) investigated protein matrix structure of reduced sodium frankfurter sausages through SEM. The image of 50% NaCl reduced sausages demonstrated an open and spongy structure with a large number of pores. This could be caused by the fact that after the sodium chloride content was reduced and not substituted by others salts, the amount of extracted proteins decreased, consequently lowering the water holding capacity and gel strength (Gordon, 1993).

However, frankfurters containing KCl as a salt substitute with an equal ionic strength revealed compact, nonporous protein matrix with dense characteristics and showed a higher emulsion stability among the treatments. In this study, using salt replacer failed to eliminate negative impact that reducing sodium brought. This might be attributed to other reagents in Saltwell replacer which disturbed the gelation of comminuted meats.

Jang, Lee & Chin (2016) analysed SEM images of heat-induced myofibrillar protein gels after the addition of 1% red bean protein isolate and noted that when protein isolate was included, the microstructure became more compact. Wang *et al*. (2018) also depicted microstructure of sausages containing non-heating collagen superfine powders as a better compact network with smaller voids and highly ordered framework and almost uniform pores size, due to the formation of strong hydrogel with meat proteins enveloping fat and moisture in the packaged network during battering and cooking. In our study, images also illustrated that LS bolognas with binders had a more compact structure with smaller cavities as compared to no binder LS control. However, most of binders still retained natural shape, or misshaped but intact, and embedded in protein aggregates, instead of gelled.

Starch consists of discrete granules with various shape, size, and composition (Li *et al*., 2003), and swell in water during heating (Rao & Tattiyakul, 1999). Comer (1979) pointed out that potato starch swelled at a faster rate than other starches such as corn, wheat and yellow field pea starches and resulted in the most stable homogenates. The author also noted that native plant proteins simply did not properly gel under low cooking temperature conditions used in manufacturing of wieners and bologna (Comer, 1979). Schoch & Maywald (1968) categorized potato starch as high swelling starch according to hot-paste viscosity patterns due to enormous swelling when cooked in water, while yellow pea and navy bean starches were classified as restricted-swelling starches due to cross-linkages within granules reducing solubilization and stabilizing swollen granules. Kim *et al*. (1996) reported that pasting temperature of three kinds of potato starches ranged from 62.7 °C to 67.8 °C, which was lower than that of navy bean starch (79 °C). Park *et al*. (2009) reported similar pasting temperature of potato starch (67.9 °C). Gujska, Reinhard & Khan (1994) compared physicochemical properties of field pea starch and navy bean starch, and reported that field pea starch had the lower initial pasting temperature (73 °C) than navy bean starch (83.5 °C). Hoover & Ratnayake (2002) investigated two cultivars of navy bean starches and two cultivars of smooth pea starches and found that navy bean starches had lower pasting temperature (70 °C and 72 °C respectively) than pea starches (74 °C and 75 °C respectively). Ratnayake *et al*. (2001) also investigated pasting properties of field pea starches, and four cultivars of starches exhibited identical pasting temperatures (~79.5 °C). Du *et al*. (2014) directly determined pasting characteristics of 10 whole legume flours, and the pasting temperature of navy bean flour was 78.3 °C. Different values varied on account of the plant cultivar, but these results showed that pea starch and the starch in navy bean flour might not swell properly and increase viscosity in meat batters before cooking end point reached (71 °C). Kumar & Khatkar (2017) indicated that gelatinization temperature of two varieties of wheat starch and their subfractions ranged from 74.5 °C to 87.1 °C. Similarly, Zeng *et al*. (1997) also suggested that the temperature at which onset gelatinization occurred differed significantly among the starches and ranged from 76.2 ºC to 88.5 ºC among 6 cultivars and 8 lines of wheats. However, protein fractions in wheat flour such as gliadin and glutenin may affect the pasting temperature by types and amount of glutens (Barak, Mudgil & Khatkar, 2013, Chen *et al*. 2010). Barak, Mudgil & Khatkar (2013) suggested that 4 varieties of wheat flour had significantly different pasting temperatures, but they were all between 66.85 ºC and 68.75 ºC, which were lower than the data of wheat starch above.

Except for temperature which might be not high enough to make binders swell or form a gel, insufficient moisture content in meat batter could be another factor influencing proper plant-meat network. Comer *et al*. (1986) showed evidence of the restriction of starch gelatinization on account of limited moisture availability in comminuted meat products even though internal temperatures of 72 °C were reached. Microscopy images demonstrated misshaped but intact wheat starch granules in wieners, which were also observed in Figure 5.2, and corn starch granules in wieners appeared to be embedded in the protein matrix instead of coated by protein films, which were similar to pea starch in our study. Zhang, Li & Shi (2006) indicated that collagen hydrolysate lost the ability of collagen fibril formation and SEM images suggested no fibrillogenesis. In Figure 5.2, the triple helical shape of collagen residue was still visible. Hydrolysed collagen played more similar role to pea starch and white navy bean flour in bolognas as "filler" rather than "binder".

In emulsion sausage system, embedded fat globules appeared to be in the dispersed phase, while the proteins, polysaccharides and water appeared to be in the continuous phase (Morin, Temelli & McMullen, 2004). Due to the alcohol washing in the preparation, fat on the surface might be removed. However, some bigger hemispherical pits in some figures might represented the fat globules. Li *et al*. (2017) investigated the chemical interactions between the overdrying potato starch and surimi. It was reported that potato starch increased the amount of hydrogen bond and non-disulphide covalent bond and decreased the amount of ionic bond, which might stabilize the network structure of protein gel.

5.3.8. Consumer acceptance of bolognas

Liking scores were demonstrated in both the form of a radar plot in Figure 5.3 and Table 5.8. It was indicated that replacing NaCl with Saltwell salt replacer significantly ($p < 0.05$) reduced flavour and aftertaste acceptability of bologna sausages. Incorporation of four non-allergen binders in LS bolognas effectively improved some sensory properties and significantly ($p < 0.05$) enhanced overall acceptability, flavour, and juiciness scores. Except for increase in firmness acceptability, addition of allergen binder (wheat flour), resulted in no significant ($p > 0.05$) influence on all sensory scores compared to no binder LS control. More specifically, potato starch, pea starch, and hydrolysed collagen provided better sensory acceptance of LS bolognas in terms of overall acceptability compared to no binder treatments. Addition of potato starch, pea starch, and hydrolysed collagen also led to greater flavour preference than wheat flour or no binder controls in LS products. Furthermore, potato starch bolognas had higher overall texture scores than no binder LS bolognas. Consumers also preferred LS bolognas containing potato starch or pea starch to no binder for juiciness, firmness, chewiness, and aftertaste. Within four allergen-free treatments, potato starch showed chewier texture of bologna than the other treatments. The impacts of all treatments on appearance liking scores were statistically indistinguishable ($p > 0.05$). Regular salt bolognas processed without binder offered more acceptable flavour and aftertaste than low sodium salt products manufactured with wheat flour or no binder. Additionally, according to the TPA results (Table 5.7), it was revealed that consumer liking scores of firmness and chewiness were positively related to instrumental hardness and chewiness. Products with harder and chewier texture were more acceptable texturally for panellists. To be more specific, bolognas processed with potato starch, pea starch, and wheat flour had higher mean values of instrumental hardness and chewiness than products with hydrolysed collagen or no binder LS. Accordingly, their sensory overall texture, firmness and chewiness scores were relatively higher as well.

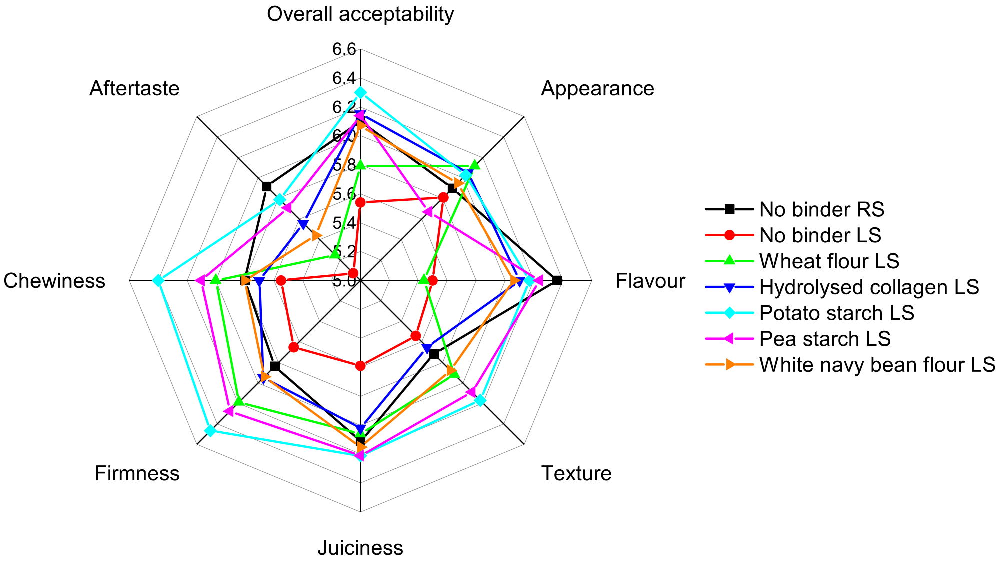


Figure 5.3. Radar plot of hedonic scores of bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 9-point hedonic scales where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like/dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.

Table 5.8. Least squares means of treatment for hedonic scores of bolognas formulated with selected salts and binders evaluated by the consumer sensory panel.

Treatment[1]	Overall	Appearance	Flavour	Texture	Juiciness	Firmness	Chewiness	Aftertaste
NB-RS	6.10^{ab}	5.90	6.36^{a}	5.72^{ab}	6.11^{ab}	5.84^{bc}	5.80^{bc}	5.92^{a}
NB-LS	5.54^{b}	5.81	5.50^{bc}	5.54^{b}	5.59^{b}	5.65^{c}	5.55^{c}	5.07^{c}
WF-LS	5.79^{ab}	6.12	5.44^{c}	5.92^{ab}	6.06^{ab}	6.19^{ab}	6.00^{abc}	5.25^{bc}
HC-LS	6.15^{a}	6.05	6.10^{a}	5.65^{ab}	6.02^{ab}	5.95^{abc}	5.70^{bc}	5.56^{abc}
WNBF-LS	6.07^{ab}	5.95	6.06^{ab}	5.88^{ab}	6.15^{a}	5.94^{abc}	5.80^{bc}	5.44^{abc}
PoS-LS	6.30^{a}	6.03	6.17^{a}	6.17^{a}	6.21^{a}	6.47^{a}	6.40^{a}	5.79^{ab}
PeS-LS	6.14^{a}	5.67	6.24^{a}	6.09^{ab}	6.21^{a}	6.28^{ab}	6.10^{ab}	5.71^{ab}
Standard error	0.14	0.12	0.14	0.13	0.13	0.13	0.12	0.14
p value	0.001	0.343	<0.001	0.005	0.022	<0.001	<0.001	<0.001

$^{a–c}$Means with different lowercase letters in the same column are significantly different (Tukey's HSD, $p < 0.05$). Scored on 9-point hedonic scales where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like/dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.

Treatment[1]: NB-RS: no binder-regular salt; NB-LS: no binder-low sodium; WF-LS: wheat flour-low sodium; HC-LS: hydrolysed collagen-low sodium; WNBF-LS: white navy bean flour-low sodium; PoS-LS: potato starch-low sodium; PeS-LS: pea starch-low sodium.

In the study of Horita *et al.* (2011), reduced-sodium mortadella, where half amount of 2% NaCl was substituted by KCl (1%), had significantly ($p < 0.05$) lower flavour acceptance while texture score did not differ from that of NaCl control, which were similar to the results observed in this study. The same conclusion was presented by dos Santos Alves *et al*. (2017), where 2.5% NaCl was replaced with 1.25% KCl and 1.25% NaCl in low-fat bologna-type sausages. In different processed meat system, the threshold of perceived bitterness from KCl varied. Keeton (1984) found that slight bitter taste could be detected in country-style hams where 33.3% replacement of sodium chloride by potassium chloride occurred. Gelabert *et al*. (2003) concluded that in fermented sausages, the bitter taste from replacing up to 40% NaCl by KCl was acceptable.

Low fat bolognas in the study of Pietrasik & Janz (2010) formulated with 4% pea starch or wheat flour showed insignificant ($p > 0.05$) distinction in terms of flavour and firmness acceptability, while in our study, pea starch bolognas had higher flavour scores than allergen bolognas. This difference might be caused by salt types incorporated in the system and consumers were more sensitive to adverse flavour in LS bologna processed with wheat flour than that incorporated with pea starch. Onweluzo *et al*. (2003) found that substituting 1.0% Dm seed flour for 3% wheat semolina in comminuted buffalo loaves with 10% fat could lead to more tender texture and lower textural liking score.

The data shown in Appendix C provides more information on sensory evaluation which might be related to the liking scores. Penalty analysis provided additional explanation about sensory properties. Treatments with net penalty>0.5 (high impact) were presented as follows. Consumers thought no binder LS (52%) and wheat flour (47%) bolognas were lack of bologna flavour. 43% consumers thought wheat flour bolognas were not salty enough. No binder RS, no binder LS, hydrolysed collagen and white navy bean flour treatments contributed to too soft texture. No binder RS and hydrolysed collagen were considered too juicy. Twenty four percent of consumers regarded no binder LS bolognas as way too crumbly products.

In addition, among 150 panellists, 62 people checked term “flavourful” for no binder RS bolognas. Although potassium salts were reported to elicit bitter taste (Sinopoli & Lawless, 2012), less than 10 panellists noted “bitter” flavour across all formulations of LS treatments. However, no binder RS bologna still had lowest check number (only 1 person). According to Saltwell product information, in commercial salt blends, sodium did not combine with potassium resulting in a bitter and metallic taste. However, in Saltwell, a natural chemical reaction combined sodium with potassium to produce a natural saltiness without a bitter edge. In addition, Gaudette & Pietrasik (2017) revealed that salt replacers containing potassium chloride could potentially replace sodium chloride in processed meats with complex flavour profiles such as spices and smoke due to their aid in masking the bitterness elicited by salt replacers, while meats with simple flavour profiles might require further flavour optimization.

5.4. Conclusions

After both preliminary test and formal experiment, we found all non-allergen binders were equivalent to wheat flour and favourably affected hydration properties and thermal stability, yielding lower cooking loss as compared to LS control. Substituting NaCl with salt replacer resulted in lower cook yield and negatively affected consumer acceptability. However, detrimental effect was partially overcome by addition of binders. Interior redness was only affected by pea starch and lightness increased during 8-week simulated retail display storage. Potato starch contributed to a harder and more springiness texture compared to low sodium treatment formulated without binder. Microstructure images illustrated protein matrix associated with potato starch. Of the ingredients tested potato starch showed the greatest potential to be utilized as gluten-free alternative to wheat flour for bologna binder applications.

In the meat industry, “clean label” ingredients have been getting more attention to meet consumer preference. Saltwell as a reduced sodium salt replacer has a potential to meet requirements for both clean label and low sodium product demand, but the adverse effects which salt replacer resulted in needed to be solved. Adding allergen-free binders in emulsion type bolognas could not only benefit consumers who are suffering from food allergy diseases, but also alleviate or even improve various processing functionalities and sensory acceptability. This research provided a vital reference for comminuted meat producers. Meanwhile, differences of functionalities between pure NaCl-KCl mix and commercial reduced-sodium salts from existing publications were also noticed in this study. Comparing binders in reduced sodium meats with natural salt substitutes has more practical values. Future studies can focus on optimisation of combinations of non-allergen binders in processed meat formulations.

Chapter 6. Conclusions, implications and future prospects

This research was conducted to evaluate selected commercially available non-allergen ingredients as low-cost and functional binders in regular and reduced sodium processed meat products. The hypothesis was that replacement of high priority allergen ingredients with non-allergenic alternatives do not negatively impact the functionality and consumer acceptability of developed products. To testify this hypothesis, research activities were organized to directly compare selected non-allergen binders to currently used allergen-containing ingredients in two model meat systems. Studies conducted in the preliminary experiments were focused on screening available commercial binders and determining their acceptable incorporation levels through internal sensory evaluation. Based on this initial screening, the potential binders including potato starch, pea starch, medium/short rice flour, and textured pea protein were selected as the best non-allergen alternatives to wheat crumb in regular coarse ground meat products. Ingredients including hydrolysed collagen, white navy bean flour, potato starch and pea starch showed the best potential in regular salt emulsion type products and were selected for further evaluation in reduced sodium products.

Results of the main experiments demonstrated that some non-allergen ingredients can effectively replace currently used binders like wheat crumb or flour while formulating meat products without negative impact on their eating quality. During refrigerated storage under simulated retail display, burgers processed with 4% textured pea protein had the best potential to replace those with 5% wheat crumb by maintaining colour stability and delaying lipid oxidation. All allergen-free binders added at 4% in beef burgers contributed to similar cooking properties as compared to the 5% wheat crumb control and had better cooking properties than treatments processed with 2% added binders. Sensory evaluation indicated that cooked burgers incorporated with 4% pea starch had relatively higher overall acceptability. Pea starch and textured pea protein at 4% had potential to substitute 5% wheat crumb as gluten-free binders in burgers.

Results also indicated that all non-allergen binders were equivalent to wheat flour and positively affected hydration properties and thermal stability in low-sodium bolognas compared to those products processed without binders. Addition of binders partially alleviated adverse effects on consumer acceptability caused by salt reduction. Among all formulations, potato starch outperformed wheat flour and presented the greatest potential to be the wheat flour alternative in emulsion type products.

Overall, this research contributed knowledge to the following areas:

a) crosswise comparison and evaluation of the effectiveness of dozens of binders in sensorial palatability for different processed meat products;
b) determination of the most potential allergen-free substitutes for commonly used wheat binders in the meat industry as functional ingredients to improve quality of processed meats.

This research determined the feasibility of manufacturing a wider selection of allergen-free meat products by replacing traditional allergenic binders with novel non-meat ingredients without compromising functionality and acceptability. It also provided processed meat producers with evidence that native ingredients could compensate the undesired impact of sodium reduction. The conclusions of this research address the challenge of developing processed meats emphasising clean label while keeping production costs low for meat processing industry, and provide a unique opportunity for meat processors to create products that differentiate themselves from other competitors on the market. The novel products with increased value would enable the meat industry to appeal to a larger consumer audience with one product line. Knowledge from this thesis will also expand opportunities for the Canadian pulse industries to target new markets for their utilization in development of value-added meat products containing Canadian-grown pulses. Moreover, elimination of allergens from meat product formulations would translate into direct savings to processors by alleviating economic burden related to product allergen testing, cross contamination, rework usage and potential product recalls, and further translate into substantial savings to meat processors and instil consumer confidence in food safety.

Last but not least, it is beneficial to consumers who are seeking clean label products for themselves or families with food allergy concerns.

Although this research provided important information on the usage levels and processing conditions for the efficient utilization of allergen-free ingredients into two processed meat products, additional work could be done to for better understanding of the results:

a) microbial analysis could be included in the shelf-life experiment of raw burgers;
b) light microscopy could be used on burgers to explore the fat and explain why the fat retention among treatments were comparable;
c) cryo-microscopy could be used on bolognas to indicate the possible interactions between fat and binders.

Future studies can focus on the following aspects:

a) optimisation of combinations of different non-allergen binders in processed meat formulations;
b) exploration of binders incorporated with other clean-label salt replacers in meats with both simple flavour profiles and complex flavour profiles;

c) application of these binders in other types of meats such as reconstructed meat products, or poultry and fish products.

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Appendix A

Figure A1. Percentage of frequency distribution for Just-about-right scale of colour for cooked burgers formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = too pale, 2 = moderately pale, 3 = slightly pale, 4 = just-about-right, 5 = slightly dark, 6 = moderately dark, and 7 = too dark. Abbreviation: PHR: a commercial clean label phosphate replacer.

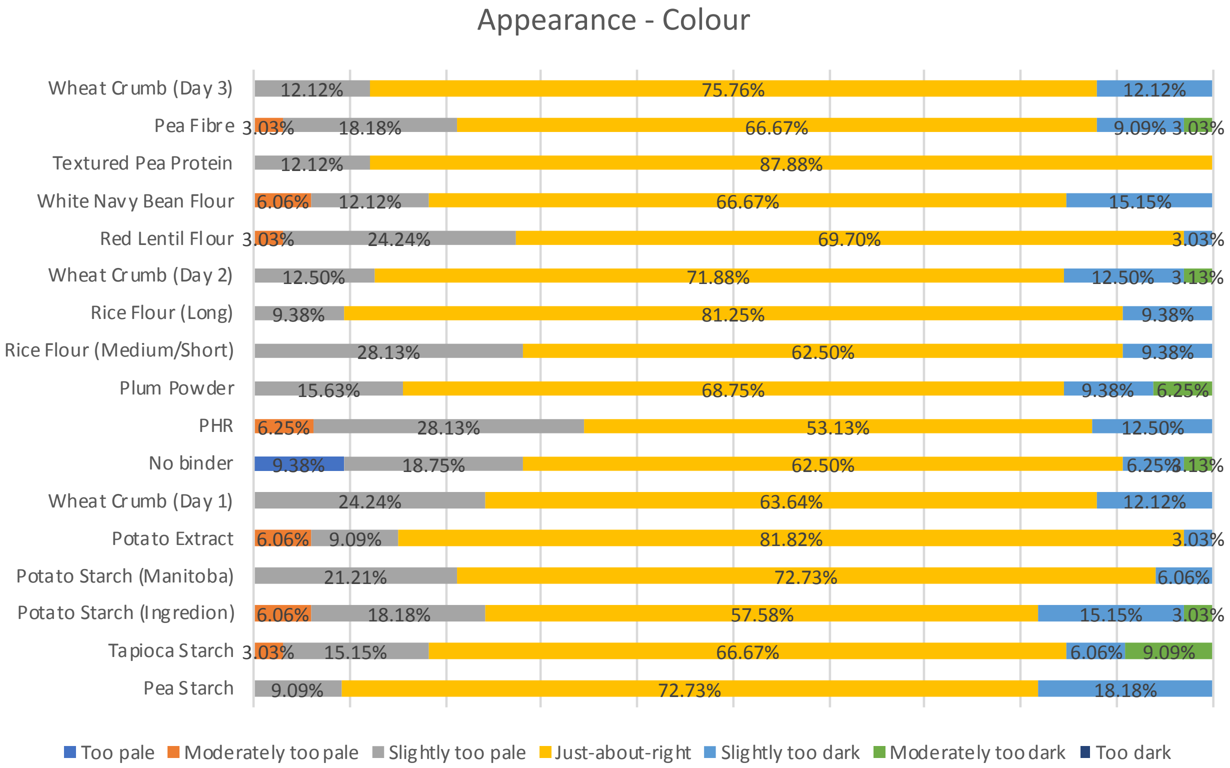
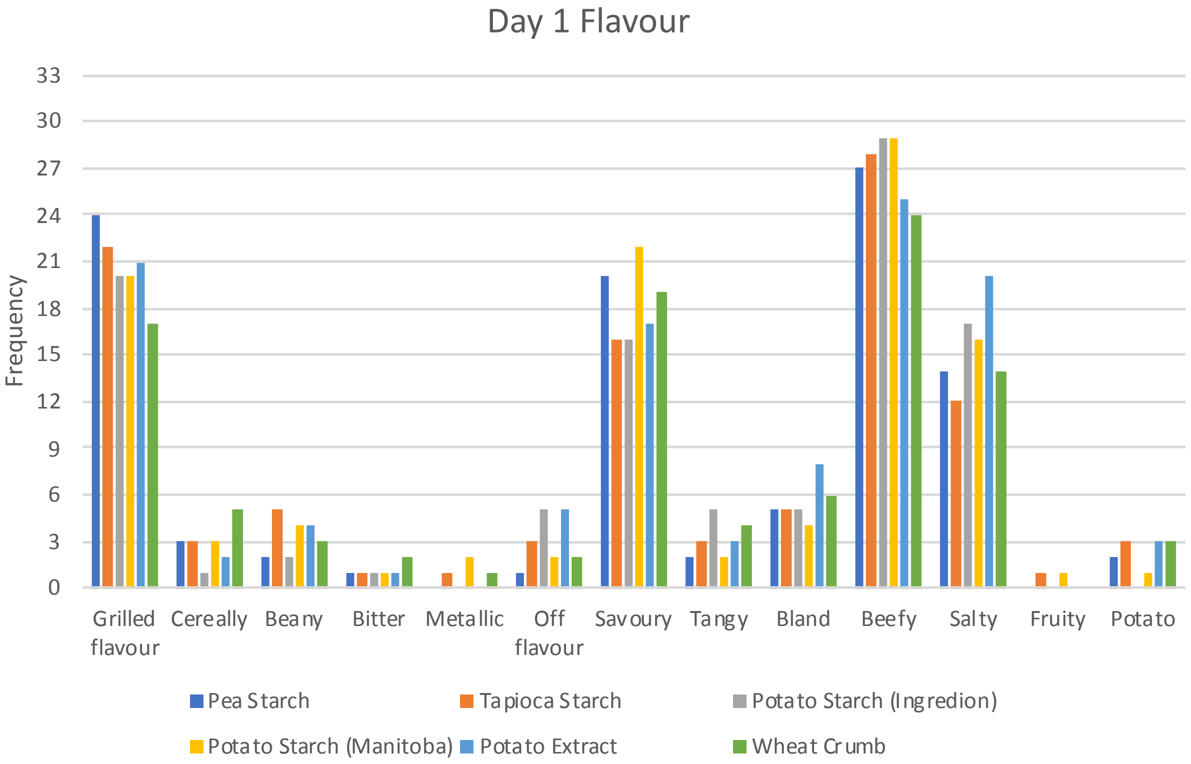


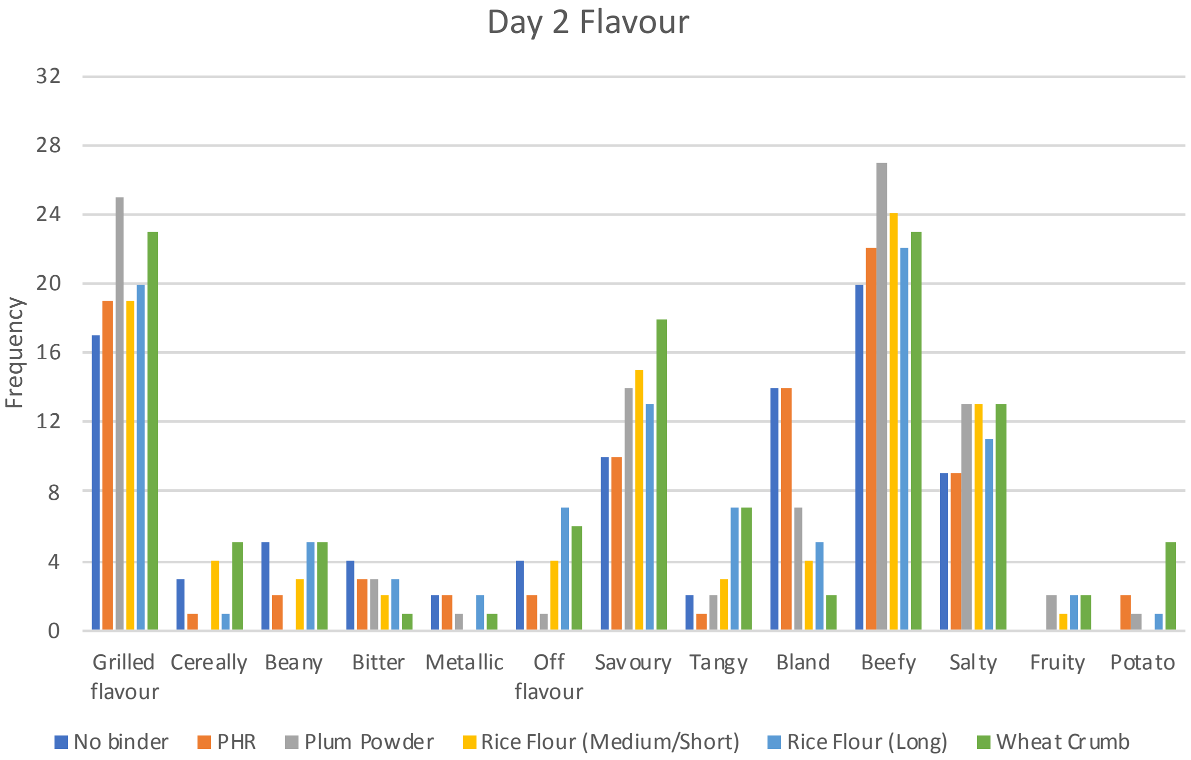
Table A1. Penalty analysis for Just-about-right scale of colour for cooked burgers formulated with different binders evaluated by the internal sensory panel. Abbreviation: PHR: a commercial clean label phosphate replacer.

Treatment	Variable	Level	Frequencies	%	Sum(appearance)	Mean(appearance)	Mean drops	Standardized difference	p-value	Significant
No Binder	colour	Too pale	9	28.13%	34.000	3.778	2.422	3.897	0.001	Yes
		Just-about-right	20	62.50%	124.000	6.200				
		Too dark	3	9.38%	18.000	6.000	0.200			
PHR	colour	Too pale	11	34.38%	49.000	4.455	1.898	3.745	0.001	Yes
		Just-about-right	17	53.13%	108.000	6.353				
		Too dark	4	12.50%	19.000	4.750	1.603			
Pea Fibre	colour	Too pale	7	21.21%	35.000	5.000	1.591	2.288	0.030	Yes
		Just-about-right	22	66.67%	145.000	6.591				
		Too dark	4	12.12%	14.000	3.500	3.091			
Pea Starch	colour	Too pale	3	9.09%	14.000	4.667	2.625			
		Just-about-right	24	72.73%	175.000	7.292				
		Too dark	6	18.18%	37.000	6.167	1.125			
Plum Powder	colour	Too pale	5	15.63%	25.000	5.000	1.591			
		Just-about-right	22	68.75%	145.000	6.591				
		Too dark	5	15.63%	27.000	5.400	1.191			
Potato Extract	colour	Too pale	5	15.15%	22.000	4.400	2.415			
		Just-about-right	27	81.82%	184.000	6.815				
		Too dark	1	3.03%	5.000	5.000	1.815			
Potato Starch (Ingredion)	colour	Too pale	8	24.24%	37.000	4.625	2.322	4.115	0.000	Yes
		Just-about-right	19	57.58%	132.000	6.947				
		Too dark	6	18.18%	31.000	5.167	1.781			
Potato Starch (Manitoba)	colour	Too pale	7	21.21%	38.000	5.429	1.363	2.784	0.009	Yes
		Just-about-right	24	72.73%	163.000	6.792				
		Too dark	2	6.06%	10.000	5.000	1.792			
Red Lentil Flour	colour	Too pale	9	27.27%	45.000	5.000	1.870	3.015	0.005	Yes
		Just-about-right	23	69.70%	158.000	6.870				
		Too dark	1	3.03%	5.000	5.000	1.870			
Rice Flour (Long)	colour	Too pale	3	9.38%	16.000	5.333	1.205			
		Just-about-right	26	81.25%	170.000	6.538				
		Too dark	3	9.38%	19.000	6.333	0.205			
Rice Flour (Medium/Short)	colour	Too pale	9	28.13%	39.000	4.333	2.617	4.570	< 0.0001	Yes
		Just-about-right	20	62.50%	139.000	6.950				
		Too dark	3	9.38%	17.000	5.667	1.283			
Tapioca Starch	colour	Too pale	6	18.18%	33.000	5.500	1.227			
		Just-about-right	22	66.67%	148.000	6.727				

		Too dark	5	15.15%	30.000	6.000	0.727			
Textured Pea Protein	colour	Too pale	4	12.12%	21.000	5.250	1.578			
		Just-about-right	29	87.88%	198.000	6.828				
		Too dark	0	0.00%						
Wheat Crumb (Day 1)	colour	Too pale	8	24.24%	44.000	5.500	1.881	2.919	0.007	Yes
		Just-about-right	21	63.64%	155.000	7.381				
		Too dark	4	12.12%	24.000	6.000	1.381			
Wheat Crumb (Day 2)	colour	Too pale	4	12.50%	20.000	5.000	2.435			
		Just-about-right	23	71.88%	171.000	7.435				
		Too dark	5	15.63%	35.000	7.000	0.435			
Wheat Crumb (Day 3)	colour	Too pale	4	12.12%	22.000	5.500	1.420			
		Just-about-right	25	75.76%	173.000	6.920				
		Too dark	4	12.12%	23.000	5.750	1.170			
White Navy Bean Flour	colour	Too pale	6	18.18%	21.000	3.500	3.136			
		Just-about-right	22	66.67%	146.000	6.636				
		Too dark	5	15.15%	29.000	5.800	0.836			

Figure A2. Frequency distribution for Check-All-That-Apply scale of flavour for cooked burgers formulated with different binders evaluated by the internal sensory panel. Abbreviation: PHR: a commercial clean label phosphate replacer.





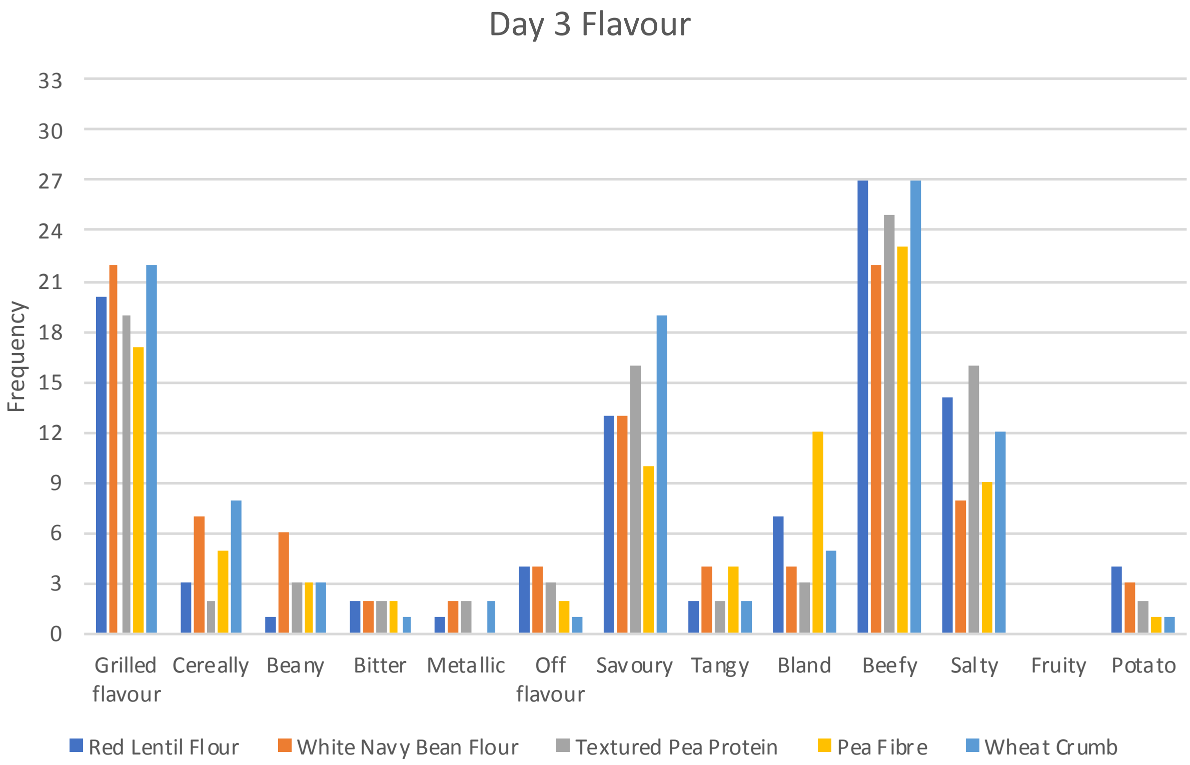

Day 3 Flavour
Frequency
33
30
27
24
21
18
15
12
9
6
3
0
Grilled flavour
Cereally
Beany
Bitter
Metallic
Off flavour
Savoury
Tangy
Bland
Beefy
Salty
Fruity
Potato
Red Lentil Flour
White Navy Bean Flour
Textured Pea Protein
Pea Fibre
Wheat Crumb

Figure A3. Percentage of frequency distribution for Just-about-right scale of firmness for cooked burgers formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = too soft, 2 = moderately soft, 3 = slightly soft, 4 = just-about-right, 5 = slightly firm, 6 = moderately firm, and 7 = too firm. Abbreviation: PHR: a commercial clean label phosphate replacer.

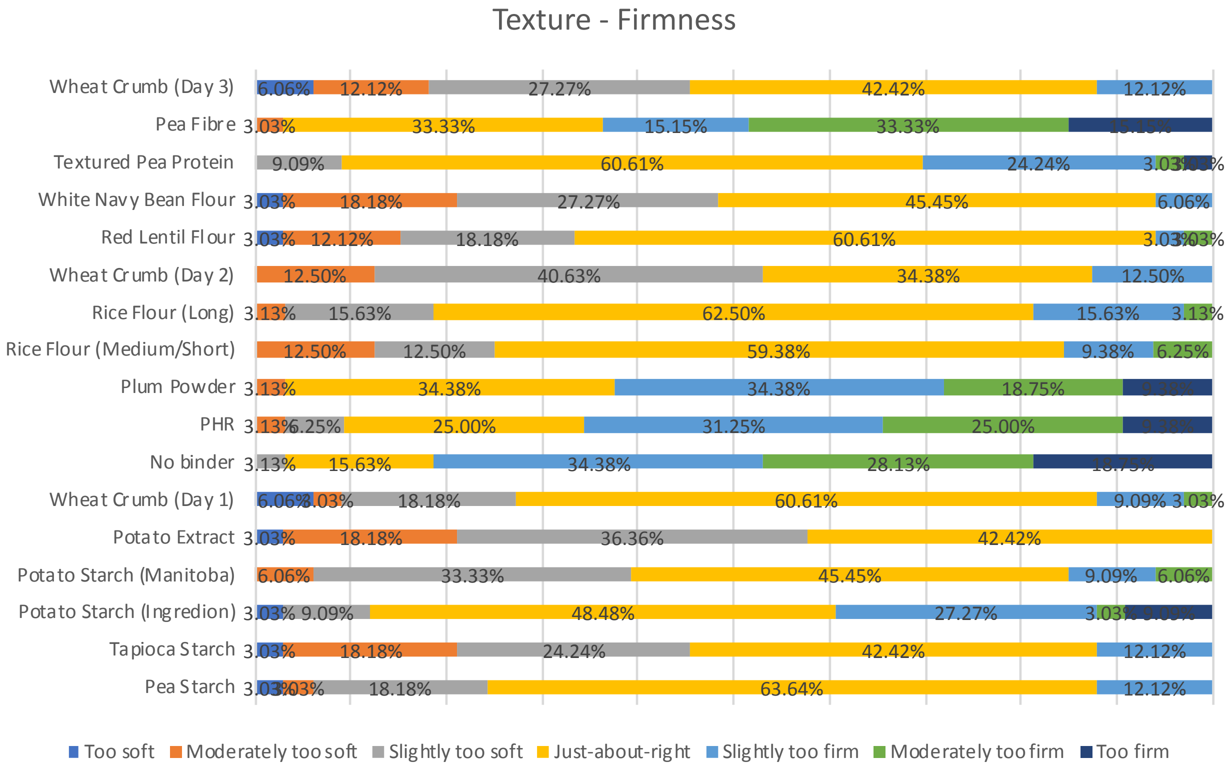


Figure A4. Percentage of frequency distribution for Just-about-right scale of juiciness for cooked burgers formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = too dry, 2 = moderately dry, 3 = slightly dry, 4 = just-about-right, 5 = slightly juicy, 6 = moderately juicy, and 7 = too juicy. Abbreviation: PHR: a commercial clean label phosphate replacer.

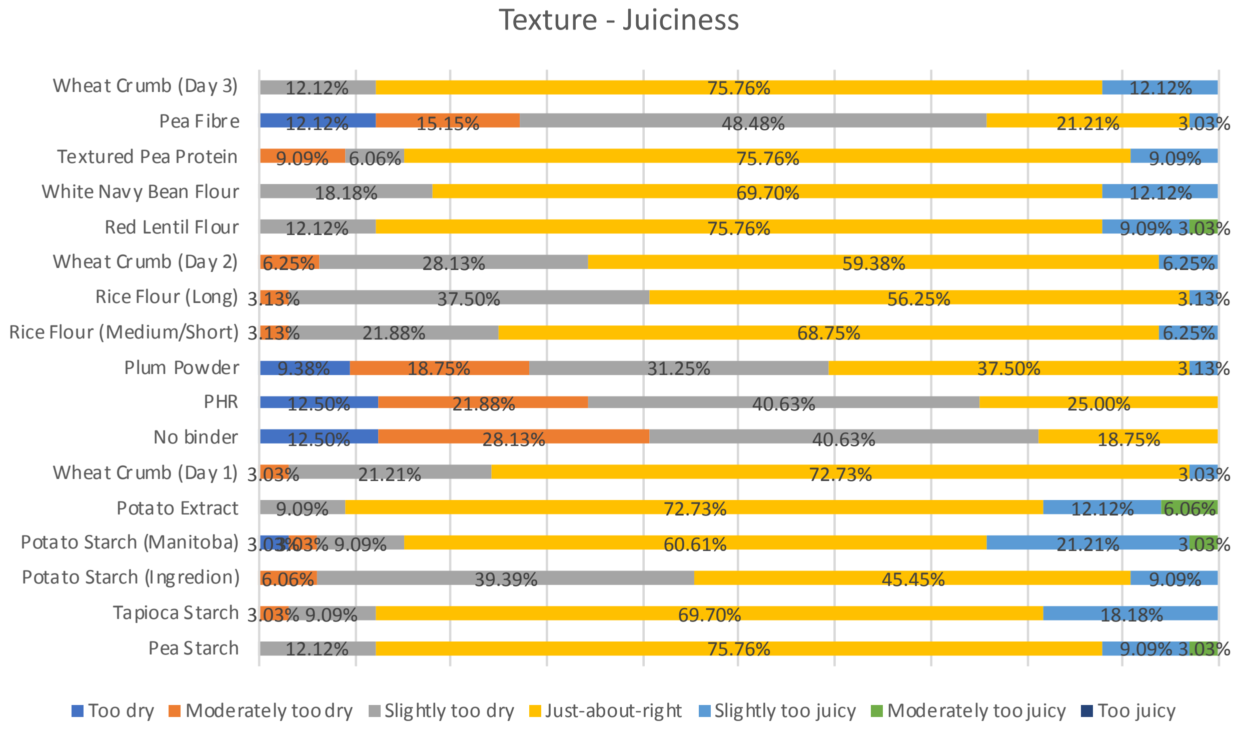


Table A2. Penalty analysis for Just-about-right scale of firmness and juiciness for cooked burgers formulated with different binders evaluated by the internal sensory panel. Abbreviation: PHR: a commercial clean label phosphate replacer.

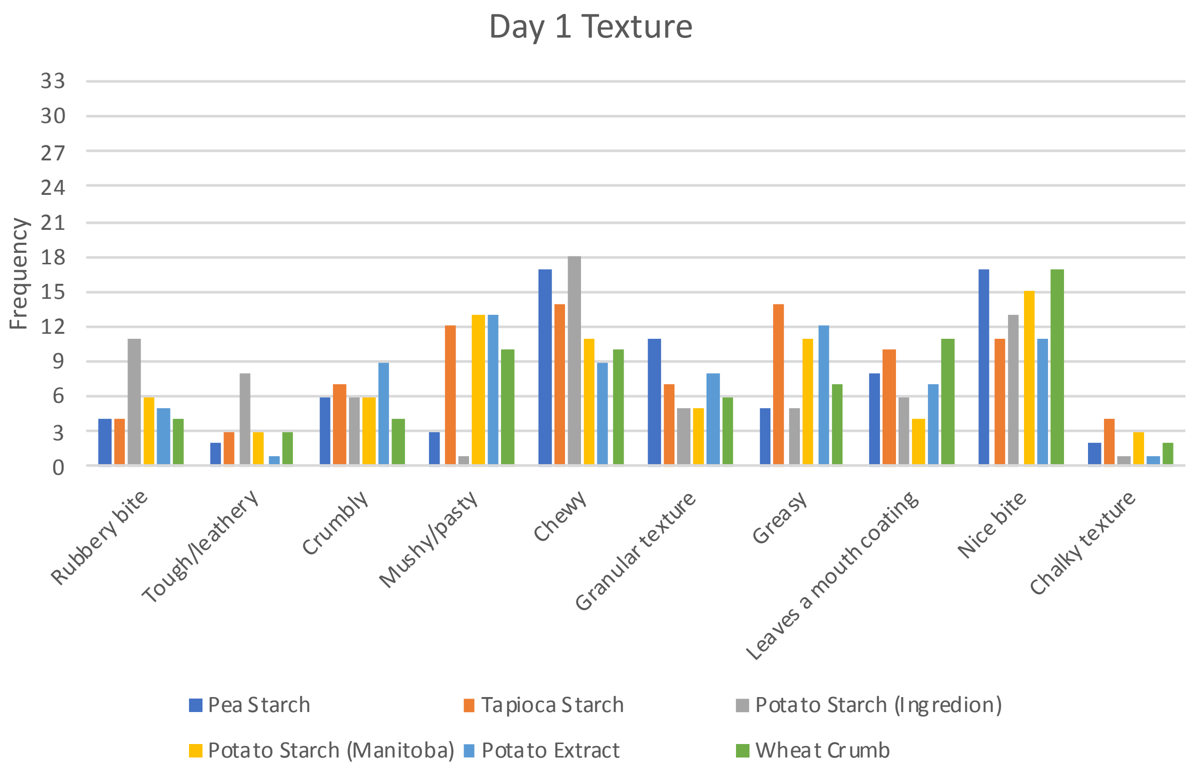
Treatment	Variable	Level	Frequencies	%	Sum(texture)	Mean(texture)	Mean drops	Standardized difference	p-value	Significant
No Binder	Firmness	Too soft	1	3.13%	4.000	4.000	3.000			
		Just-about-right	5	15.63 %	35.000	7.000				
		Too firm	26	81.25 %	91.000	3.500	3.500	4.733	< 0.0001	Yes
	Juiciness	Too dry	26	81.25 %	97.000	3.731	1.769	2.112	0.043	Yes
		Just-about-right	6	18.75 %	33.000	5.500				
		Too juicy	0	0.00%						
PHR	Firmness	Too soft	3	9.38%	8.000	2.667	3.708			
		Just-about-right	8	25.00 %	51.000	6.375				
		Too firm	21	65.63 %	79.000	3.762	2.613	4.835	< 0.0001	Yes
	Juiciness	Too dry	24	75.00 %	93.000	3.875	1.750	2.684	0.012	Yes
		Just-about-right	8	25.00 %	45.000	5.625				
		Too juicy	0	0.00%						
Pea Fibre	Firmness	Too soft	1	3.03%	2.000	2.000	5.273			
		Just-about-right	11	33.33 %	80.000	7.273				
		Too firm	21	63.64 %	81.000	3.857	3.416	6.793	< 0.0001	Yes
	Juiciness	Too dry	25	75.76 %	103.000	4.120	3.309	4.788	< 0.0001	Yes
		Just-about-right	7	21.21 %	52.000	7.429				
		Too juicy	1	3.03%	8.000	8.000	-0.571			
Pea Starch	Firmness	Too soft	8	24.24 %	44.000	5.500	1.738	2.640	0.014	Yes
		Just-about-right	21	63.64 %	152.000	7.238				
		Too firm	4	12.12 %	26.000	6.500	0.738			
	Juiciness	Too dry	4	12.12 %	19.000	4.750	2.290			
		Just-about-right	25	75.76 %	176.000	7.040				
		Too juicy	4	12.12 %	27.000	6.750	0.290			
Plum Powder	Firmness	Too soft	1	3.13%	3.000	3.000	4.545			
		Just-about-right	11	34.38 %	83.000	7.545				
		Too firm	20	62.50 %	74.000	3.700	3.845	7.730	< 0.0001	Yes
	Juiciness	Too dry	19	59.38 %	75.000	3.947	2.469	3.505	0.002	Yes
		Just-about-right	12	37.50 %	77.000	6.417				
		Too juicy	1	3.13%	8.000	8.000	-1.583			

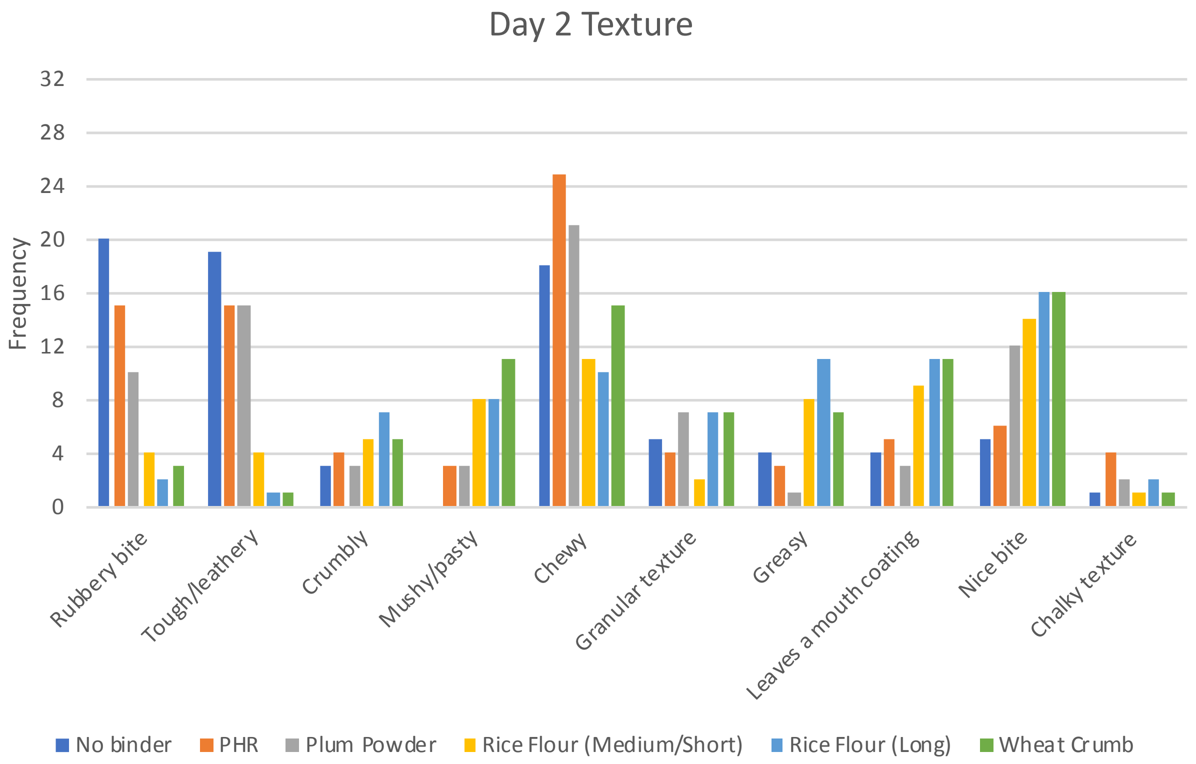
Potato Extract	Firmness	Too soft	19	57.58%	77.000	4.053	3.447	7.385	< 0.0001	Yes
		Just-about-right	14	42.42%	105.000	7.500				
		Too firm	0	0.00%						
	Juiciness	Too dry	3	9.09%	16.000	5.333	0.833			
		Just-about-right	24	72.73%	148.000	6.167				
		Too juicy	6	18.18%	18.000	3.000	3.167			
Potato Starch (Ingredion)	Firmness	Too soft	4	12.12%	17.000	4.250	3.250			
		Just-about-right	16	48.48%	120.000	7.500				
		Too firm	13	39.39%	65.000	5.000	2.500	4.491	0.000	Yes
	Juiciness	Too dry	15	45.45%	79.000	5.267	1.600	2.288	0.030	Yes
		Just-about-right	15	45.45%	103.000	6.867				
		Too juicy	3	9.09%	20.000	6.667	0.200			
Potato Starch (Manitoba)	Firmness	Too soft	13	39.39%	72.000	5.538	1.062	1.674	0.106	No
		Just-about-right	15	45.45%	99.000	6.600				
		Too firm	5	15.15%	26.000	5.200	1.400			
	Juiciness	Too dry	5	15.15%	23.000	4.600	2.000			
		Just-about-right	20	60.61%	132.000	6.600				
		Too juicy	8	24.24%	42.000	5.250	1.350	1.935	0.064	No
Red Lentil Flour	Firmness	Too soft	11	33.33%	44.000	4.000	2.600	4.497	0.000	Yes
		Just-about-right	20	60.61%	132.000	6.600				
		Too firm	2	6.06%	8.000	4.000	2.600			
	Juiciness	Too dry	4	12.12%	18.000	4.500	1.580			
		Just-about-right	25	75.76%	152.000	6.080				
		Too juicy	4	12.12%	14.000	3.500	2.580			
Rice Flour (Long)	Firmness	Too soft	6	18.75%	25.000	4.167	2.533			
		Just-about-right	20	62.50%	134.000	6.700				
		Too firm	6	18.75%	29.000	4.833	1.867			
	Juiciness	Too dry	13	40.63%	61.000	4.692	2.030	3.482	0.002	Yes
		Just-about-right	18	56.25%	121.000	6.722				
		Too juicy	1	3.13%	6.000	6.000	0.722			
Rice Flour (Medium/Short)	Firmness	Too soft	8	25.00%	32.000	4.000	3.211	5.546	< 0.0001	Yes
		Just-about-right	19	59.38%	137.000	7.211				
		Too firm	5	15.63%	26.000	5.200	2.011			

	Juiciness	Too dry	8	25.00%	39.000	4.875	1.898	2.473	0.020	Yes
		Just-about-right	22	68.75%	149.000	6.773				
		Too juicy	2	6.25%	7.000	3.500	3.273			
Tapioca Starch	Firmness	Too soft	15	45.45%	73.000	4.867	2.133	3.341	0.002	Yes
		Just-about-right	14	42.42%	98.000	7.000				
		Too firm	4	12.12%	18.000	4.500	2.500			
	Juiciness	Too dry	4	12.12%	17.000	4.250	1.967			
		Just-about-right	23	69.70%	143.000	6.217				
		Too juicy	6	18.18%	29.000	4.833	1.384			
Textured Pea Protein	Firmness	Too soft	3	9.09%	13.000	4.333	2.667			
		Just-about-right	20	60.61%	140.000	7.000				
		Too firm	10	30.30%	51.000	5.100	1.900	2.785	0.009	Yes
	Juiciness	Too dry	5	15.15%	19.000	3.800	2.960			
		Just-about-right	25	75.76%	169.000	6.760				
		Too juicy	3	9.09%	16.000	5.333	1.427			
Wheat Crumb (Day 1)	Firmness	Too soft	9	27.27%	40.000	4.444	2.556	3.210	0.003	Yes
		Just-about-right	20	60.61%	140.000	7.000				
		Too firm	4	12.12%	20.000	5.000	2.000			
	Juiciness	Too dry	8	24.24%	36.000	4.500	2.125	2.504	0.018	Yes
		Just-about-right	24	72.73%	159.000	6.625				
		Too juicy	1	3.03%	5.000	5.000	1.625			
Wheat Crumb (Day 2)	Firmness	Too soft	17	53.13%	75.000	4.412	3.316	4.548	0.000	Yes
		Just-about-right	11	34.38%	85.000	7.727				
		Too firm	4	12.50%	24.000	6.000	1.727			
	Juiciness	Too dry	11	34.38%	48.000	4.364	2.478	3.285	0.003	Yes
		Just-about-right	19	59.38%	130.000	6.842				
		Too juicy	2	6.25%	6.000	3.000	3.842			
Wheat Crumb (Day 3)	Firmness	Too soft	15	45.45%	70.000	4.667	2.762	4.054	0.000	Yes
		Just-about-right	14	42.42%	104.000	7.429				
		Too firm	4	12.12%	21.000	5.250	2.179			
	Juiciness	Too dry	4	12.12%	24.000	6.000	0.040			
		Just-about-right	25	75.76%	151.000	6.040				
		Too juicy	4	12.12%	20.000	5.000	1.040			

White Navy Bean Flour	Firmness	Too soft	16	48.48 %	68.000	4.250	2.817	5.047	< 0.0001	Yes
		Just-about-right	15	45.45 %	106.000	7.067				
		Too firm	2	6.06%	11.000	5.500	1.567			
	Juiciness	Too dry	6	18.18 %	28.000	4.667	1.420			
		Just-about-right	23	69.70 %	140.000	6.087				
		Too juicy	4	12.12 %	17.000	4.250	1.837			

Figure A5. Frequency distribution for Check-All-That-Apply scale of texture for cooked burgers formulated with different binders evaluated by the internal sensory panel. Abbreviation: PHR: a commercial clean label phosphate replacer.




Day 2 Texture
Frequency
0
4
8
12
16
20
24
28
32
Rubbery bite
Tough/leathery
Crumbly
Mushy/pasty
Chewy
Granular texture
Greasy
Leaves a mouth coating
Nice bite
Chalky texture
No binder
PHR
Plum Powder
Rice Flour (Medium/Short)
Rice Flour (Long)
Wheat Crumb

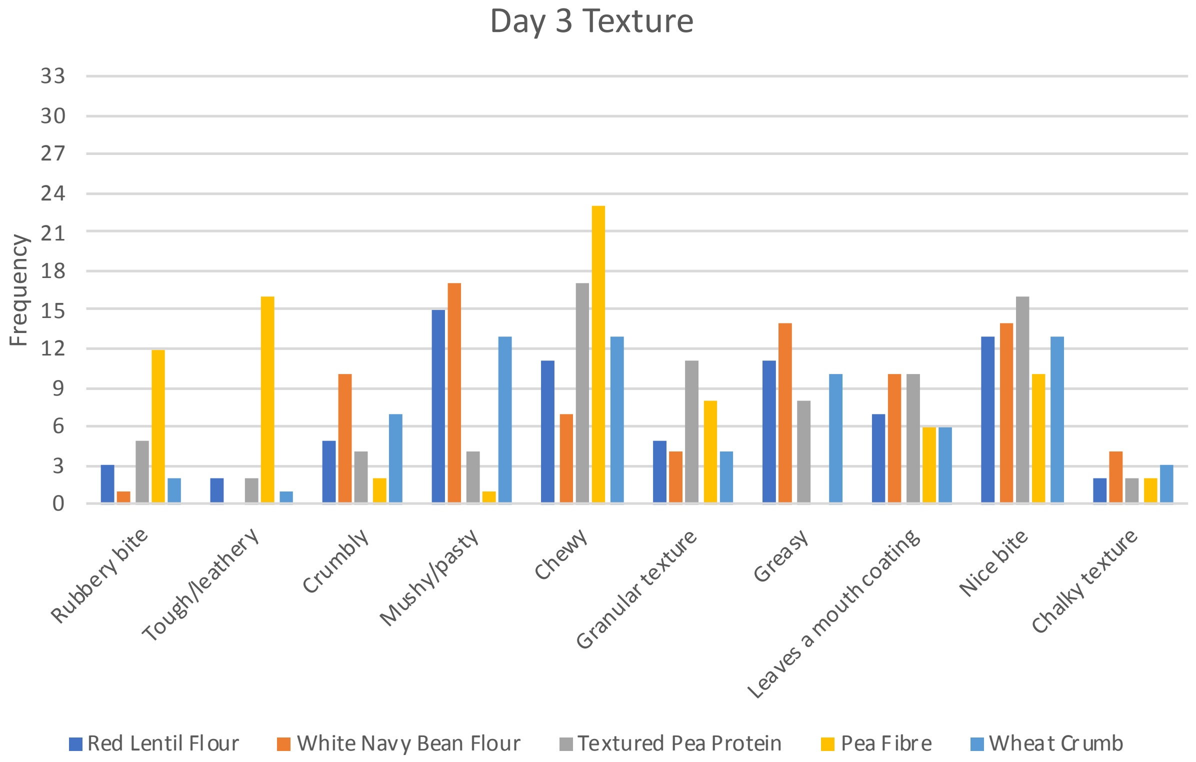

Day 3 Texture
Frequency
0
3
6
9
12
15
18
21
24
27
30
33
Rubbery bite
Tough/leathery
Crumbly
Mushy/pasty
Chewy
Granular texture
Greasy
Leaves a mouth coating
Nice bite
Chalky texture
Red Lentil Flour
White Navy Bean Flour
Textured Pea Protein
Pea Fibre
Wheat Crumb

Figure A6. Frequency distribution for Check-All-That-Apply scale of flavour for bolognas formulated with different binders evaluated by the internal sensory panel.

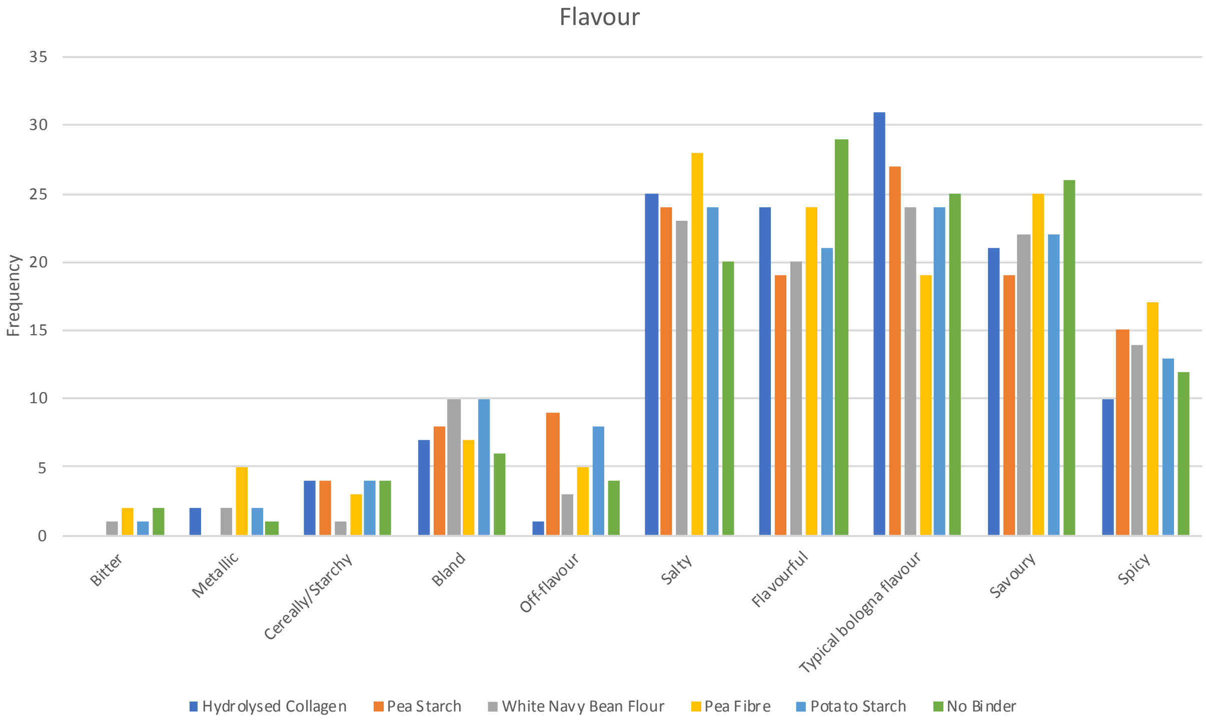


Figure A7. Percentage of frequency distribution for Just-about-right scale of firmness for bolognas formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = too soft, 2 = moderately soft, 3 = slightly soft, 4 = just-about-right, 5 = slightly firm, 6 = moderately firm, and 7 = too firm.

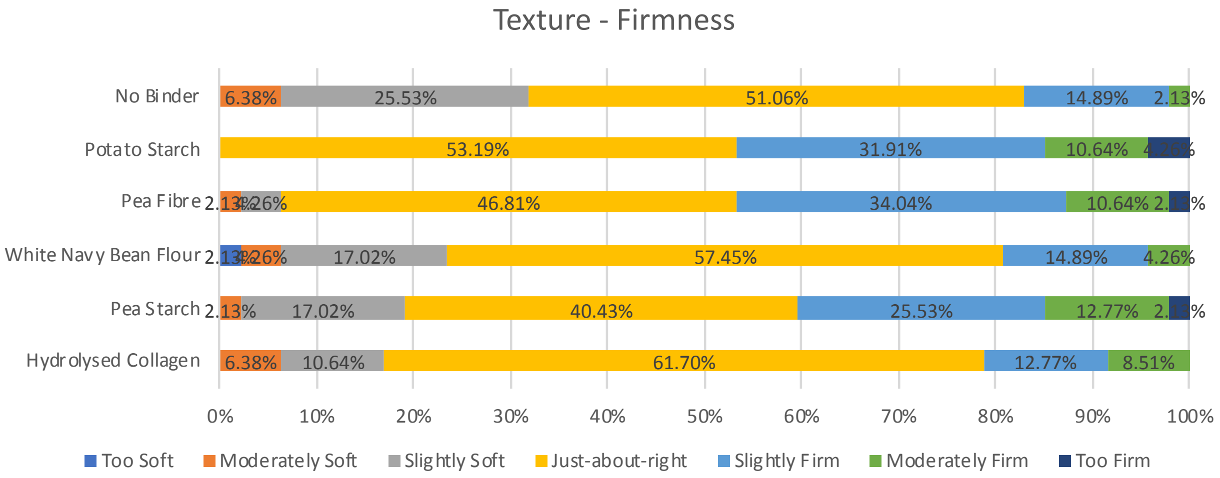


Table A3. Penalty analysis for Just-about-right scale of firmness for bolognas formulated with different binders evaluated by the internal sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum(firmness)	Mean(firmness)	Mean drops	Standardized difference	p-value	Significant
Hydrolysed Collagen	firmness	Too soft	8	17.02 %	41.000	5.125	1.841			
		Just-about-right	29	61.70 %	202.000	6.966				
		Too firm	10	21.28 %	64.000	6.400	0.566	1.160	0.253	No
No Binder	firmness	Too soft	15	31.91 %	72.000	4.800	2.200	4.990	< 0.0001	Yes
		Just-about-right	24	51.06 %	168.000	7.000				
		Too firm	8	17.02 %	48.000	6.000	1.000			
Pea Fibre	firmness	Too soft	3	6.38%	17.000	5.667	1.424			
		Just-about-right	22	46.81 %	156.000	7.091				
		Too firm	22	46.81 %	121.000	5.500	1.591	3.792	0.000	Yes
Pea Starch	firmness	Too soft	9	19.15 %	45.000	5.000	2.000			
		Just-about-right	19	40.43 %	133.000	7.000				
		Too firm	19	40.43 %	101.000	5.316	1.684	3.480	0.001	Yes
Potato Starch	firmness	Too soft	0	0.00%						
		Just-about-right	25	53.19 %	184.000	7.360				
		Too firm	22	46.81 %	118.000	5.364	1.996	4.608	< 0.0001	Yes
White Navy Bean Flour	firmness	Too soft	11	23.40 %	51.000	4.636	2.401	5.505	< 0.0001	Yes
		Just-about-right	27	57.45 %	190.000	7.037				
		Too firm	9	19.15 %	51.000	5.667	1.370			

Figure A8. Percentage of frequency distribution for Just-about-right scale of juiciness for bolognas formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = too dry, 2 = moderately dry, 3 = slightly dry, 4 = just-about-right, 5 = slightly juicy, 6 = moderately juicy, and 7 = too juicy.

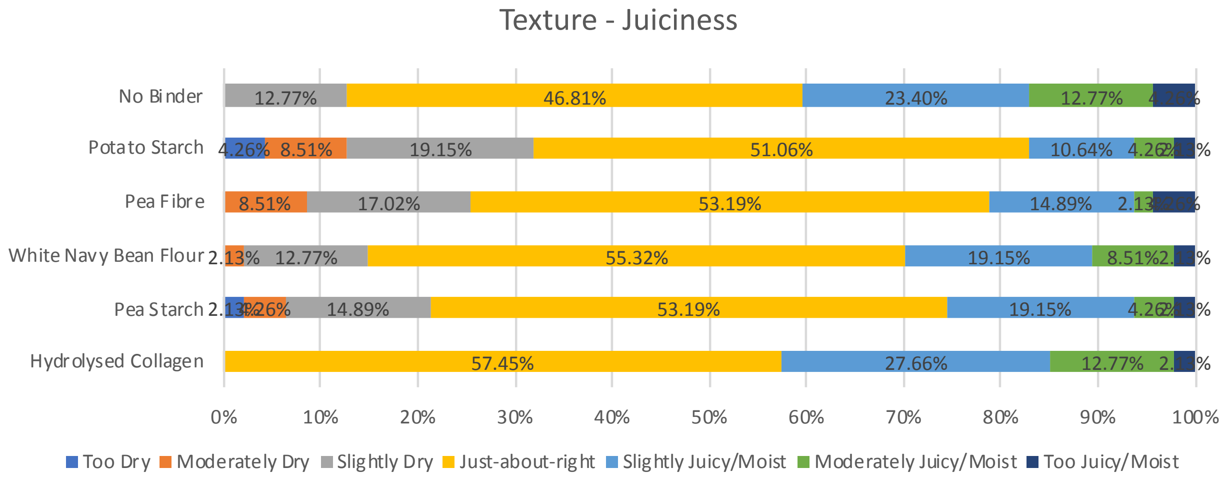


Table A4. Penalty analysis for Just-about-right scale of juiciness for bolognas formulated with different binders evaluated by the internal sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum(juiciness)	Mean(juiciness)	Mean drops	Standardized difference	p-value	Significant
Hydrolysed Collagen	juiciness	Too dry	0	0.00%						
		Just-about-right	27	57.45%	190.000	7.037				
		Too juicy	20	42.55%	116.000	5.800	1.237	2.868	0.006	Yes
No Binder	juiciness	Too dry	6	12.77%	31.000	5.167	1.879			
		Just-about-right	22	46.81%	155.000	7.045				
		Too juicy	19	40.43%	110.000	5.789	1.256	2.609	0.013	Yes
Pea Fibre	juiciness	Too dry	12	25.53%	63.000	5.250	1.390	2.928	0.015	Yes
		Just-about-right	25	53.19%	166.000	6.640				
		Too juicy	10	21.28%	64.000	6.400	0.240	0.474	0.884	No
Pea Starch	juiciness	Too dry	10	21.28%	47.000	4.700	2.180	3.889	0.001	Yes
		Just-about-right	25	53.19%	172.000	6.880				
		Too juicy	12	25.53%	72.000	6.000	0.880	1.673	0.227	No
Potato Starch	juiciness	Too dry	15	31.91%	75.000	5.000	2.208	5.361	< 0.0001	Yes
		Just-about-right	24	51.06%	173.000	7.208				
		Too juicy	8	17.02%	53.000	6.625	0.583			
White Navy Bean Flour	juiciness	Too dry	7	14.89%	28.000	4.000	2.808			
		Just-about-right	26	55.32%	177.000	6.808				
		Too juicy	14	29.79%	94.000	6.714	0.093	0.198	0.844	No

Figure A9. Percentage of frequency distribution for Just-about-right scale of chewiness for bolognas formulated with different binders evaluated by the internal sensory panel. Scored on 7-point scales where 1 = too crumbly, 2 = moderately crumbly, 3 = slightly crumbly, 4 = just-about-right, 5 = slightly rubbery, 6 = moderately rubbery, and 7 = too rubbery.

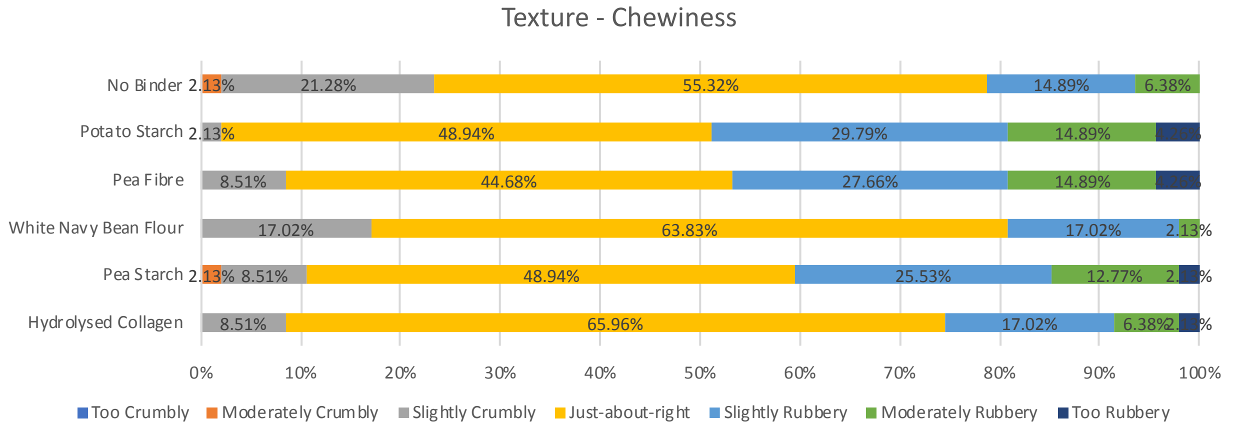


Table A5. Penalty analysis for Just-about-right scale of chewiness for bolognas formulated with different binders evaluated by the internal sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum(chewiness)	Mean(chewiness)	Mean drops	Standardized difference	p-value	Significant
Hydrolysed Collagen	chewiness	Too crumbly	4	8.51%	22.000	5.500	1.435			
		Just-about-right	31	65.96%	215.000	6.935				
		Too rubbery	12	25.53%	71.000	5.917	1.019	2.384	0.022	Yes
No Binder	chewiness	Too crumbly	11	23.40%	57.000	5.182	1.780	3.760	0.001	Yes
		Just-about-right	26	55.32%	181.000	6.962				
		Too rubbery	10	21.28%	52.000	5.200	1.762	3.597	0.002	Yes
Pea Fibre	chewiness	Too crumbly	4	8.51%	18.000	4.500	2.357			
		Just-about-right	21	44.68%	144.000	6.857				
		Too rubbery	22	46.81%	119.000	5.409	1.448	3.359	0.002	Yes
Pea Starch	chewiness	Too crumbly	5	10.64%	19.000	3.800	3.070			
		Just-about-right	23	48.94%	158.000	6.870				
		Too rubbery	19	40.43%	101.000	5.316	1.554	3.769	0.001	Yes
Potato Starch	chewiness	Too crumbly	1	2.13%	4.000	4.000	3.304			
		Just-about-right	23	48.94%	168.000	7.304				
		Too rubbery	23	48.94%	120.000	5.217	2.087	4.772	< 0.0001	Yes
White Navy Bean Flour	chewiness	Too crumbly	8	17.02%	41.000	5.125	1.575			
		Just-about-right	30	63.83%	201.000	6.700				
		Too rubbery	9	19.15%	53.000	5.889	0.811			

Appendix B

Figure B1. Percentage of frequency distribution for Just-about-right scale of colour for cooked burgers formulated with selected binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too pale, 2 = moderately pale, 3 = slightly pale, 4 = just-about-right, 5 = slightly dark, 6 = moderately dark, and 7 = too dark.

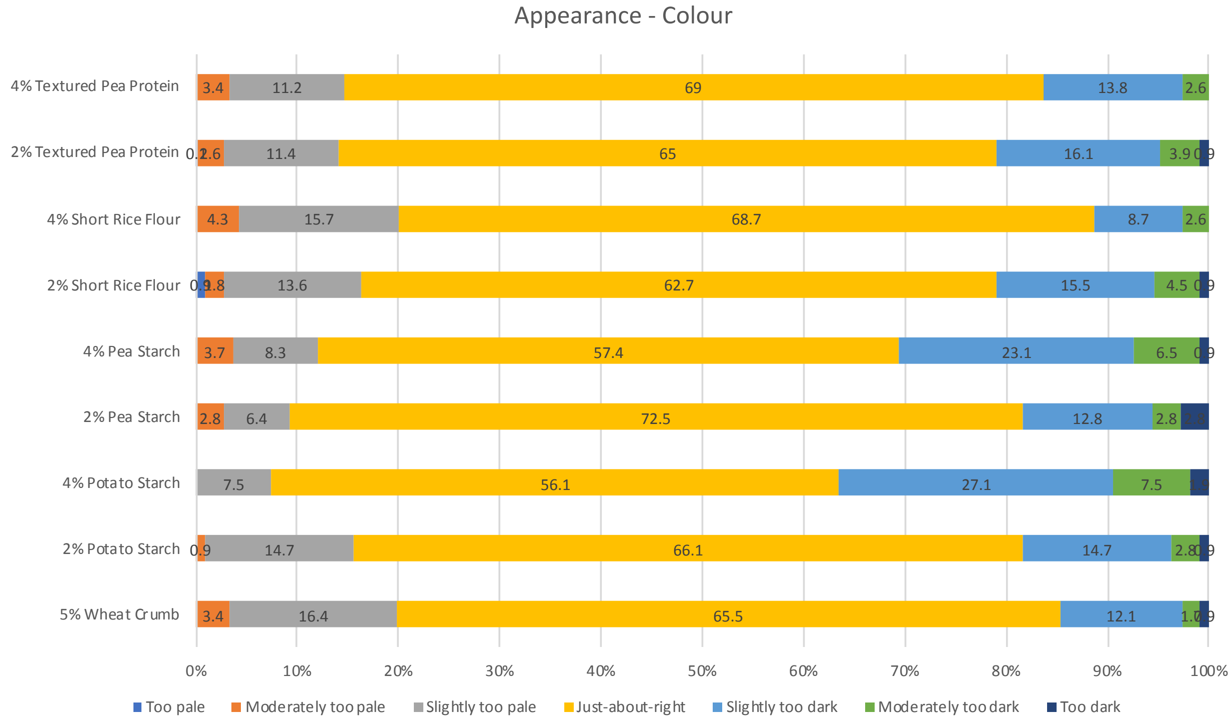


Table B1. Penalty analysis for Just-about-right scale of colour for cooked burgers formulated with selected binders evaluated by the consumer sensory panel.

Sample	Variable	Level	Frequencies	%	Sum Liking Scores	Mean Liking Scores	Mean drops	p-value	Significant
5% Wheat Crumb	Interior colour	Too little	23	19.83%	128.000	5.565	0.935		
		JAR	76	65.52%	494.000	6.500			
		Too much	17	14.66%	93.000	5.471	1.029		
2% Potato Starch	Interior colour	Too little	17	15.60%	89.000	5.235	1.501		
		JAR	72	66.06%	485.000	6.736			
		Too much	20	18.35%	99.000	4.950	1.786		
4% Potato Starch	Interior colour	Too little	8	7.48%	33.000	4.125	2.442		
		JAR	60	56.07%	394.000	6.567			
		Too much	39	36.45%	211.000	5.410	1.156	0.001	Yes
2% Pea Starch	Interior colour	Too little	10	9.17%	45.000	4.500	2.285		
		JAR	79	72.48%	536.000	6.785			
		Too much	20	18.35%	92.000	4.600	2.185		
4% Pea Starch	Interior colour	Too little	10	9.17%	45.000	4.500	2.285		
		JAR	79	72.48%	536.000	6.785			
		Too much	20	18.35%	92.000	4.600	2.185		
2% Short Rice Flour	Interior colour	Too little	18	16.36%	95.000	5.278	1.258		
		JAR	69	62.73%	451.000	6.536			
		Too much	23	20.91%	125.000	5.435	1.101	0.014	Yes
4% Short Rice Flour	Interior colour	Too little	23	20.00%	114.000	4.957	1.854	< 0.0001	Yes
		JAR	79	68.70%	538.000	6.810			
		Too much	13	11.30%	72.000	5.538	1.272		
2% Textured Pea Protein	Interior colour	Too little	12	10.71%	58.000	4.833	1.513		
		JAR	75	66.96%	476.000	6.347			
		Too much	25	22.32%	127.000	5.080	1.267	0.003	Yes
4% Textured Pea Protein	Interior colour	Too little	17	14.66%	97.000	5.706	1.332		
		JAR	80	68.97%	563.000	7.038			
		Too much	19	16.38%	97.000	5.105	1.932		

Figure B2. Frequency distribution for Check-All-That-Apply scale of flavour for cooked burgers formulated with selected binders evaluated by the consumer sensory panel.

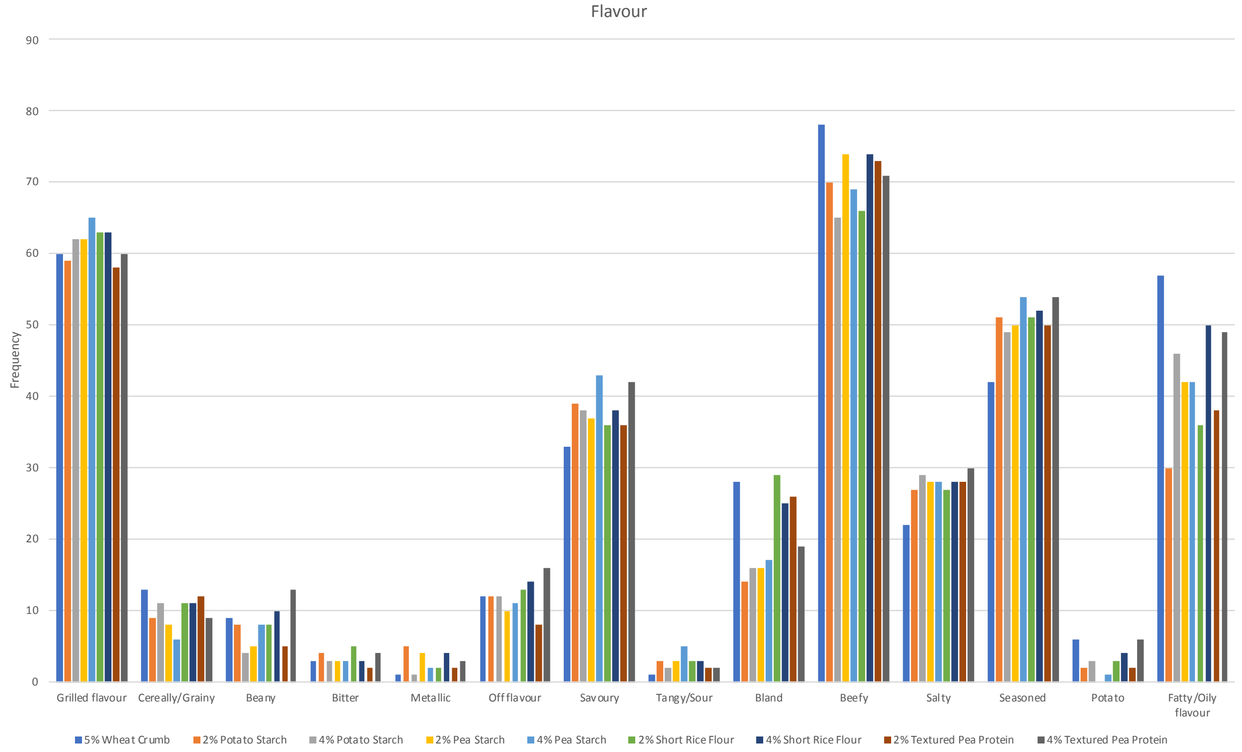


Figure B3. Percentage of frequency distribution for Just-about-right scale of firmness for cooked burgers formulated with selected binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too soft, 2 = moderately soft, 3 = slightly soft, 4 = just-about-right, 5 = slightly firm, 6 = moderately firm, and 7 = too firm.

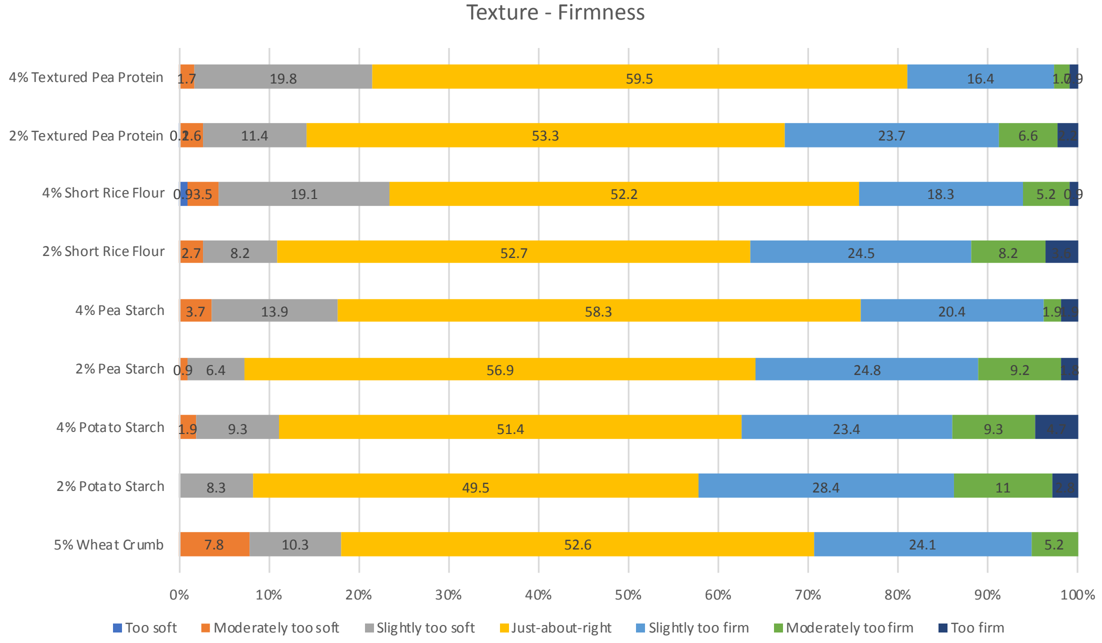


Figure B4. Percentage of frequency distribution for Just-about-right scale of juiciness for cooked burgers formulated with selected binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too dry, 2 = moderately dry, 3 = slightly dry, 4 = just-about-right, 5 = slightly juicy, 6 = moderately juicy, and 7 = too juicy.

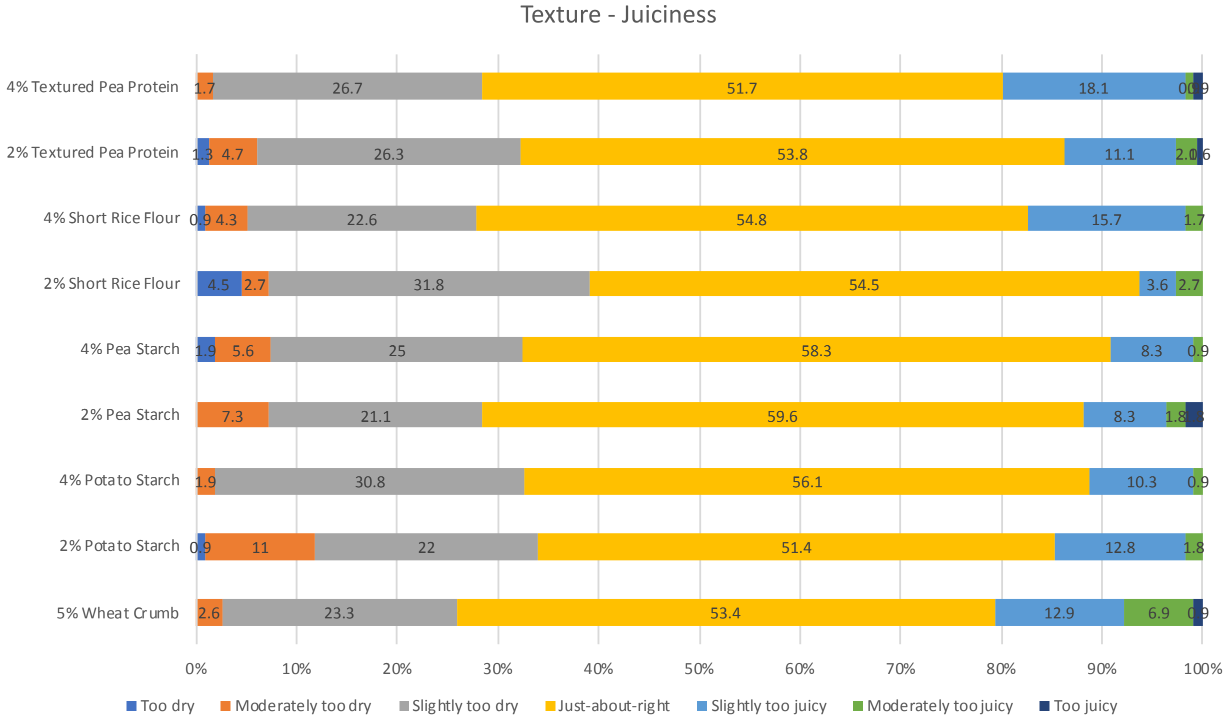


Table B2. Penalty analysis for Just-about-right scale of firmness and juiciness for cooked burgers formulated with selected binders evaluated by the consumer sensory panel.

Sample	Variable	Level	Frequencies	%	Sum Liking Scores	Mean Liking Scores	Mean drops	p-value	Significant
5% Wheat Crumb	Firmness	Too soft	21	18.10%	94.000	4.476	1.950		
		Just-about-right	61	52.59%	392.000	6.426			
		Too firm	34	29.31%	170.000	5.000	1.426	0.000	Yes
	Juiciness	Too dry	30	25.86%	142.000	4.733	1.573	0.000	Yes
		Just-about-right	62	53.45%	391.000	6.306			
		Too juicy	24	20.69%	123.000	5.125	1.181	0.017	Yes
2% Potato Starch	Firmness	Too soft	9	8.26%	39.000	4.333	2.556		
		Just-about-right	54	49.54%	372.000	6.889			
		Too firm	46	42.20%	225.000	4.891	1.998	< 0.0001	Yes
	Juiciness	Too dry	37	33.94%	173.000	4.676	2.021	< 0.0001	Yes
		Just-about-right	56	51.38%	375.000	6.696			
		Too juicy	16	14.68%	88.000	5.500	1.196		
4% Potato Starch	Firmness	Too soft	12	11.21%	68.000	5.667	1.206		
		Just-about-right	55	51.40%	378.000	6.873			
		Too firm	40	37.38%	179.000	4.475	2.398	< 0.0001	Yes
	Juiciness	Too dry	35	32.71%	166.000	4.743	1.974	< 0.0001	Yes
		Just-about-right	60	56.07%	403.000	6.717			
		Too juicy	12	11.21%	56.000	4.667	2.050		
2% Pea Starch	Firmness	Too soft	8	7.34%	38.000	4.750	2.089		
		Just-about-right	62	56.88%	424.000	6.839			
		Too firm	39	35.78%	188.000	4.821	2.018	< 0.0001	Yes
	Juiciness	Too dry	31	28.44%	169.000	5.452	1.010	0.005	Yes
		Just-about-right	65	59.63%	420.000	6.462			
		Too juicy	13	11.93%	61.000	4.692	1.769		
4% Pea Starch	Firmness	Too soft	19	17.59%	100.000	5.263	1.594		
		Just-about-right	63	58.33%	432.000	6.857			
		Too firm	26	24.07%	137.000	5.269	1.588	< 0.0001	Yes
	Juiciness	Too dry	35	32.41%	188.000	5.371	1.470	< 0.0001	Yes
		Just-about-right	63	58.33%	431.000	6.841			
		Too juicy	10	9.26%	50.000	5.000	1.841		
2% Short Rice Flour	Firmness	Too soft	12	10.91%	53.000	4.417	2.497		
		Just-about-right	58	52.73%	401.000	6.914			
		Too firm	40	36.36%	186.000	4.650	2.264	< 0.0001	Yes
	Juiciness	Too dry	43	39.09%	214.000	4.977	1.573	< 0.0001	Yes
		Just-about-right	60	54.55%	393.000	6.550			

		Too juicy	7	6.36%	33.000	4.714	1.836		
4% Short Rice Flour	Firmness	Too soft	27	23.48%	118.000	4.370	2.446	< 0.0001	Yes
		Just-about-right	60	52.17%	409.000	6.817			
		Too firm	28	24.35%	128.000	4.571	2.245	< 0.0001	Yes
	Juiciness	Too dry	32	27.83%	148.000	4.625	1.994	< 0.0001	Yes
		Just-about-right	63	54.78%	417.000	6.619			
		Too juicy	20	17.39%	90.000	4.500	2.119		
2% Textured Pea Protein	Firmness	Too soft	8	7.14%	40.000	5.000	2.036		
		Just-about-right	55	49.11%	387.000	7.036			
		Too firm	49	43.75%	226.000	4.612	2.424	< 0.0001	Yes
	Juiciness	Too dry	44	39.29%	224.000	5.091	1.789	< 0.0001	Yes
		Just-about-right	50	44.64%	344.000	6.880			
		Too juicy	18	16.07%	85.000	4.722	2.158		
4% Textured Pea Protein	Firmness	Too soft	25	21.55%	130.000	5.200	1.612	< 0.0001	Yes
		Just-about-right	69	59.48%	470.000	6.812			
		Too firm	22	18.97%	115.000	5.227	1.584		
	Juiciness	Too dry	33	28.45%	183.000	5.545	1.188	0.001	Yes
		Just-about-right	60	51.72%	404.000	6.733			
		Too juicy	23	19.83%	128.000	5.565	1.168		

Figure B5. Frequency distribution for Check-All-That-Apply scale of texture for cooked burgers formulated with selected binders evaluated by the consumer sensory panel.

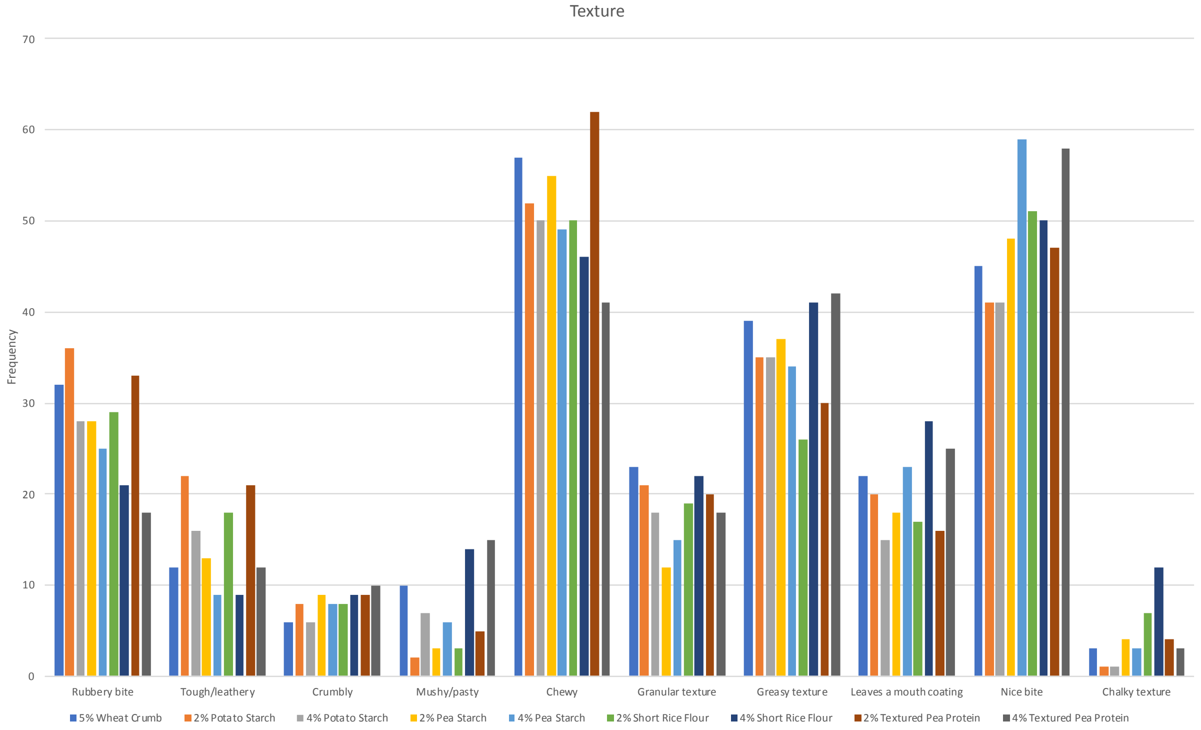


Figure B6. Percentage of frequency distribution for linear intensity of lingering aftertaste for cooked burgers formulated with selected binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = no aftertaste, 7 = lingering.

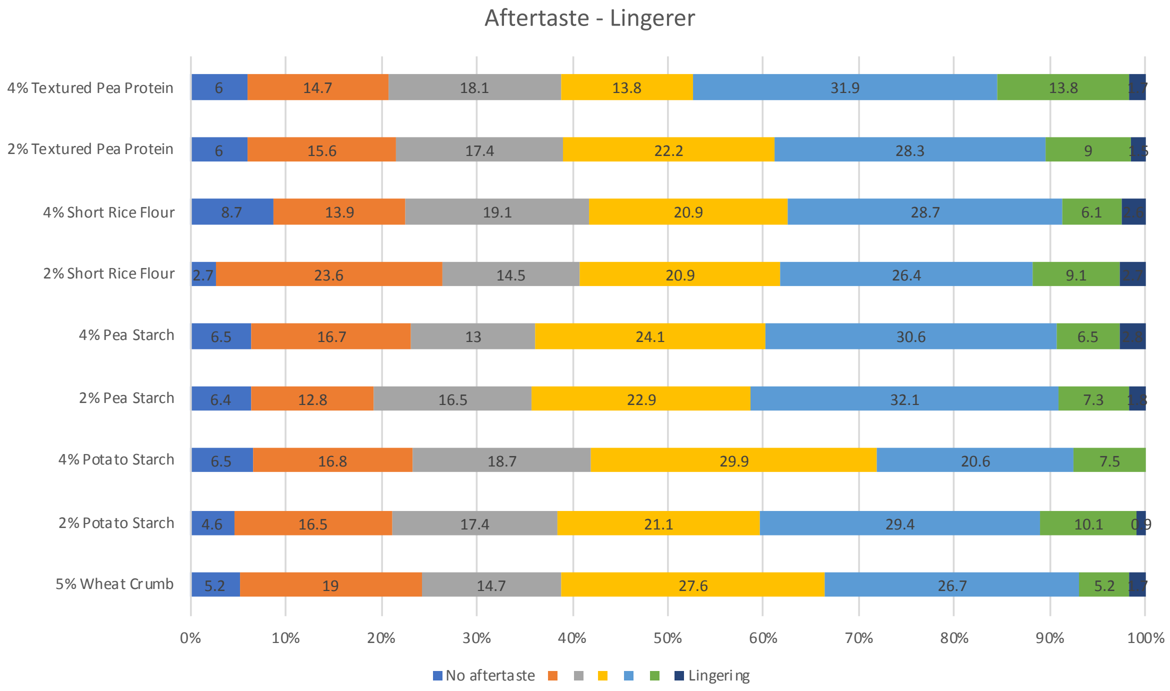


Figure B7. Percentage of frequency distribution for Just-about-right scale of pleasant aftertaste for cooked burgers formulated with selected binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = very unpleasant, 4 = just-about-right, 7 = lingering/very pleasant.

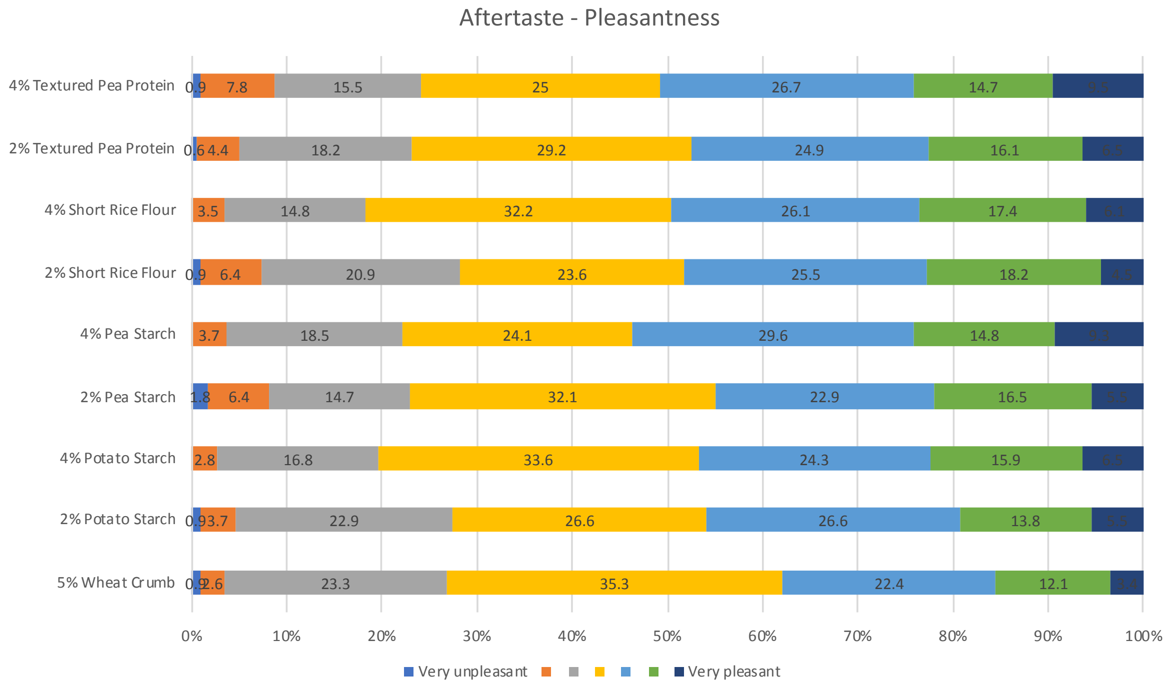
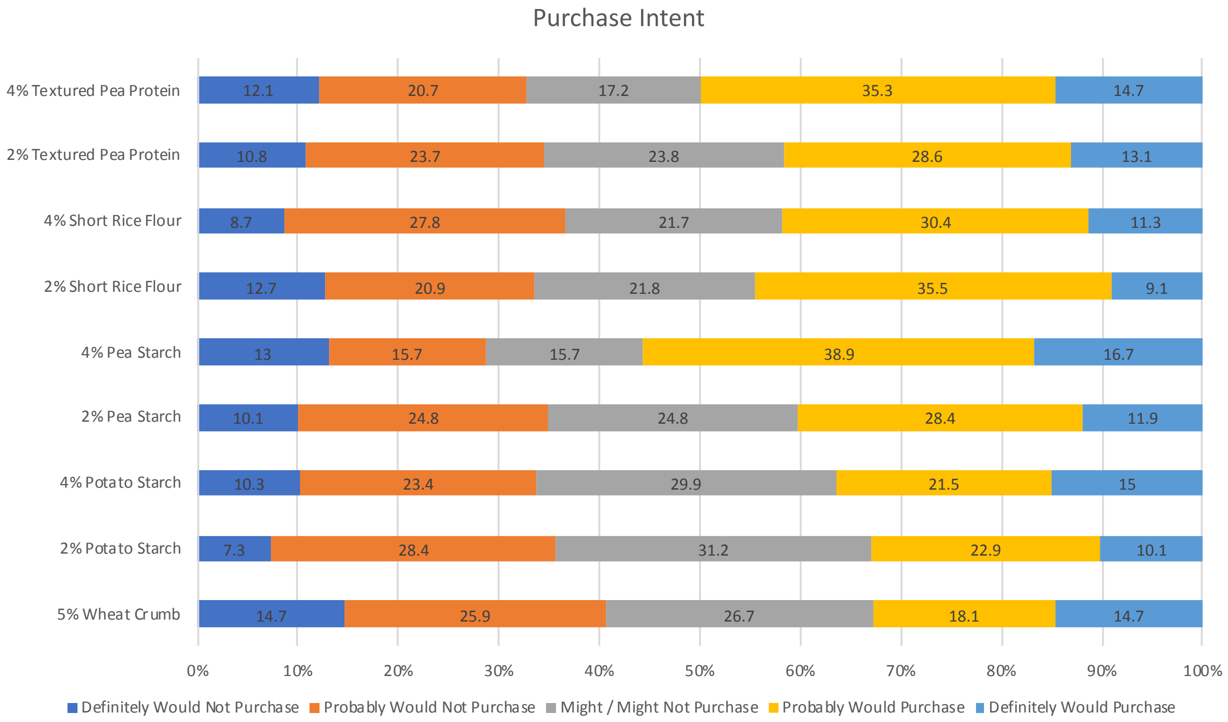


Figure B8. Percentage of frequency distribution of purchase intent for cooked burgers formulated with selected binders evaluated by the consumer sensory panel. Scored on 5-point scales where 1 = definitely would not purchase, 2 = probably would not purchase, 3 = might/might not purchase, 4 = probably would purchase, 5 = definitely would purchase.



Appendix C

Figure C1. Percentage of frequency distribution for Just-about-right scale of colour for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too grey and dull, 2 = moderately grey and dull, 3 = slightly grey and dull, 4 = just-about-right, 5 = slightly pink and bright, 6 = moderately pink and bright, and 7 = too pink and bright.

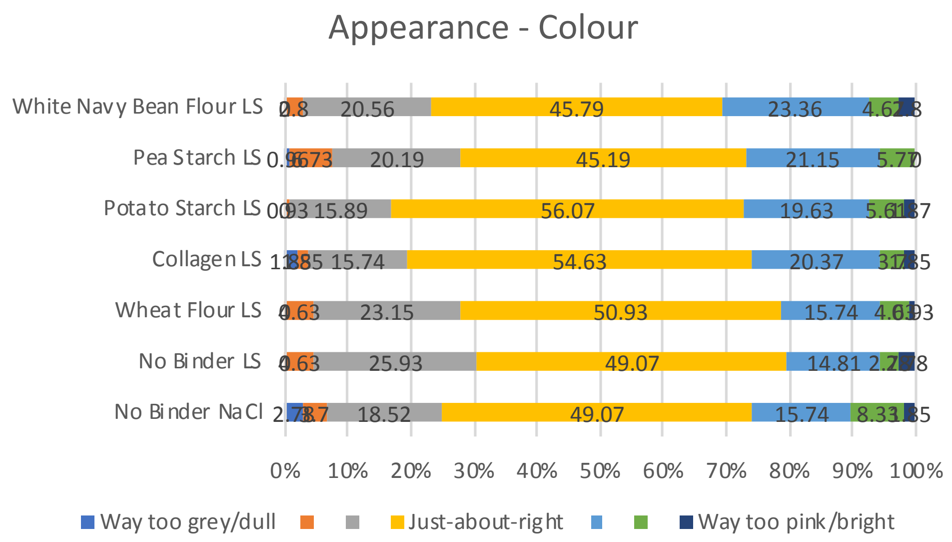


Figure C2. Percentage of frequency distribution for Just-about-right scale of surface moisture for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too dry surface, 2 = moderately dry surface, 3 = slightly dry surface, 4 = just-about-right, 5 = slightly moist surface, 6 = moderately moist surface, and 7 = too moist surface.

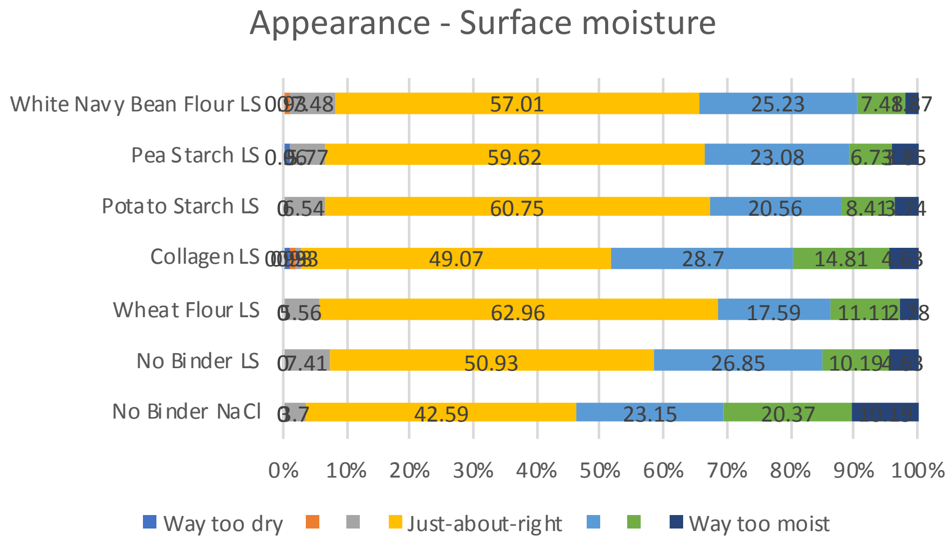


Table C1. Penalty analysis for Just-about-right scale of colour and surface moisture for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum	Mean	Mean drops	Standardized difference	p-value	Significant
No Binder NaCl	Colour	Way too grey/dull	27	25.00%	132.000	4.889	1.677	4.404	< 0.0001	Yes
		Just-about-right	53	49.07%	348.000	6.566				
		Way too pink/bright	28	25.93%	157.000	5.607	0.959	2.548	0.033	Yes
	Surface moisture	Way too dry	4	3.70%	24.000	6.000	0.457			
		Just-about-right	46	42.59%	297.000	6.457				
		Way too moist	58	53.70%	316.000	5.448	1.008	3.009	0.003	Yes
No Binder LS	Colour	Way too grey/dull	33	30.56%	166.000	5.030	1.479	4.706	< 0.0001	Yes
		Just-about-right	53	49.07%	345.000	6.509				
		Way too pink/bright	22	20.37%	117.000	5.318	1.191	3.314	0.004	Yes
	Surface moisture	Way too dry	8	7.41%	37.000	4.625	1.575			
		Just-about-right	55	50.93%	341.000	6.200				
		Way too moist	45	41.67%	250.000	5.556	0.644	2.102	0.038	Yes
Wheat Flour LS	Colour	Way too grey/dull	30	27.78%	160.000	5.333	1.230	3.797	0.001	Yes
		Just-about-right	55	50.93%	361.000	6.564				
		Way too pink/bright	23	21.30%	140.000	6.087	0.477	1.345	0.374	No
	Surface moisture	Way too dry	6	5.56%	31.000	5.167	1.083			
		Just-about-right	68	62.96%	425.000	6.250				
		Way too moist	34	31.48%	205.000	6.029	0.221	0.693	0.490	No
Collagen LS	Colour	Way too grey/dull	21	19.44%	104.000	4.952	1.539			
		Just-about-right	59	54.63%	383.000	6.492				
		Way too pink/bright	28	25.93%	166.000	5.929	0.563	1.558	0.123	No
	Surface moisture	Way too dry	3	2.78%	15.000	5.000	1.604			
		Just-about-right	53	49.07%	350.000	6.604				
		Way too moist	52	48.15%	288.000	5.538	1.065	3.361	0.001	Yes
Potato Starch LS	Colour	Way too grey/dull	18	16.82%	89.000	4.944	1.539			
		Just-about-right	60	56.07%	389.000	6.483				
		Way too pink/bright	29	27.10%	167.000	5.759	0.725	1.860	0.066	No
	Surface moisture	Way too dry	7	6.54%	31.000	4.429	1.971			
		Just-about-right	65	60.75%	416.000	6.400				
		Way too moist	35	32.71%	198.000	5.657	0.743	2.177	0.032	Yes
Pea Starch LS	Colour	Way too grey/dull	29	27.88%	134.000	4.621	1.613	4.438	< 0.0001	Yes
		Just-about-right	47	45.19%	293.000	6.234				
		Way too pink/bright	28	26.92%	163.000	5.821	0.413	1.123	0.502	No
	Surface moisture	Way too dry	7	6.73%	27.000	3.857	2.111			
		Just-about-right	62	59.62%	370.000	5.968				

		Way too moist	35	33.65%	193.000	5.514	0.453	1.320	0.190	No
White Navy Bean Flour LS	Colour	Way too grey/dull	25	23.36%	131.000	5.240	1.270	3.012	0.009	Yes
		Just-about-right	49	45.79%	319.000	6.510				
		Way too pink/bright	33	30.84%	187.000	5.667	0.844	2.183	0.079	No
	Surface moisture	Way too dry	9	8.41%	51.000	5.667	0.481			
		Just-about-right	61	57.01%	375.000	6.148				
		Way too moist	37	34.58%	211.000	5.703	0.445	1.182	0.240	No

Figure C3. Percentage of frequency distribution for Just-about-right scale of saltiness for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too blend, 2 = moderately blend, 3 = slightly blend, 4 = just-about-right, 5 = slightly strong, 6 = moderately strong, and 7 = too strong.

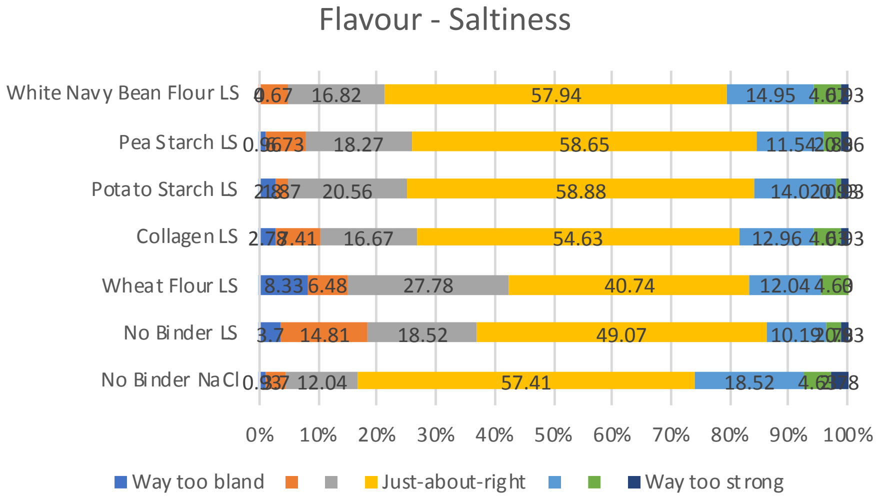


Figure C4. Percentage of frequency distribution for Just-about-right scale of bologna flavour intensity for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too blend, 2 = moderately blend, 3 = slightly blend, 4 = just-about-right, 5 = slightly strong, 6 = moderately strong, and 7 = too strong.

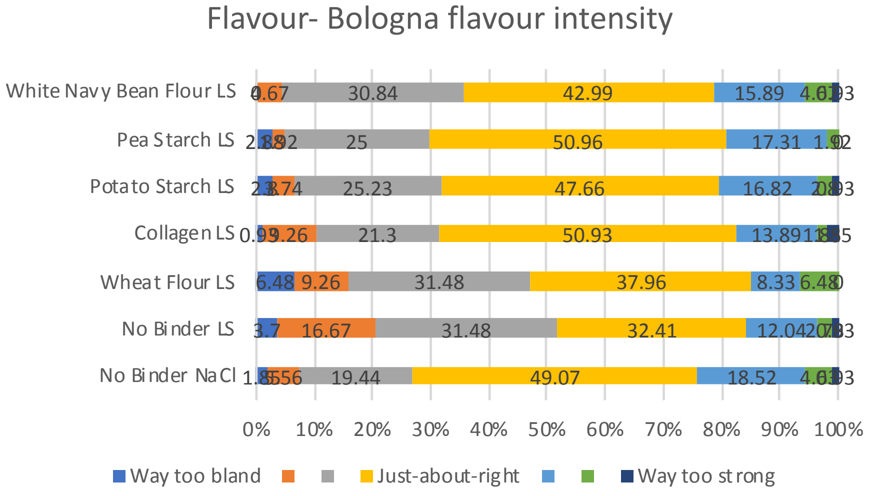


Table C2. Penalty analysis for Just-about-right scale of saltiness and bologna flavour intensity for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum	Mean	Mean drops	Standardized difference	p-value	Significant
No Binder NaCl	Saltiness	Way too bland	18	16.67%	102.000	5.667	1.220			
		Just-about-right	62	57.41%	427.000	6.887				
		Way too strong	28	25.93%	158.000	5.643	1.244	3.093	0.003	Yes
	Bologna flavour intensity	Way too bland	29	26.85%	159.000	5.483	1.687	4.393	< 0.0001	Yes
		Just-about-right	53	49.07%	380.000	7.170				
		Way too strong	26	24.07%	148.000	5.692	1.478	3.711	0.001	Yes
No Binder LS	Saltiness	Way too bland	40	37.04%	194.000	4.850	1.188	3.470	0.001	Yes
		Just-about-right	53	49.07%	320.000	6.038				
		Way too strong	15	13.89%	80.000	5.333	0.704			
	Bologna flavour intensity	Way too bland	56	51.85%	278.000	4.964	1.579	4.612	< 0.0001	Yes
		Just-about-right	35	32.41%	229.000	6.543				
		Way too strong	17	15.74%	87.000	5.118	1.425			
Wheat Flour LS	Saltiness	Way too bland	46	42.59%	212.000	4.609	1.778	5.177	< 0.0001	Yes
		Just-about-right	44	40.74%	281.000	6.386				
		Way too strong	18	16.67%	95.000	5.278	1.109			
	Bologna flavour intensity	Way too bland	51	47.22%	234.000	4.588	1.729	5.098	< 0.0001	Yes
		Just-about-right	41	37.96%	259.000	6.317				
		Way too strong	16	14.81%	95.000	5.938	0.380			
Collagen LS	Saltiness	Way too bland	29	26.85%	139.000	4.793	1.970	5.883	< 0.0001	Yes
		Just-about-right	59	54.63%	399.000	6.763				
		Way too strong	20	18.52%	121.000	6.050	0.713			
	Bologna flavour intensity	Way too bland	34	31.48%	167.000	4.912	1.997	6.240	< 0.0001	Yes
		Just-about-right	55	50.93%	380.000	6.909				
		Way too strong	19	17.59%	112.000	5.895	1.014			
Potato Starch LS	Saltiness	Way too bland	27	25.23%	146.000	5.407	1.116	2.822	0.006	Yes
		Just-about-right	63	58.88%	411.000	6.524				
		Way too strong	17	15.89%	103.000	6.059	0.465			
	Bologna flavour intensity	Way too bland	34	31.78%	177.000	5.206	1.716	4.731	< 0.0001	Yes
		Just-about-right	51	47.66%	353.000	6.922				
		Way too strong	22	20.56%	130.000	5.909	1.012	2.423	0.045	Yes
Pea Starch LS	Saltiness	Way too bland	27	25.96%	142.000	5.259	1.528	4.709	< 0.0001	Yes
		Just-about-right	61	58.65%	414.000	6.787				
		Way too strong	16	15.38%	93.000	5.813	0.974			
	Bologna flavour intensity	Way too bland	31	29.81%	167.000	5.387	1.594	5.172	< 0.0001	Yes
		Just-about-right	53	50.96%	370.000	6.981				

		Way too strong	20	19.23%	112.000	5.600	1.381			
White Navy Bean Flour LS	Saltiness	Way too bland	23	21.50%	117.000	5.087	1.574	3.804	0.001	Yes
		Just-about-right	62	57.94%	413.000	6.661				
		Way too strong	22	20.56%	118.000	5.364	1.298	3.085	0.007	Yes
	Bologna flavour intensity	Way too bland	38	35.51%	187.000	4.921	2.253	6.706	< 0.0001	Yes
		Just-about-right	46	42.99%	330.000	7.174				
		Way too strong	23	21.50%	131.000	5.696	1.478	3.777	0.001	Yes

Figure C5. Frequency distribution for Check-All-That-Apply scale of flavour for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel.

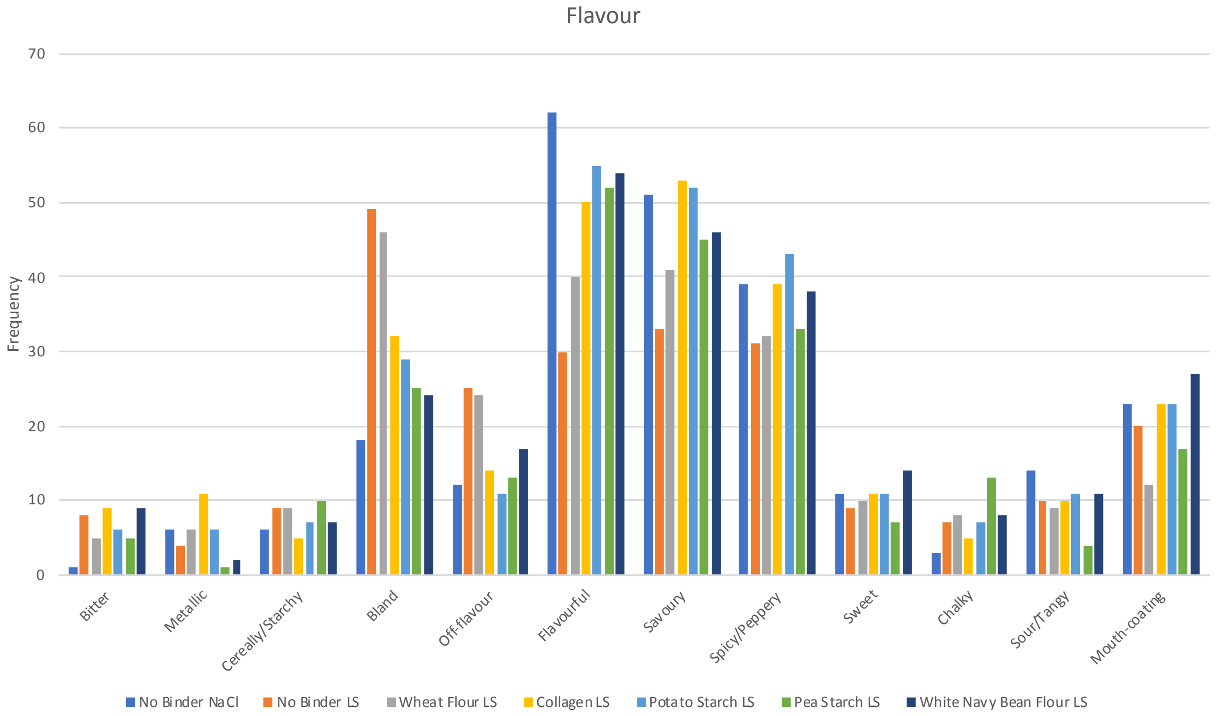


Figure C6. Percentage of frequency distribution for Just-about-right scale of juiciness for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too dry, 2 = moderately dry, 3 = slightly dry, 4 = just-about-right, 5 = slightly juicy/moist, 6 = moderately juicy/moist, and 7 = too juicy/moist.

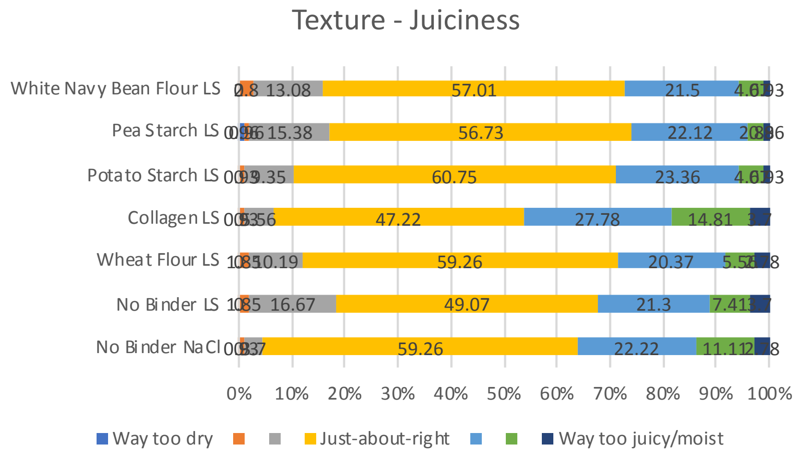


Table C3. Penalty analysis for Just-about-right scale of juiciness for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum	Mean	Mean drops	Standardized difference	p-value	Significant
No Binder NaCl	Juiciness	Way too dry	5	4.63%	19.000	3.800	3.028			
		Just-about-right	64	59.26%	437.000	6.828				
		Way too juicy/moist	39	36.11%	204.000	5.231	1.597	4.998	< 0.0001	Yes
No Binder LS	Juiciness	Way too dry	20	18.52%	88.000	4.400	2.015			
		Just-about-right	53	49.07%	340.000	6.415				
		Way too juicy/moist	35	32.41%	176.000	5.029	1.387	3.928	0.000	Yes
Wheat Flour LS	Juiciness	Way too dry	13	12.04%	52.000	4.000	2.750			
		Just-about-right	64	59.26%	432.000	6.750				
		Way too juicy/moist	31	28.70%	170.000	5.484	1.266	4.162	< 0.0001	Yes
Collagen LS	Juiciness	Way too dry	7	6.48%	32.000	4.571	2.331			
		Just-about-right	51	47.22%	352.000	6.902				
		Way too juicy/moist	50	46.30%	266.000	5.320	1.582	5.070	< 0.0001	Yes
Potato Starch LS	Juiciness	Way too dry	11	10.28%	50.000	4.545	2.285			
		Just-about-right	65	60.75%	444.000	6.831				
		Way too juicy/moist	31	28.97%	170.000	5.484	1.347	4.631	< 0.0001	Yes
Pea Starch LS	Juiciness	Way too dry	18	17.31%	87.000	4.833	1.946			
		Just-about-right	59	56.73%	400.000	6.780				
		Way too juicy/moist	27	25.96%	159.000	5.889	0.891	2.961	0.004	Yes
White Navy Bean Flour LS	Juiciness	Way too dry	17	15.89%	84.000	4.941	1.600			
		Just-about-right	61	57.01%	399.000	6.541				
		Way too juicy/moist	29	27.10%	175.000	6.034	0.507	1.520	0.132	No

Figure C7. Percentage of frequency distribution for Just-about-right scale of firmness for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too soft, 2 = moderately soft, 3 = slightly soft, 4 = just-about-right, 5 = slightly firm, 6 = moderately firm, and 7 = too firm.

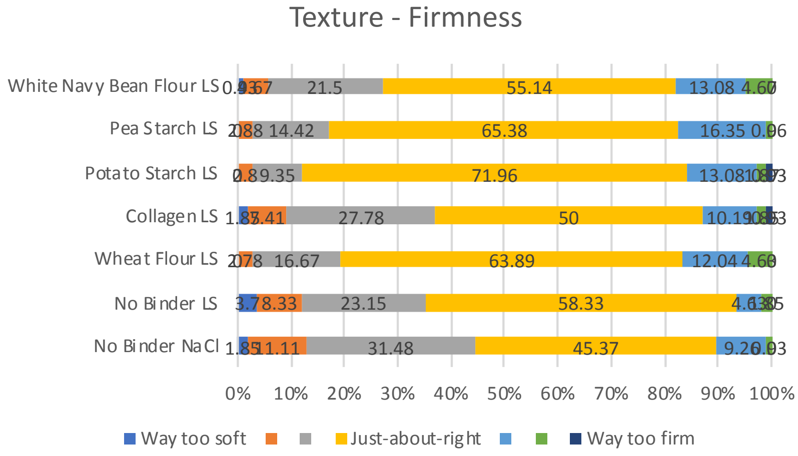


Table C4. Penalty analysis for Just-about-right scale of firmness for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum	Mean	Mean drops	Standardized difference	p-value	Significant
No Binder NaCl	Firmness	Way too soft	48	44.44%	229.000	4.771	2.045	6.562	< 0.0001	Yes
		Just-about-right	49	45.37%	334.000	6.816				
		Way too firm	11	10.19%	68.000	6.182	0.635			
No Binder LS	Firmness	Way too soft	38	35.19%	156.000	4.105	2.450	9.181	< 0.0001	Yes
		Just-about-right	63	58.33%	413.000	6.556				
		Way too firm	7	6.48%	41.000	5.857	0.698			
Wheat Flour LS	Firmness	Way too soft	21	19.44%	99.000	4.714	2.025			
		Just-about-right	69	63.89%	465.000	6.739				
		Way too firm	18	16.67%	104.000	5.778	0.961			
Collagen LS	Firmness	Way too soft	40	37.04%	178.000	4.450	2.476	9.370	< 0.0001	Yes
		Just-about-right	54	50.00%	374.000	6.926				
		Way too firm	14	12.96%	91.000	6.500	0.426			
Potato Starch LS	Firmness	Way too soft	13	12.15%	59.000	4.538	2.371			
		Just-about-right	77	71.96%	532.000	6.909				
		Way too firm	17	15.89%	101.000	5.941	0.968			
Pea Starch LS	Firmness	Way too soft	18	17.31%	77.000	4.278	2.546			
		Just-about-right	68	65.38%	464.000	6.824				
		Way too firm	18	17.31%	112.000	6.222	0.601			
White Navy Bean Flour LS	Firmness	Way too soft	29	27.10%	136.000	4.690	2.039	6.636	< 0.0001	Yes
		Just-about-right	59	55.14%	397.000	6.729				
		Way too firm	19	17.76%	103.000	5.421	1.308			

Figure C9. Percentage of frequency distribution for Just-about-right scale of chewiness for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = too crumbly, 2 = moderately crumbly, 3 = slightly crumbly, 4 = just-about-right, 5 = slightly rubbery, 6 = moderately rubbery, and 7 = too rubbery.

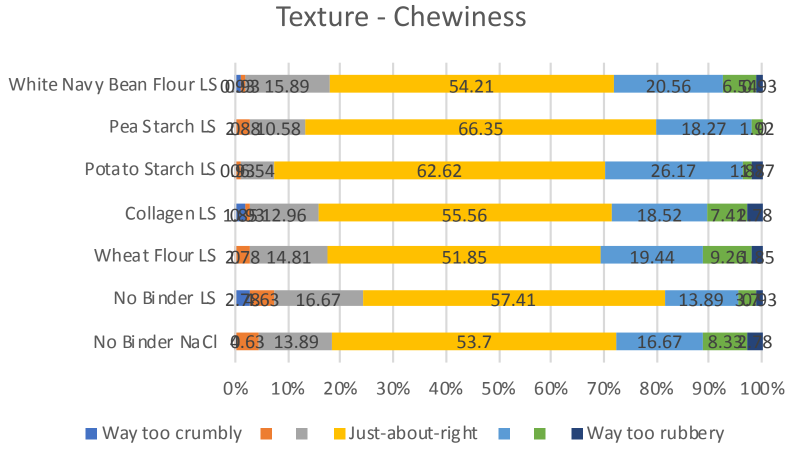


Table C5. Penalty analysis for Just-about-right scale of chewiness for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel.

Treatment	Variable	Level	Frequencies	%	Sum	Mean	Mean drops	Standardized difference	p-value	Significant
No Binder NaCl	Chewiness	Way too crumbly	20	18.52%	89.000	4.450	2.240			
		Just-about-right	58	53.70%	388.000	6.690				
		Way too rubbery	30	27.78%	149.000	4.967	1.723	4.864	< 0.0001	Yes
No Binder LS	Chewiness	Way too crumbly	26	24.07%	104.000	4.000	2.435	8.928	< 0.0001	Yes
		Just-about-right	62	57.41%	399.000	6.435				
		Way too rubbery	20	18.52%	96.000	4.800	1.635			
Wheat Flour LS	Chewiness	Way too crumbly	19	17.59%	95.000	5.000	1.714			
		Just-about-right	56	51.85%	376.000	6.714				
		Way too rubbery	33	30.56%	177.000	5.364	1.351	4.339	< 0.0001	Yes
Collagen LS	Chewiness	Way too crumbly	17	15.74%	68.000	4.000	2.600			
		Just-about-right	60	55.56%	396.000	6.600				
		Way too rubbery	31	28.70%	152.000	4.903	1.697	4.867	< 0.0001	Yes
Potato Starch LS	Chewiness	Way too crumbly	8	7.48%	40.000	5.000	1.925			
		Just-about-right	67	62.62%	464.000	6.925				
		Way too rubbery	32	29.91%	181.000	5.656	1.269	4.362	< 0.0001	Yes
Pea Starch LS	Chewiness	Way too crumbly	14	13.46%	59.000	4.214	2.438			
		Just-about-right	69	66.35%	459.000	6.652				
		Way too rubbery	21	20.19%	116.000	5.524	1.128	3.288	0.001	Yes
White Navy Bean Flour LS	Chewiness	Way too crumbly	19	17.76%	83.000	4.368	2.201			
		Just-about-right	58	54.21%	381.000	6.569				
		Way too rubbery	30	28.04%	157.000	5.233	1.336	4.229	< 0.0001	Yes

Figure C10. Percentage of frequency distribution for linear intensity of lingering aftertaste for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = no aftertaste, 7 = lingering.

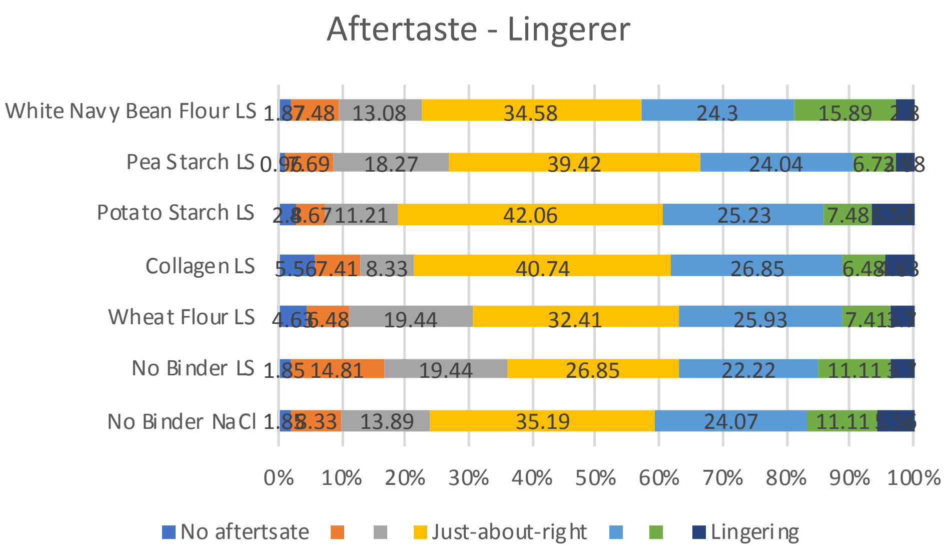


Figure C11. Percentage of frequency distribution for Just-about-right scale of pleasant aftertaste for bolognas formulated with selected salts and binders evaluated by the consumer sensory panel. Scored on 7-point scales where 1 = very unpleasant, 4 = just-about-right, 7 = lingering/very pleasant.