Insights into Wheat Grain Microstructure and Composition for the Development of Novel Flour with Slow Digestion Properties and Enhanced Functional Characteristics

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Abstract

Wheat has been consumed as whole grains, broken grains, flattened format, and puffed format, other than the flour format, which has a wide application in different types of food preparations. Wheat flour possesses a unique ability to form a cohesive dough that has viscoelastic properties. A range of products with wheat as their major ingredient are high glycaemic index (GI) foods as wheat flour contains highly digestible starch. However, the consumption of high GI foods is associated with chronic diseases such as diabetes, coronary heart disease, and obesity due to a rapid increase in blood glucose levels and secretion of insulin. The major objective of the research studies of this thesis included creating slowly digestible flour with improved functionality using slowly digested starch sources and non-starch components.

Modifying wheat grain through different processing techniques alters the microstructure, and therefore, starch digestibility is impacted. Microstructure modification through various processing techniques, which can control the access of digestive enzymes to starch, could help develop products with controlled starch digestibility. To advance the understanding of the impact of wheat grain microstructure on starch hydrolysis, Chapter 3 explored a study on whole wheat grain in different commercially available forms (kibbled, cut grains, and flour) to understand the influence of microstructural changes on *in vitro* starch digestibility. The process of size reduction from raw intact grains to kibbled grains and flour caused an increase in overall starch hydrolysis (%) during simulated digestion in the order of flour>kibbled>cut>intact whole wheat grains. Cooking of these formats further increased their starch hydrolysis. However, both cooked cut and intact grains were low glycaemic with the expected glycaemic indices (eGI) of values of 54.08±0.03 and 41.98±0.04, respectively, revealing the role of intact microstructure in starch hydrolysis of wheat grains.

Based on the role of intact microstructure, Chapter 4 investigated the possibility of reducing the starch hydrolysis in wheat grain formats (whole, flakes, and flour) by hydrothermal

treatment and low-temperature storage of whole wheat grains. The extent of starch hydrolysis after oral-gastro-small intestinal digestion *in vitro* was significantly lower *($p<0.05$)* in intact grains, flakes, and flours from the cold-stored grains than their non-cold-stored counterparts. In this study, scanning electron micrographs, pasting properties, water retention capacities, and relative crystallinity of the resulting flours revealed an enhanced degree of gelatinisation with the treatment temperature; however, cold-storage of treated grains resulted in a change in these properties due to the retrogradation of the starch. This study indicates that hydrothermal pre-treatment of grains followed by low-temperature storage for prolonged periods might help to reduce the starch digestibility of wheat grains and their resulting products and could be an effective strategy in developing reduced glycaemic impact grain products. However, in our preliminary trials, the flours from hydrothermally treated and low-temperature stored grains resulted in doughs of inferior viscoelastic properties.

Furthermore, intending to create slowly digestible flour, Chapter 5 employed two approaches to modify a resistant starch: one involving soluble extracts from wheat flour and vital gluten (water solubles, salt-assisted water-solubles, and acid-solubles) and the other utilising hydrocolloids (guar gum, xanthan gum, locust bean gum, and carboxymethyl cellulose). Modifications from both approaches resulted in modified starch morphology with the formation of starch clusters mimicking the wheat flour. Moreover, the modification with hydrocolloids resulted in an improved pasting profile. Furthermore, *in vitro* digestion studies revealed that the starch hydrolysis rate was decreased for most of the cooked modified starches with wheat solubles and a slower starch hydrolysis profile until 60 min of simulated small intestinal digestion for most of the hydrocolloids used, carboxymethyl cellulose being the least effective in slowing the starch hydrolysis rate.

Additionally, Chapter 6 evaluates the functionality and starch digestibility of a wheat flour system (dough and flatbread-chapatti) by utilising the modified starches created in Chapter 5

as low glycaemic ingredients. The interaction of the modified starches with vital gluten and wheat flour components resulted in improved viscosity of the functional flour. The microstructure of the functional flour dough indicated that the modified starches with wheat solubles (soluble extracts from wheat flour and vital gluten) and hydrocolloids improved the starch-protein matrix and gluten network. Furthermore, the *in vitro* digestion study revealed the overall starch hydrolysis of chapattis from all the functional flour formulations was significantly lower than the wheat flour chapatti.

In conclusion, structural modifications of wheat grain could help reduce the overall starch hydrolysis of wheat grain products. Moreover, the wheat grain components have the potential to modify resistant starch sources to improve their functionality while retaining their slow digestion property. Also, utilising hydrocolloids to modify resistant starch sources could be an effective strategy to enhance the functionality of resistant starches in wheat-based systems. Modified resistant starches created using wheat solubles (soluble extracts from wheat flour and vital gluten) and hydrocolloids have potential applications with slow digestibility and improved functionality in wheat-based products.

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Abbreviations

Aa	Area of the amorphous peak
Ac	Area of the crystalline peak
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of variance
AX	Arabinoxylan
CLSM	Confocal laser scanning microscopy
CMC	Carboxymethylcellulose
cP	Centipoise
DS	Damaged starch
dwb	Dry weight basis
ECW	Endosperm cell wall
eGI	Estimated glycaemic index
FITC	Fluorescein isothiocyanate
FL	Flour
FV	Final viscosity
g	Gram
g	Relative centrifugal force
G'	Storage modulus
G"	Loss modulus
GI	Glycaemic index
Gp	Glucose produced
h	Hours
HACS	High amylose corn starch

HACS FL	Functional flour with high amylose corn starch
HASF	High amylose corn starch modified with acid-soluble extract from wheat flour
HASF FL	Functional flour with HACS modified with acid-soluble extract from wheat flour
HASV	High amylose corn starch modified with acid-soluble extract from vital gluten
HASV FL	Functional flour with HACS modified with acid-soluble extract from vital gluten
HCl	Hydrochloric acid
HCMC	High amylose corn starch modified with carboxymethyl cellulose
HCMC FL	Functional flour with HACS modified with carboxymethyl cellulose
HGG	High amylose corn starch modified with guar gum
HGG FL	Functional flour with HACS modified with guar gum
HI	Hydrolysis index
HLBG	High amylose corn starch modified with locust bean gum
HLBG FL	Functional flour with HACS modified with locust bean gum
HWS	High amylose corn starch modified with water-soluble wheat flour extract
HWS FL	Functional flour with HACS modified with water-soluble wheat flour extract
HWSS	High amylose corn starch modified with salt-assisted water-soluble wheat flour extract
HWSS FL	Functional flour with HACS modified with salt-assisted water-soluble wheat flour

HXG	High amylose corn starch modified with xanthan gum
HXG FL	Functional flour with HACS modified with xanthan gum
LASRC	Lactic acid retention capacity
LMW	Low molecular weight
M	Molar
mg	Milligram
min	Minutes
ml	Mililitre
mm	Millimetre
NaCl	Sodium chloride
NaOH	Sodium hydroxide
nm	Nanometre
NMR	Nuclear magnetic resonance
PM	Protein matrix
PV	Peak viscosity
RDS	Rapidly digestible starch
RO	Reverse Osmosis
rpm	Rotation per minute
RS	Resistant starch
RVA	Rapid visco-analyser
s	Seconds
S	Starch
SCSRC	Sodium carbonate retention capacity
SD	Standard deviation
SDS	Slowly digestible starch

SEM	Scanning electron microscopy
SFL	Flour from hydrothermally treated and cold-stored grains
SG	Hydrothermally treated and cold-stored grains
SGF	Simulated gastric fluid
SH	Starch hydrolysis
Si	Initial amount of starch
SIF	Simulated intestinal fluid
SRC	Solvent retention capacities
SSF	Simulated salivary fluid
STD	Standard method
SuSRC	Sucrose retention capacity
U	Units of enzymes
WSRC	Water retention capacity
w/v	Weight by volume
w/w	Weight by weight
XRD	X-ray diffraction
%	Percent
μm	Micrometre
°C	Degree Centigrade

List of peer-reviewed publications

Some of the chapters have either been published, in preparation or were presented at a conference.

Publications

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2. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. (2022). Effects of hydrothermal treatment and low-temperature storage of whole wheat grains on in vitro starch hydrolysis and flour properties. *Food Chemistry*, *395*, 133516. https://doi.org/10.1016/j.foodchem.2022.133516
3. Abhilasha, A., Kaur, L., & Singh, J. (2025). Wheat Grain Microstructure and Starch Digestion. Book Chapter, Editors Vega-Castro et al., 2025, In *Selected Topics in Food Process Engineering*. Springer Nature Switzerland (in press).
4. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. Effect of water solubles, salt-assisted waters-solubles and acid-solubles from wheat flour on functionality and starch digestibility of high amylose corn starch: Physico-chemical, microstructural, rheological characteristics and in vitro starch hydrolysis. *(Foods, to be submitted).*
5. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. Effect of guar gum, xanthan gum, locust bean gum and carboxymethyl cellulose on functionality and starch digestibility of high amylose corn starch: Physico-chemical, microstructural, rheological characteristics and in vitro starch hydrolysis. *(to be submitted).*

Conference proceedings

1. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. (2019, 30 Spetember), Biomimetic wheat flour: An artificial wheat flour with low glycaemic features. Presented at Riddet Institute Student Colloquium, Rotorua, New Zealand
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3. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. (2020, 25 October), Development of biomimetic wheat flour with low glycaemic features. Presented at Postgraduate Food Science Symposium, Massey University, Palmerston North, New Zealand
4. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. (2021,7-9 April), Hydrothermal treatment and low-temperature storage of wheat grains: An investigation into their role in developing low glycaemic flours. Poster presented at Riddet Institute Conference/Student Colloquium, Wellington, New Zealand
5. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. (2021, 6-8 July), *In vitro* gastro-small intestinal starch digestion behaviour of whole wheat grains. Poster presented at NZIFST Annual Conference, Palmerston North, New Zealand
6. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. (2021, 6-8 July), *In vitro* gastro-small intestinal starch digestion behaviour of whole wheat grains. Presented at 3MP NZIFST Annual Conference, Palmerston North, New Zealand
7. Abhilasha, A., Kaur, L., Monro, J., Hardacre, A., & Singh, J. (2021, 16-19 November), Does hydrothermal treatment and low-temperature storage of wheat grains impact the in vitro starch hydrolysis and flour properties. Poster presented at Food Structure, Digestion and Health 6th International Conference, virtual event.

Chapter 1

Chapter 1 Introduction and Thesis Overview

1.1 Background

Cereals have been an important part of the world's food consumption. The share of dietary energy supplied by cereals is more than 55% of global food consumption (Palacios & Ruiz-Vanoye, 2018). Among all the cereals, wheat holds a special place being a key cereal grain with regard to human nutrition. Carbohydrates are a very important source of dietary energy (45-70% of the total energy intake) (Lafiandra et al., 2014), and the energy provided by wheat is between 1220 and 1450 kJ per 100g (Rosell, 2012), the majority of which is contributed by the starch. Along with being an important source of calories, wheat is also a significant source of dietary protein.

Wheat has been consumed as whole grains, broken grains, flattened format, and puffed format, other than the flour format, which has a wide application in different types of food preparations. Along with these products, gluten and starch are commercially important products from wheat grain. Wheat flour possesses a unique ability to form a cohesive dough that has viscoelastic properties. The viscoelastic properties of wheat are important for the processing steps such as mixing, fermentation, sheeting, and molding, making it a major ingredient in products such as pasta, noodles, breakfast cereals, bread, biscuits, cake, pastry, doughnuts, pizza, and many other bakery products.

Based on their glycaemic index (GI), foods are generally categorised as low GI (<55), medium GI (56-69), and high GI (>70). The GI of a food is the postprandial blood glucose response to available carbohydrates in a food expressed as the percentage of the response to an equivalent weight of carbohydrate reference glucose or available carbohydrate in white bread (Wolever et al., 1991). A range of products with wheat as their major ingredient are high-GI foods (Atkinson et al., 2021; Kumar & Prabhasankar, 2014) as wheat flour contains highly digestible starch.

However, the consumption of high GI foods is associated with chronic diseases such as diabetes, coronary heart disease, and obesity due to a rapid increase in blood glucose levels and secretion of insulin (Augustin et al., 2002; Riccardi et al., 2008; Wolever, 2003). The low GI diets have been reported to impart benefits concerning several metabolic consequences such as insulin resistance and glucose and lipid metabolism, managing obesity, lowering the risk of heart diseases and managing cholesterol levels, reducing the risk of different cancers, reducing polycystic ovarian syndrome as well as age-related macular degeneration (Kaur et al., 2022). Multiple formulations have been studied for low GI breads and other wheat products. For instance, wheat and finger millet flour composite flour has been studied for chapati making (Sharma et al., 2017), buckwheat flour has been incorporated for the development of Chinese steamed bread (Liu et al., 2017), and part of wheat flour was replaced by chickpea flour for bread making (Shrivastava & Chakraborty, 2018; Utrilla-Coello et al., 2007). The uniqueness of wheat flour is attributed to the presence of the network-forming gluten proteins and wheat starch, contributing to their unique interactions in the dough. However, flours of non-wheat cereals cause rheological changes as these flours have different techno-functional properties and do not produce viscoelastic dough (Sullivan et al., 2013). The unavailability of the right type of raw material is limiting the production of high-quality low-glycaemic and satiety-enhancing foods. Therefore, it is of prime importance to understand the role of components of wheat and other ingredients in starch digestibility as well as functionality, and develop slowly digestible raw materials with improved functional properties to overcome the above-mentioned limitations.

1.2 Research aims and objectives

The present study aims to better understand the starch digestibility in wheat and develop slowly digestible starches that could be used in wheat-based products to lower their starch digestibility and improve functionality.

The research aim of this study included:

- Creating slowly digestible flour with improved functionality using slowly digested starch sources and non-starch components.

To achieve the research aim, the following objectives have been studied:

Objective 1. To determine the effect of wheat grain microstructure and the influence of processing on *in vitro* gastro-small intestinal starch digestibility.

Objective 2. To explore the impact of cooking and cooling whole wheat grain to lower the starch digestibility.

Objective 3. To investigate the treatment of a resistant starch source with soluble components of wheat and food-grade hydrocolloids, and characterise the resulting impact on starch digestibility and functional properties.

Objective 4. To assess the functionality and starch digestibility of the resistant starches modified with wheat solubles (soluble extracts from wheat flour and vital gluten), and hydrocolloids in a wheat flour system (flatbread).

1.3 Research questions, hypotheses, and organisation of the thesis

The research questions and corresponding hypotheses addressed in this thesis are presented in the schematic Figure 1.1.

Below is the overview of the thesis:

Chapter 1 outlines the background and objectives of this study.

Chapter 2 contains a detailed review of the literature about the microstructure of wheat grains, factors impacting their functionality and digestibility, and formats of low-glycaemic ingredients in wheat-based products.

Chapter 3 explores a study on whole wheat grain in different commercially available forms (kibbled and milled) to understand the influence of microstructural changes on *in vitro* starch digestibility.

Based on the role of intact microstructure, Chapter 4 investigates the possibility of reducing the starch hydrolysis in wheat grain formats (whole, flakes and flour) by hydrothermal treatment and low-temperature storage of whole wheat grains. This chapter also explores the impact of hydrothermal treatment and low-temperature storage of whole wheat grains on the functional properties and digestibility of wheat flour.

Furthermore, intending to create slowly digestible flour, Chapter 5 utilised soluble extracts from wheat, and food-grade hydrocolloids to modify resistant starch (high amylose corn starch) and characterised their microstructural and starch hydrolysis properties.

Chapter 6 consists of the evaluation of the functionality and starch digestibility of a wheat flour system (dough and flatbread-chapatti) by utilising the modified starches developed in Chapter 5 as low glycaemic ingredients.

Chapter 7 concludes the general findings of the studies and further recommendations.

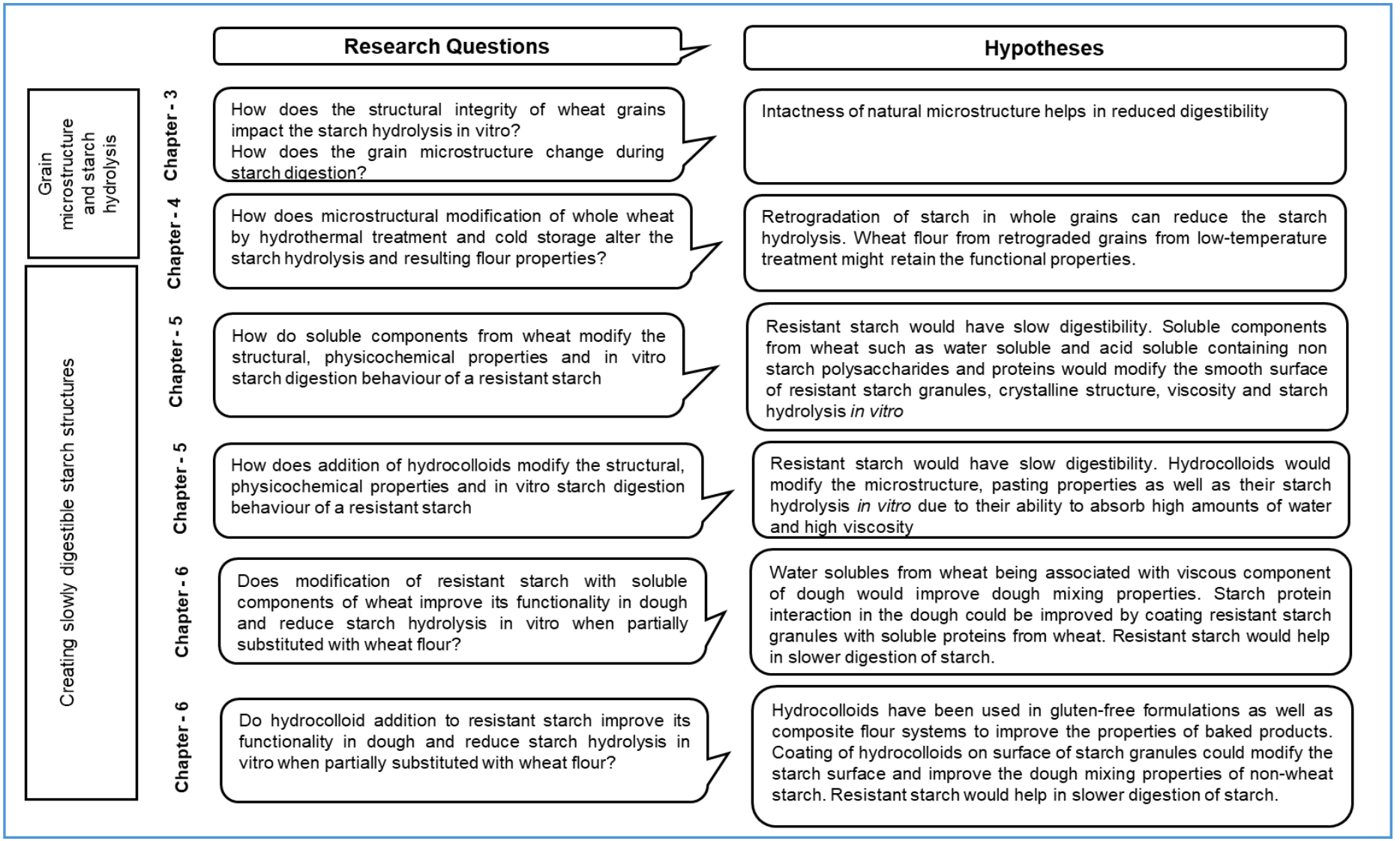

Figure 1. 1 *Schematic overview of research objectives, questions, and hypotheses of the thesis*

Chapter 2

Chapter 2 Review of Literature

2.1 Introduction

Cereals are the most used grains to prepare food products. In global food consumption, cereals supply approximately 50% of the dietary energy (Bruinsma (2002). Wheat is a major cereal for human nutrition, and global consumption of wheat has increased at a faster rate compared to all other cereals (Kearney, 2010). Carbohydrates are a significant source of dietary energy (45-70% of the total energy intake) (Lafiandra et al., 2014), and the energy provided by wheat is between 1220 and 1450 kJ per 100 g of cereal (Rosell, 2012), the majority of which is contributed by starch. World wheat production was 757.2 million tons in 2017, around 29% of world cereal production.

World wheat production was 757.2 million tonnes in 2017, around 29% of world cereal production. Moreover, global per capita consumption of wheat is accounted to be 66.7 kg per year (FAO, 2018). Wheat is the most favored staple diet in the world, along with rice. It is a major ingredient in products such as breakfast cereals, cracked grain meals, pasta, noodles, bread, biscuits, cake, pastry, doughnuts, pizza, and many other bakery products in the form of flour.

Digestion of starch is an important factor in designing food products due to its association with blood glucose levels and insulin secretion. In addition, the matrix in which starch is present in the intestine governs the contact of starch with digestive enzymes, affecting digestibility (J. Parada & J. Aguilera, 2011). Singh et al. (2013a) provide detailed information on the starch digestion process of starchy foods.

Wheat is a commonly used ingredient for various food products in its milled format, i.e. flour. There are many factors such as protein content and particle size, that impact the functionality of wheat flour when used for food product applications. Therefore, it is important to understand

these factors to create flours and products with particular applications such as creating new products with slowly digestible properties.

This chapter provides a basic understanding of wheat grain microstructure, factors impacting wheat starch digestion, and the factors influencing the functional properties of wheat flour to prepare dough and wheat-based products. Furthermore, recent trends in designing slowly digestible wheat-based products have also been reviewed in this chapter.

2.2 Wheat grain microstructure

The average length, width, and thickness of wheat grain are 6-7 mm, 3-3.5 mm and 2.5-3 mm, respectively. Wheat grains are rounded on one side (dorsal side), and a crease is present on the other side (ventral side) (Figure 2.1). This crease occupies 0.7-1.9% of the grain volume. In addition, several hairs (brush) are present at one end of the grain, called trichomes. The average weight of the wheat grains is 30-35 mg. The grain colour varies from light buff or yellow to red-brown depending upon the seed coat's absence or presence of red pigmentation (Grundas, 2003; Pomeranz, 1982). Wheat is a single-seeded fruit called a caryopsis or kernel. It consists of caryopsis coats, endosperm, and embryo. In terms of millers, wheat kernel is divided into bran, endosperm (white flour), and germ.

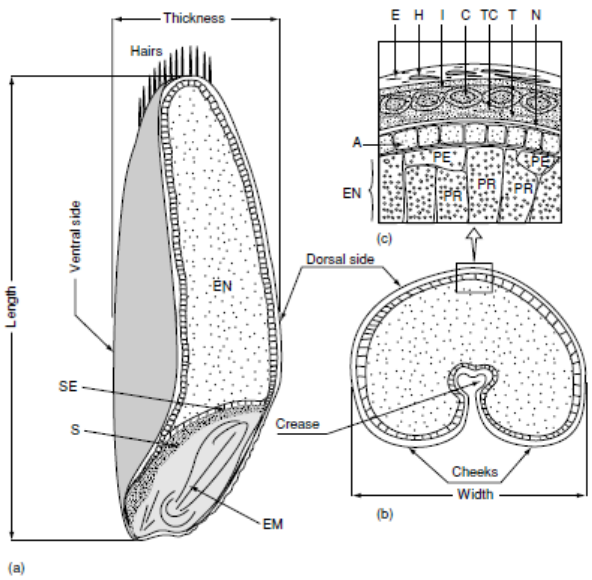

Figure 2. 1 *Structure of a wheat kernel (a) longitudinal section; (b) cross-section; (c) segment of the bran layer with aleurone and endosperm cells. (E-epidermis; H-hypodermis; I-inner pericarp; C-cross cells; TC- tube cells; T-testa; N-nucellar layer; A-aleurone layer; EN-endosperm; PE-peripheral cells of endosperm; PR-prismatic cells of endosperm; SE-scutellar epithelium; S-scutellum; EM-embryo). Reproduced with copyright permission from Grundas (2003).*

The caryopsis coats comprise the pericarp, seed coat, nucellar epidermis, and nuclear projection, which are reported to comprise about 5% of the kernel volume. When the seed is cut transversely, the following layers are observed: Epidermis, Hypodermis, cross cells, tube cells, seed coat, nucellar epidermis, aleurone layer, and endosperm. The seed colour is due to any pigmentation present in the seed coat. The endosperm contains aleurone cells and starchy endosperm. The aleurone cells are generally one cell thick and surround the starchy endosperm and part of the embryo. They contain protein bodies, lipid droplets and are rich in mineral matter. Although the aleurone layer is part of the endosperm, it is considered part of the bran by millers. The contamination of the aleurone layer in the flour is generally determined by the high ash content (Grundas, 2003; Pomeranz, 1982).

Starchy endosperm cells are classified according to shape, size, and site of occurrence as peripheral, prismatic, and central cells (Greer et al., 1951). The peripheral cells, also known as

sub-aleurone cells, are adjacent to aleurone cells. They are 60 μm in diameter and 20-60 μm radially, resemble in size, and have a thick cell wall. Next to peripheral cells, from peripheral to central cells, are prismatic cells which are 128-200 μm in length, and 40-60 μm in width, in the form of columns. In the centre of the cheeks are central cells, first to be formed during grain development, have irregular shape and size, 72-144 μm in length and 69-120 μm in width (Grundas, 2003).

Endosperm cells contain starch granules embedded in a protein matrix within the cell walls. Endosperm cell walls are composed of arabinoxylan (AX) (67%), β-glucan (27%), phenolic acids, cellulose, heteromannans, and proteins (Fincher & Stone, 1986). The protein content is lower near the endosperm cavity and increases radially until the outer region, which is nearly twice that of the endosperm cavity. On the other hand, the sub-aleurone cells are rich in protein and contain significantly less starch (Tosi et al., 2011). Albumin and globulin are mainly found in the grain's outer layers and are relatively low in the endosperm (Goesaert et al., 2005).

Starch and proteins are the major components of the wheat grain and form most of the endosperm (Figure 2.2). The microstructural properties of starch and proteins are discussed below.

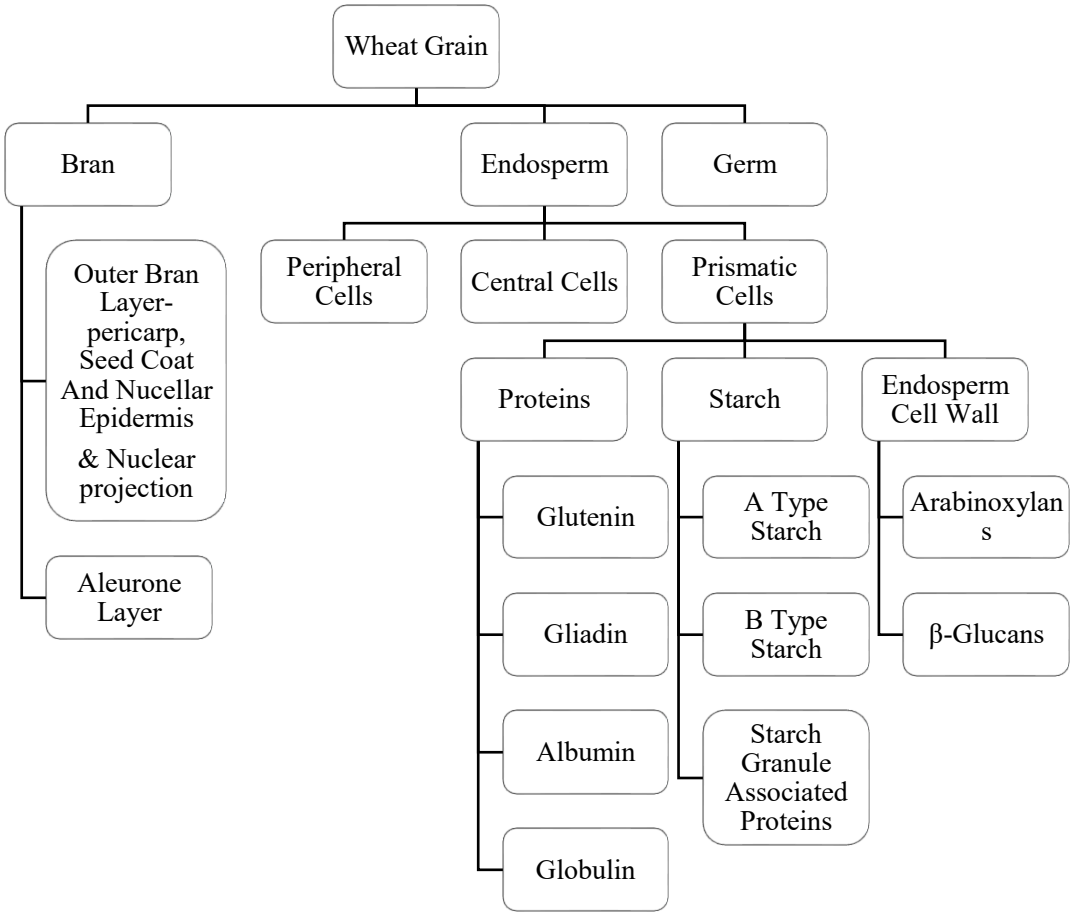

Figure 2. 2 *Overview of wheat grain structure*

2.2.1 The organisation of starch in wheat grain

Starch is synthesised and deposited in the grain in organelles called amyloplasts. Large type A granules (diameters greater than 10 µm) are initiated early in development, smaller type B granules (diameters between 5 and 10 µm) are initiated during mid-development (Figure 2.3), and much smaller type C granules (diameters less than 5 µm) are initiated late in development (Bechtel et al., 1990). The starch granules contain intrinsic proteins embedded in the starch matrix and proteins associated with the granule surface. The intrinsic proteins are mainly enzymes associated with starch synthesis. In addition, there is the presence of storage proteins on the surface of starch granules because of the breakdown of the organelle's structure during development (Rahman et al., 1995).

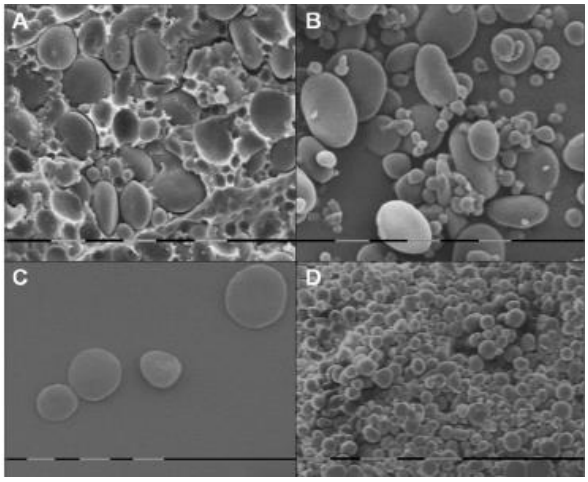

Figure 2. 3 *Scanning electron microscope (SEM) images (A) floury endosperm, (B) isolated A and B granules, (C) purified A-type, and (D) B-type starch granules. Scale bar: 10 µm. Reproduced with copyright permission from Bancel et al. (2010).*

The major components of starch are glucose polymers, amylose, and amylopectin (Figure 2.4). Amylose is considered a linear molecule, consisting of α-(1,4) linked D-glucopyranosyl units with a degree of polymerisation (DP) in the range of 500-6000 glucose residues. In addition, a fraction of the amylose molecules are slightly branched by α-(1,6) linkages (Hizukuri et al., 1981; Shibanuma et al., 1994). In contrast, amylopectin is a very large, highly branched polysaccharide composed of chains of α-(1,4) linked D-glucopyranosyl residues which are interlinked by α-(1,6) bonds, with a DP ranging from 3×10^5 to 3×10^6 glucose units (Zobel, 1988). The typical levels of amylose and amylopectin in starches are 25-28% and 72-75%, respectively (Colonna & Buleon, 1992).

The native starch granules are birefringent when viewed in polarised light, indicating a degree of order in the starch granules and an orientation of the macromolecules perpendicular to the surface of the granule (Buleon et al., 1998; French, 1984). Native starch has a degree of crystallinity in the range of 20-40% (Hizukuri et al., 1981). During milling, a significant fraction of the starch granules are damaged depending upon the type of milling. As a result, the

damaged starch loses its birefringence, has higher water absorption, and is more susceptible to enzymatic hydrolysis (Hoseney, 1994).

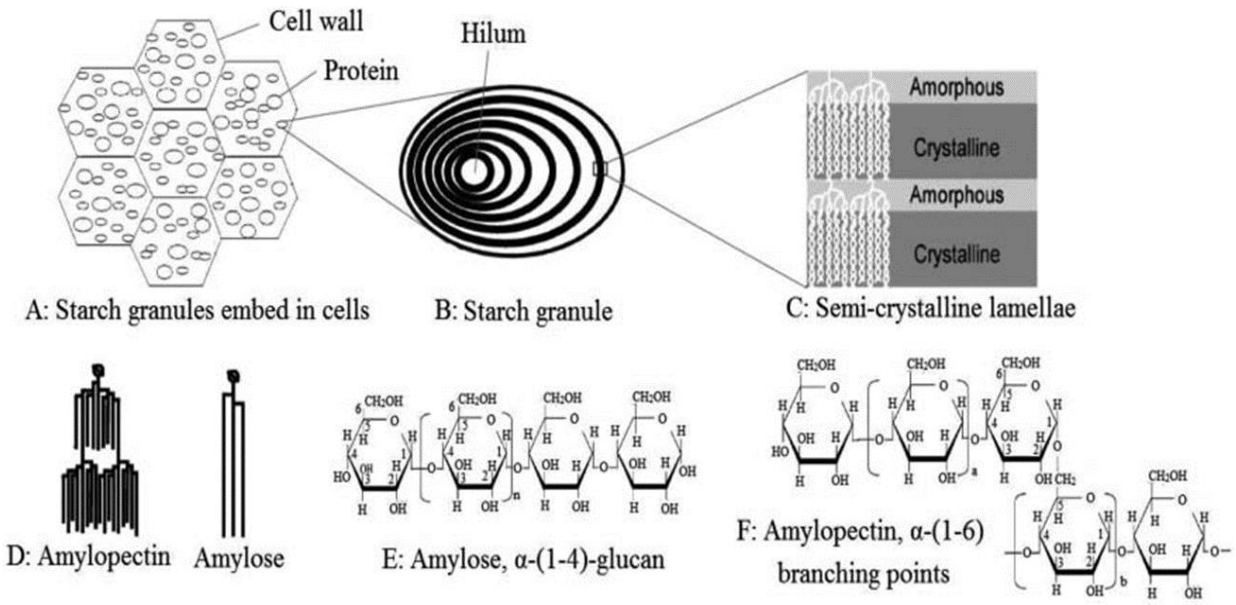

Figure 2. 4 *Architecture of starch. Reproduced with copyright permission from Tian et al. (2019).*

2.2.2 The organisation of protein in wheat grain

Protein is found to be deposited in vacuoles during development, and after development, water evaporates, starch granules enlarge and result in the protein matrix (Pomeranz, 1982). Therefore, mealy wheat cultivars have a fragmented protein matrix, while vitreous wheat has a continuous protein matrix (Stenvert & Kingswood, 1977). According to Osborne (1924), wheat proteins were classified into albumins (extractable in water), globulins (extractable in dilute salt), gliadins (extractable in aqueous alcohol), and glutenins (extractable in dilute acetic acid). However, a fraction of wheat proteins is unextractable in the aforementioned solvents. The wheat protein can be divided into two groups based on a functional point of view: non-gluten proteins and gluten proteins. The non-gluten proteins (15-20% of total wheat protein) mainly occur in the outer layers of the wheat kernel with lower concentrations in the

endosperm; most of these are albumin and globulin fractions (Goesaert et al., 2005). On the other hand, gluten proteins (80-85% of total wheat protein) are wheat's major storage proteins and belong to the prolamin class of seed storage proteins (Shewry & Halford, 2002).

Gluten proteins are classified as glutenins and gliadins. Glutenins are huge molecules with sizes ranging from 500000 to more than 10 million Da. Glutenins contain interchain disulfide bonds, and after the reduction of disulfide bonds, they are soluble in aqueous alcohol. Glutenins are further divided into high molecular weight subunits (HMW-GS) and low molecular weight subunits (LMW-GS) having a molecular weight of 67000-88000 Da and 320000-35000 Da, respectively (Wieser et al., 2006). On the other hand, gliadins are monomeric proteins with molecular weights around 28000-55000, and the disulfide bonds are either absent or present as intrachain crosslinks. Gliadins are classified into α, β, γ, and ω-type according to their different primary structures (Wieser, 2007; Wieser et al., 2006).

2.2.3 Proteins associated with starch granules

Starch granule-associated proteins and storage proteins remain adsorbed to starch granules (Figure 2.5) even after extraction (Li et al., 2016). Most of the starch surface proteins that Kasarda et al. (2008) found were storage proteins and proteins associated with protecting the grain from biotic and abiotic stresses. Storage proteins included both gluten (HMW and LMW glutenins, plus gliadins) and non-gluten proteins (albumins and globulins), while stress/ defence proteins included thaumatin, α-amylase, and α-amylase/subtilisin inhibitors, chitinase, pathogenesis-related protein, serpin, tritin, xylanase inhibitor, peroxidase, and peroxiredoxin. By different microscopic techniques, it has been seen that the starch granules are surrounded by protein. On the position immediately surrounding the starch granule, water-soluble proteins are present. The water-soluble material may act as a cementing substance between starch and the storage proteins (Barlow et al., 1973).

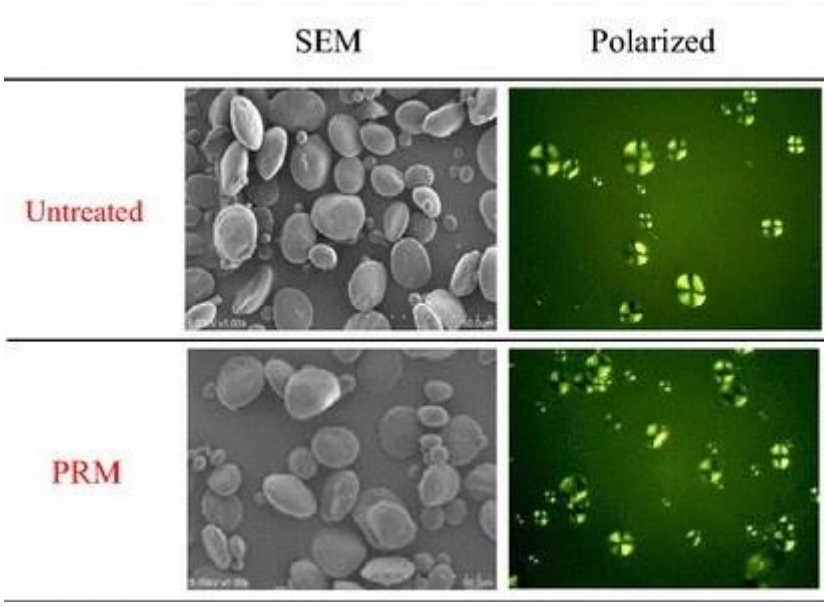

Figure 2. 5 *Wheat starch granules observed under SEM and polarised light before and after surface protein removal treatment (PRM). Reproduced with copyright permission from Li et al. (2016).*

2.3 Overview of starch digestion in wheat

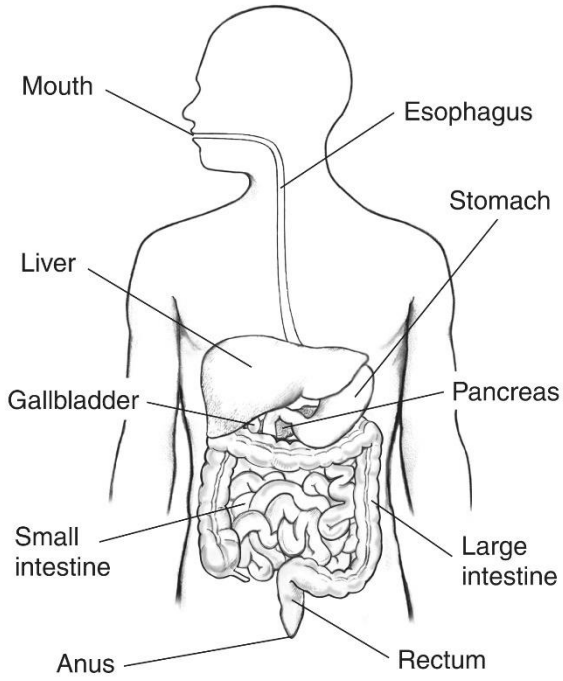

Figure 2. 6 *Human digestive system. Source: National Institute of Diabetes and Digestive and Kidney Diseases , National Institute of Health.*

Physiological digestion of starch and starch-based foods is a complex process where ingested food is broken down into smaller components involving a series of mechanical and enzymatic processes in the human digestive system (Figure 2.6). The digestion process initiates from the oral cavity, and continues through the stomach, small intestine and large intestine. In the mouth, mastication and salivation reduce the particle size of the foods and initiate the enzymatic digestion with salivary enzymes, forming the bolus, which is then swallowed and passed through oesophageal peristalsis to the stomach (Li et al., 2020). Salivary α-amylase (ptyalin) initiates the digestion of starch by breakdown of α-1,4-glycosidic linkages, but its activity is ceased in the stomach due to a change in the pH from neutral to acidic (Patricia & Dhamoon, 2019). In the stomach, the motor functions such as storage, mixing, grinding and emptying happen (Li et al., 2020). While gastric juices, salts and digestive enzymes such as pepsin and lipase are secreted, little to no enzymatic hydrolysis of starch occurs in the stomach (Patricia & Dhamoon, 2019). The majority of starch digestion happens in the small intestine by pancreatic enzymes (containing amylases) under near-neutral pH conditions, transforming starch into shorter oligosaccharides (Li et al., 2020). At the brush border of the small intestine, disaccharidases such as maltase and sucrase-isomaltase hydrolyse these oligosaccharides into glucose, which is then absorbed and transported into the bloodstream (Patricia & Dhamoon, 2019). The undigested starch from small intestine, also known as resistant starch, reaches the large intestine for colonic fermentation and is converted into short-chain fatty acids beneficial for gut health (C. Li et al., 2023).

The digestibility of starchy foods in terms of the rate and extent of starch digestion is correlated to various factors such as state of starch, food microstructure, processing conditions and presence of other macronutrients (Singh et al., 2013a; Tian et al., 2018). These factors collectively determine the glycemic response and physiological impact of starchy foods.

Studies have shown that the same raw material processed into different product forms (pasta or white bread) could also lead to significantly different blood glucose responses (Barkeling et al., 1995; Granfeldt et al., 1991). Processing may affect the interaction among the components of food, affecting the digestibility of the whole system (Parada & Santos, 2016). Different technologies used for studying the microstructure and digestibility of starchy foods are mentioned in Table 2.1.

Table 2. 1 *Technologies to study microstructure and digestibility of starch-rich foods*

Technology	Function/properties	Reference
Rapid Visco-Analyser (RVA)	Rheological characteristics	(Bordoloi et al., 2012)
X-ray micro-CT	3D structure	(Renshaw et al., 2016)
Scanning Electron Microscopy (SEM)	Surface properties	(Do et al., 2019)
Confocal Laser Scanning Microscopy (CLSM)	Spatial distribution	(Tamura et al., 2016a; Zou et al., 2015)
X-Ray Diffraction (XRD)	Crystal type and order	(Warren et al., 2016)
Differential Scanning Calorimetry (DSC)	Thermal properties	(Do et al., 2019)
Fourier transform infrared spectroscopy (FTIR)	Functional groups absorbance	(Warren et al., 2016)
Nuclear Magnetic Resonance (NMR)	Conformational features	(Warren et al., 2016)
Laser Confocal Micro-Raman	Molecular order characterisation	(Wang, Wang, Guo, et al., 2017)
***In vitro* and *in vivo* starch digestion**	Starch digestion	(Akila et al., 2019; Dartois et al., 2010; Srikaeo et al., 2005)

2.3.1 Particle size

The digestibility of wheat starch is greatly affected by the particle size of the flour. The effect of particle size on starch digestion has been studied extensively. Jenkins et al. (1988) found that bread prepared with coarse cracked whole wheat showed a significantly lower glycaemic response than milled wheat. Heaton et al. (1988) obtained similar results where the plasma insulin responses were in increasing order with the consumption of meals containing whole grains, cracked grains, coarse flour, and fine flour, respectively. Holt et al. (1995) also found that the particle size of the food affects the glycaemic-insulin response and satiety rating; as the particle size of food increased, the glycaemic-inulin response decreased, and satiety rating increased. However, Behall et al. (1999) reported that particle size exerts a more significant effect on glycaemic and insulin response when large food or grain particles are present, not the regular white and whole-wheat flours.

A recent study by Guo, Yu, Wang, et al. (2018) supports the effect of particle size on starch digestibility where raw and cooked milled wheat with three different particle sizes were investigated for *in vitro* starch digestibility. For both raw and cooked flours, the digestibility was highest in particle size <0.15mm, followed by 0.15-0.25 mm and 0.25-0.5 mm.

The difference in the degree of milling of wholegrain wheat flour has been reported to alter the glycaemic response of wholegrain wheat flour bread, where the postprandial glycemia in adults with type 2 diabetes was reduced by the bread containing more intact and coarsely ground grains compared with bread containing finely milled grains (Reynolds et al., 2020). Moreover, a study by Monro and Mishra (2022) has also shown that particle size significantly impacts the glycaemic response of wheat products. The *in vitro* glycaemic index of cooked finely-ground porridge was found to be 63.9, while it dropped to 44.1 for cooked kibbled porridge. Also, the bread containing intact+kibbled grains had a lower in vitro glycaemic index (49.5) compared to bread prepared with roller-milled flour (67.4) (Monro & Mishra, 2022).

2.3.2 State of starch

Cooking significantly increases the starch hydrolysis compared to the raw or native state (Guo, Yu, Wang, et al., 2018). A recent study by Monro and Mishra (2022) has demonstrated that raw porridge prepared with finely ground wheat had much lower glucose release compared to its cooked version. Similar results were also obtained for porridge prepared with kibbled grains, where cooking resulted in an increase in the in vitro glycaemic index from 12.6 to 44.1 (Monro & Mishra, 2022). When starch granules are exposed to heat in the presence of water, they undergo an irreversible swelling and destruction of crystalline structure, and this process is known as gelatinisation of starch. The degree of gelatinisation is known to affect starch digestibility (Chung et al., 2006; Holm et al., 1988). The rate of starch digestion *in vitro* and glucose and insulin response *in vivo* increased to different extents with the increase in the degree of gelatinisation (Holm et al., 1988).

However, studies by Wang, Wang, Liu, et al. (2017) and Guo, Yu, Copeland, et al. (2018) showed that the degree of gelatinisation does not affect wheat starch digestion. Wheat starch with a degree of gelatinisation from 0-100% was evaluated for *in vitro* starch hydrolysis, and it was found that the extent of starch hydrolysis did not change significantly after 6% degree of gelatinisation (Wang, Wang, Liu, et al., 2017). When wheat flour was cooked, the crystalline structure of starch was disrupted, and the disruption increased with increasing water content and gelatinised fully at a water content of 40%. The *in vitro* starch digestibility increased when the flour was cooked, but it did not change with increasing water content and cooking time. These results suggested that the loss of crystalline structure in gelatinised starch does not determine the rate of starch digestion (Guo, Yu, Copeland, et al., 2018).

Wang, Wang, Liu, et al. (2017) proposed a mechanism to explain that the residual structures do not determine the rate of starch hydrolysis. They suggested that the outer layer of the native starch containing glucan chains acts as a barrier to enzyme binding. Once this barrier is

disrupted, even at a low degree of gelatinisation, the access of enzymes to starch is increased significantly. A further increase in the degree of gelatinisation does not increase the binding of enzymes to starch. Once the enzymes bind to the substrate, subsequent catalysis is not rate-limiting (Wang, Wang, Liu, et al., 2017). Zhang et al. (2006) highlighted that the crystalline and amorphous structures are digested evenly by amylases.

2.3.3 Presence of proteins

Starch hydrolysis is affected by the presence of other components, such as proteins. In a study by Jenkins et al. (1987), the digestibility of white bread, gluten-free bread and gluten-free bread plus gluten were evaluated by *in vitro* and blood glucose studies. The results of *in vitro* studies indicated that the concentration of total starch digestion products was significantly lower for white bread than the other breads. Furthermore, in blood glucose studies, the peak rise in blood glucose for white bread was lower than that of the mean of the other two breads. Hence, it was suggested that natural starch-protein interaction in white bread might account for a decreased glycaemic response.

The presence of protein affected the starch hydrolysis in a starch-gluten mixture (raw form) in a study by Bhattarai et al. (2016). When a part of wheat starch (20%) was replaced with gluten, the starch hydrolysis decreased from 26% to 18%, indicating that protein hindrance affects the starch hydrolysis. When wheat flour (raw form) was subjected to amylase, protease, and lipase, the starch hydrolysis after 6 hours was 25%, whereas it was reduced to 16.5% in the presence of protease inhibitors (Bhattarai et al., 2016). Similarly, when gluten was removed from wheat flour by treatment with pepsin, the rate and extent of starch hydrolysis increased (Bhattarai et al., 2016). Hence, the presence of protein helps decrease the starch hydrolysis, but external addition of gluten to starch does not reduce the starch hydrolysis as much as the presence of gluten in intact flour.

2.3.4 Presence of starch surface proteins and lipids

Bhattarai et al. (2016) observed that when the surface proteins of wheat starch (raw form) are effectively removed by pepsin treatment and then subjected to hydrolysis by α-amylase, the reducing sugar release increased by almost 10% as compared to starch without pepsin treatment. Also, when gluten was added to starch granules with and without pepsin treatment, starch hydrolysis was higher in gluten added to starch with pepsin treatment. It shows that the presence of surface-associated proteins of starch hinders the enzyme binding to starch.

Also, the presence of lipids in raw wheat flour affects starch hydrolysis. When wheat flour was subjected to amylase, protease, and lipase, the starch hydrolysis after 6 h was 25%, whereas it was affected in the presence of a lipase inhibitor. The starch hydrolysis was reduced to 20% in the presence of a lipase inhibitor (Bhattarai et al., 2016).

Another recent study by Hou et al. (2024) has also revealed similar findings that the removal of endogenous components of wheat flour, such as protein and lipid, leads to increased rates of starch hydrolysis in the cooked wheat flour.

2.3.5 Presence of bran components

Arabinoxylan (AX) is a major fibre component present in wheat bran (Henry, 1985). In a study by Lu et al. (2004), the postprandial glucose and insulin responses significantly improved when bread and muffins with 16% AX fibre were consumed. In a similar study by Garcia et al. (2007), bread supplemented with 15 g of AX along with the consumption of 5 g AX powder resulted in lower postprandial responses in serum glucose and insulin.

In another study by Hardacre et al. (2015), fibres derived from wheat were mixed with potato and corn starch, and their *in vitro* digestibility was checked after cooking. They observed that the presence of these fibres at physiological concentrations slows down the rate of starch digestion.

It has been suggested that when AX behaves as soluble dietary fibre, it increases the viscosity of the food in the stomach, delays gastric emptying, and reduces the digestibility of food (Garcia et al., 2007; Hardacre et al., 2015; Lu et al., 2004; Lu et al., 2000).

Wheat secures a significant position in cereal consumption; however, most of the products from wheat possess faster digestion properties. Numerous studies captured in this literature review have demonstrated that factors such as the particle size of flour, state of starch, presence of proteins and lipids, and presence of bran components could change the starch digestibility of wheat-based products. Modification of whole wheat grains through treatments such as hydrothermal treatment and retrogradation could be useful to modify the microstructure and, in turn, would be helpful to create a broad range of products with improved starch digestibility. Studies on the role of the individual components of the grain microstructure would be beneficial to further understand and create products with controlled starch digestibility.

2.4 Functionality of wheat flour and dough

2.4.1 Overview of wheat flour and dough

Wheat flour is a product of the milling of wheat grains (Figure 2.7). To facilitate the separation of bran and endosperm, conditioning of wheat grains is done where the moisture of grains is maintained between 15-17%. To obtain white flour commercially, the conditioned grains are passed through fluted rollers to break open the grain and scrape the endosperm from the bran. The endosperm particles are reduced to flour using smooth rollers (Grundas, 2003).

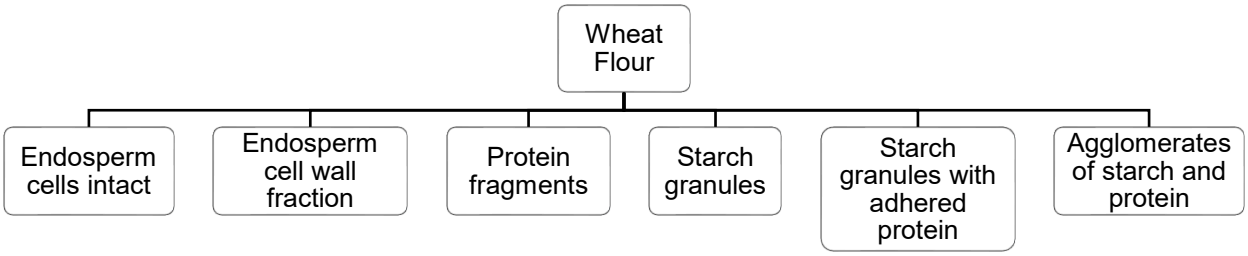

Figure 2. 7 *Overview of wheat flour components*

During milling, the endosperm is reduced to small-sized particles, leading to fragmentation of the protein matrix, cell wall, and starch granules (Figure 2.8). Wheat flour preserves some characteristics of the intact endosperm tissue, but the original, compact structure of the endosperm is broken into aggregates of the protein matrix that is embedded in groups of cellular components, mainly starch granules (Gangadharappa et al., 2008). Generally, wheat flour is composed of starch (70-75%), protein (8-16%), moisture (12-14%), fibre (2-3%), lipids (2%) and minerals (1%) (Scheuer et al., 2011).

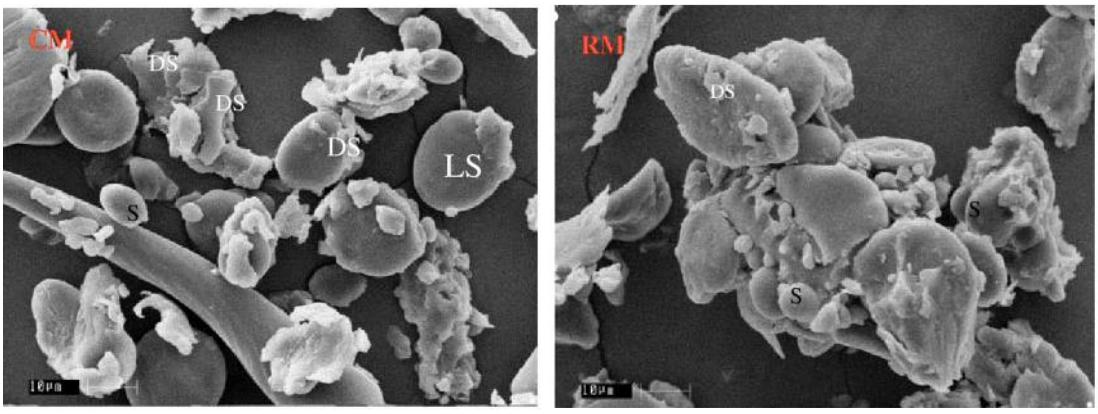

Figure 2. 8 *SEM images of wheat flour from different mills: Chakki mill (CM) and Roller mill (RM). LS-large starch; S-starch; DS-damaged starch. Reproduced with copyright permission from Inamdar et al. (2015).*

The protein content of different mill streams increases as the milling progresses through the break streams, due to an increasing concentration of the peripheral endosperm portion which is rich in protein (Prabhasankar et al., 2000). It has been reported that the protein content and ash content increase with the increase in break and reduction passages. The reduction streams have a higher content of protein and ash in general (Pomeranz et al., 1988).

In a study by Zhou et al. (2018), the grains were pearled to observe the spatial gradients of protein and starch within the grain. Albumin and globulin were found to decrease from the surface layer to the centre of the grain. Glutenin and gliadin decreased from the outer to inner layers, whereas starch content rapidly increased towards the centre with a slight decrease in the core.

The functionality of flour is affected by factors such as particle size, damaged starch content, and protein content, and with the knowledge of these, speciality flours with specific applications can be created. The typical functional properties of flour are water-holding capacity, solubility, emulsion capacity, swelling volume, and pasting properties (Belorio et al., 2019; Suresh, 2013).

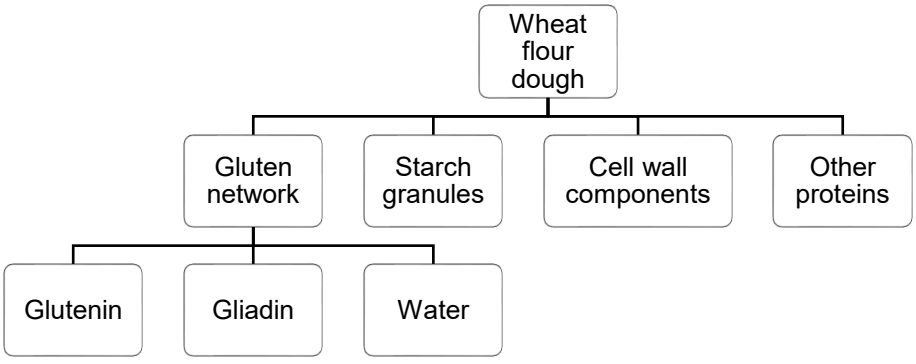

Figure 2. 9 *Overview of components of wheat flour dough*

When water is added to wheat flour and applied with mechanical energy, gluten forms a network through intra and inter-disulfide bonds between glutenin and gliadin, and the dough is

developed (Figure 2.9). A continuous cohesive visco-elastic gluten protein network (Figure 2.10) is formed by the gluten proteins, and for the aggregation of glutenins and gliadins, non-covalent bonds such as ionic bonds, hydrogen bonds, and hydrophobic bonds are important (Wieser, 2007).

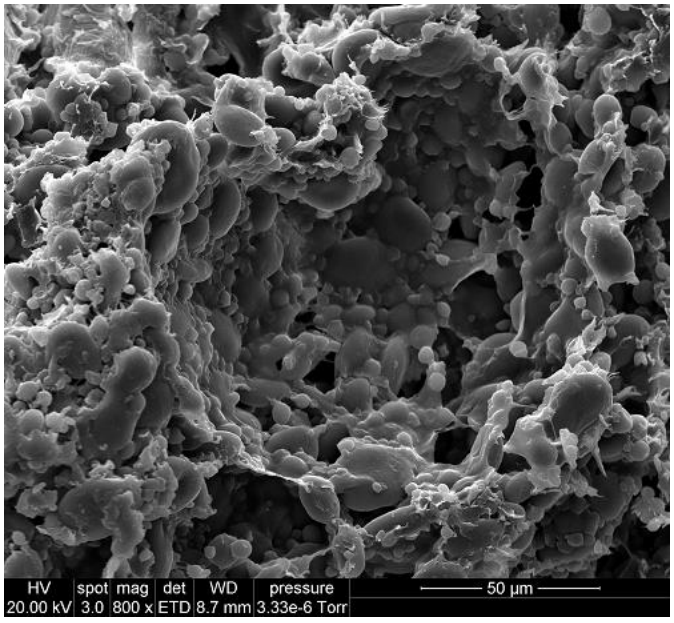

Figure 2. 10 *Scanning electron micrograph of gluten network in wheat flour dough*

The development of the gluten network during dough mixing can be monitored by recording dough mixers such as the Farinograph and the Mixograph (Walker, 1996). To study the function of dough ingredients and properties of dough, rheological testing, especially in the linear viscoelastic region, has been used (Janssen et al., 1996; Miller & Hoseney, 1999). The viscoelastic characteristics of dough are expressed in storage and loss moduli, G' and G'', and loss tangent tan δ. It is generally found that tan δ values are lower for doughs made from good-quality flour than doughs made from poor-quality flour (Safari-Ardi & Phan-Thien, 1998).

2.4.2 Role of protein and starch from wheat in dough functionality

In broader terms, the protein content of flour shows an inverse relationship with storage and loss moduli up to 14% protein, as shown in a study by Khatkar (2005). Furthermore, gluten contributes to the viscoelastic properties of dough to varying degrees depending on glutenins, gliadin/glutenin ratio, HMW-GS, and LMW-GS of the protein source (Edwards et al., 2003; Edwards et al., 2001). It is widely accepted that gliadins contribute to viscous properties, while glutenins impart strength and elasticity to the dough (Shewry et al., 1986). The viscoelasticity is influenced by HMW-GS and subunit composition by modifying the size distribution and protein aggregation through cross-linking. Increasing the gliadin/glutenin ratio causes a decrease in elasticity (Popineau et al., 1994). Khatkar et al. (2002) found that the addition of α-, β-, γ- and ω-gliadins containing cysteine residues to gluten causes an unexpected increase in G' and G''.

The contribution of starch to dough functional properties has received much less attention as compared to the role of gluten. Since starch is a major part of the dough, it can significantly affect the dough's properties. Starch has been suggested to act as an inert filler in the continuous protein matrix of the dough (Bloksma, 1990), while Eliasson and Larsson (1993) described dough as a bi-continuous network of starch and protein. Another study reported that the rheological behaviour of wheat dough is influenced by the specific properties of the starch granule surface (Larsson & Eliasson, 1997).

Studies indicate that G′ increases rapidly with increasing the protein content in starch-gluten blend with constant water content. Increasing the starch content enhances the elasticity of dough when starch granules are homogeneously dispersed in the gluten network (Edwards et al., 2002; Watanabe et al., 2002).

When starches from different wheat cultivars were mixed with constant gluten content to make a dough, it led to large rheological differences, indicating an active role of starch in starch-

gluten interactions (Petrofsky & Hoseney, 1995). A high proportion of smaller starch granules were found to increase the elastic character of dough by Edwards et al. (2002).

To confirm the importance of interface interactions between matrix and filler in dough functional properties, the starch granules were replaced by glass beads of similar particle size and it was observed that the surface interaction plays an important role in determining the viscoelastic properties of dough (Edwards et al., 2002). In a recent study by Zhang et al. (2018), doughs prepared with vital gluten and starches from different botanical sources were evaluated. It was observed that wheat starch (WS)-gluten dough had the highest water absorption value, followed by tapioca starch, corn starch, sweet potato starch, and potato starch-gluten dough. A correlation of swelling power was found in a way that the higher swelling power of starch needs lower water absorption to produce uniform starch-gluten dough. The greater the disulfide bond content, the more stable is dough and network structure (Zhang et al., 2018). WS-gluten dough formed the strongest and stable dough among the different starch-gluten blends(Zhang et al., 2018), indicating that there is a unique interaction between the starch and gluten in the dough, and more extensive study is needed to understand it.

In a study by Wang et al. (2014), it has been demonstrated that starch surface characteristics determine the rate of reaction with water, chemical reagents, and enzymes. Also, it has been reported that surface proteins and lipid removal can alter the physiochemical and structural properties of starch (Chan et al., 2010; Debet & Gidley, 2006; Wang et al., 2014). A study by Li et al. (2016) also indicated that the removal of surface proteins from wheat starches alters their relative crystallinity, swelling power, and thermal and pasting characteristics, affecting their functionality overall; however, their role in dough development was not studied.

2.4.3 Role of water-soluble components of wheat in dough functionality

In a fractionation and reconstitution study of flour, water-soluble components have been found to play a role in the modification of gluten extensibility (Hoseney et al., 1969). In a similar

study by Miller and Hoseney (1999), it was found that the flour that was reconstituted without water-soluble components had a higher optimum water absorption and a much longer optimum mixing time than the original unfractionated flour. The higher water absorption presumably results from the need to compensate for the reducing effect of the water-soluble components. Also, adding water-soluble components shortened the mixing time, decreased the elastic modulus, and made the dough relatively viscous (Miller & Hoseney, 1999). Moreover, water-extractable pentosans have been reported to interfere with gluten formation and affect the extensibility of gluten (Wang et al., 2002).

2.4.4 Role of bran components from wheat in dough functionality

A comparative study was conducted by Schmiele et al. (2012) on the effect of blending whole wheat flour or wheat bran into white wheat flour. It was observed that the increasing bran content in the blend caused a linear increase in water absorption. The increase in the amount of dietary fibre, mainly AX, caused increased water absorption. Due to the decreased starch content, the gelling ability of the blends decreased. Moreover, the bran addition resulted in lower viscosity values in the blend compared to the addition of whole wheat flour at the same level. These results showed that the presence of fibres that do not swell (cellulose, lignin, and hemicellulose), impaired the gelling ability (Schmiele et al., 2012). In another study by Bucsella et al. (2016), the rheological properties of the flour enriched with aleurone were significantly different as compared to the bread wheat flour, with increased dough development time, dough stability and mixing tolerance.

Different components in bran fractions, such as AX, β-glucans, arabinogalactans, galactomannans, phytates, and lipids can interact with the gluten network. The AX can bind covalently to the gluten proteins via the esterified phenolic components (Bucsella et al., 2016). The non-swelling cellulose fibres delay the formation of the gluten network. These structural effects contribute to the decrease of elasticity and stability of the dough and the reduction of

the gelling ability, thereby altering the dough structure (Bucsella et al., 2016; Noort et al., 2010).

Furthermore, the particle size of the bran added to flour has a significant impact on rheological behaviour along with end-product performance. The addition of fine bran fraction provides a larger surface for interaction, resulting in shorter dough stability and smaller bread loaves compared to the addition of coarse bran (Noort et al., 2010). However, Curti et al. (2013) have reported no effect of bran particle size on water activity and loaf volume of bread.

Isolated water-extractable arabinoxylans (WEAX) addition in white wheat flour up to 1.3% increased the water absorption and dough development time via the high water-binding capacity of the polysaccharides (Biliaderis et al., 1995). Furthermore, the presence of WEAX up to 0.9% improved bread volumes; however, a higher dosage resulted in decreased volumes compared to the control white flour bread (Biliaderis et al., 1995).

Overall, the wheat grain components play an important role in the functionality of flour and therefore need more attention to modify the functionality of speciality products.

2.5 Recent trends in designing slowly digestible wheat-based foods

The glycaemic index (GI) is the ranking of carbohydrates in food in terms of their relative effect on postprandial blood glucose levels. A few examples of low, medium, and high GI foods are shown in Table 2.2. Low GI foods discharge glucose slowly in the blood, while high GI foods release glucose rapidly in the blood.

The GI of foods is affected by susceptibility, digestive enzyme action interfering substances, the microstructure of foods, and molecular arrangements in starch (J. Parada & J. M. Aguilera, 2011). GI values for a wide range of food products are available in the literature (Atkinson et al., 2021; Foster-Powell et al., 2002b). To underpin the knowledge of GI, extensive research has been carried out through *in vitro, in vivo,* and human clinical studies to understand the

glucose release during the digestion of starch-rich foods (Azeredo et al., 2021). This section is aimed at covering the three recent approaches in designing slowly digestible wheat-based food products.

Table 2. 2 *Examples of foods based on GI (Foster-Powell et al., 2002b)*

GI classification	Examples
Low GI (<55)	Most whole intact grains- wheat, millet, oat, rye, barley, lentil, soy, chickpea, most vegetables
Medium GI (55-70)	Non-intact whole wheat or enriched wheat, pita bread, basmati rice, raisins, prunes, banana
High GI (>70)	White bread, noodles, most white rice, corn flakes, extruded breakfast cereals, glucose, maltodextrins, potato

2.5.1 Use of whole grains

The global burden of metabolic disorders, including diabetes, has heightened interest in the GI of foods (Augustin et al., 2015). Whole grains, rich in dietary fibre, bioactive compounds, and complex carbohydrates, have emerged as pivotal ingredients in developing low-GI foods. Whole grains, including Wheat, Rye, Barley, Oats, and Brown rice, have gained prominence in nutritional science due to their potential to lower GI values in food products (Tosh & Bordenave, 2020). The presence of bran, germ, and endosperm in whole grains contributes to the complex structural integrity of the carbohydrate profile and provides dietary fibre, resistant starch, and bioactive compounds.

Whole grains influence glucose and insulin responses due to their slow digestibility behaviour. Foods with a low GI cause smaller increases in blood sugar and insulin levels. Numerous studies have linked higher cereal fibre intake to a reduced risk of diabetes mellitus. For

example, greater consumption of whole grains has been associated with lower fasting insulin levels (Pereira et al., 1998), and fibre from whole-grain cereals has shown an inverse relationship with type 2 diabetes mellitus. Further, the presence of magnesium, dietary fibre, and vitamin E in whole grain foods improves insulin sensitivity independent of body weight change in overweight hyperinsulinemic individuals (Pereira et al., 2002). Some strategies to incorporate whole grains into the diet are mentioned in Table 2.3.

Research comparing the effects of refined wheat and rye breads on glucose and insulin responses demonstrates the benefits of rye-based breads in reducing postprandial insulin levels. In a study conducted on 20 non-diabetic, postmenopausal women, Juntunen et al. (2003) revealed that the compact matrix of Rye bread significantly reduced the insulin, glucose-dependent insulinotropic polypeptide and C-peptide levels than the wheat bread due to the differences in starch structure (Juntunen et al., 2003).

Table 2. 3 *Some strategies to incorporate whole grains into the diet*

Food products	**Strategies to incorporate whole grains**	**Reference**
Breakfast Cereals	Utilising whole grain cereals. Fortification with additional fibre sources.	(Rawat et al., 2023)
Bakery Products	Substituting refined flour with whole grain flour in cereals lowers the GI without compromising palatability. Fortification with additional fibre sources can further enhance the effect.	(Borczak et al., 2018)
Snacks and Convenience Foods	Whole grain bars, crackers, and snacks offer a low-GI alternative to traditional refined options, promoting sustained energy release.	(Vardhan et al., 2024)

Sagnelli et al. (2018) explored the low GI foods from two varieties of barley grains, wild barley Hordeum vulgare Subsp. *spontaneum* (Hs) and Amylose-only barley grain (AO). The *in vitro* digestion study of grains and bread from Hs and AO showed low predicted GI. The GI of Hs barley was attributed to the viscosity effects of its high β-glucan content, while the AO barley's low pGI is due to the slow digestibility of its >99% amylose starch (Sagnelli et al., 2018).

2.5.2 Incorporation of Resistant starches

Resistant starch has gained a lot of attention for its beneficial effect on digestive function, including resident gut microbiota, control of glycaemic index, and control of fasting plasma triglyceride and blood cholesterol levels (DeMartino & Cockburn, 2020). Briefly, starch can be classified into three categories based on the time taken for digestion: Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS is measured as starch, which can be digested into subsequent glucose units within 20 minutes of enzymatic digestion, while SDS can be digested after a further 100 minutes of enzymatic digestion (Berry, 1986). Resistant starch is the fraction of starch that is not digested by 120 minutes of exposure to *in vitro* digestion conditions (Englyst et al., 1992). RS are resistant to intestinal pancreatic enzymes, and they reach the colon and are fermented by colonic microflora (Niba, 2002).

Resistant starches can be prepared using various methods such as heat treatment, enzyme treatment, combined heat and enzyme treatment, and chemical treatment (Sajilata et al., 2006) and can be further divided into five types, as mentioned in Table 2.4.

The overall digestibility of RS depends on the source and category of RS. About 89% of RS2 from raw potato and 83% of RS3 from corn are degraded by bacterial fermentation in the colon. The degradation of RS is affected by various food processing conditions, and the digestibility varies per individual due to individual differences regarding enzymatic responses (Sharma et al., 2008). Various food processing techniques, including cooking and grinding, and changes

in food processing conditions like pH, temperature, duration, heating and cooling cycles, and freezing and drying can influence the RS content in food products (Zaman & Sarbini, 2016).

Table 2. 4 *Classification of resistant starches (Sajilata et al., 2006; Fuentes-Zaragoza, et al., 2011)*

Type of RS	Description	Food source
RS1	Physically inaccessible to digestion	Whole or partly milled grains, legumes
RS2	Ungelatinized resistant starch granules with type B crystallinity, slowly digested by α-amylase	Raw potatoes, green bananas, high amylose corn
RS3	Retrograded starch	Cooked and cooled-potatoes, bread, cornflakes, and foods with a repeated heat moisture treatment
RS4	Chemically modified starches by cross-linking with chemical agents	Application products of modified starches
RS5	Amylo-lipid complexes	Products of high amylose starches-lipid complexes

To obtain the physiological benefits of RS, it should be 10-20% of daily carbohydrate intake; however, most conventional foods have less than 5% RS per serving, and the conventional processing of foods decreases the RS in foods (Alsaffar, 2011; Foster-Powell et al., 2002a). Stephen et al. (2017) reported that the replacement of digestible starch with resistant starch lowers the postprandial blood glucose levels, while this only occurs when the total resistant starch content in foods has at least 14% of the starch. It has been reported that intake of 6-12 grams of RS type II in a meal had lowered the postprandial glucose and insulin levels (Homayouni et al., 2014). These RS can be used to develop a wide range of commercial food products, and their impact on postprandial blood glucose is given in Table 2.5.

Xian et.al., 2006 developed a low glycaemic index starch by treating maize with partial α-amylase treatment. Such enzymatic modification of starch was known to be responsible for the slowly digestible and resistant properties of the starch. The enzyme treatment significantly reduced the molecular size of native amylopectin and amylose to a narrower molecular weight range, which reduced the postprandial glycaemic response in rats (Han et al., 2006).

Moreover, two forms of resistant starches, cross-linked (Fsym) and pregelatinized cross-linked (FRite) RS from wheat were evaluated by Alviola et al. (2010) their effect on dough and tortilla quality. Dough made with 15% FRite was significantly harder and less extensible, and the tortillas made were less puffed and denser than the control, whereas dough and tortillas made with 15% Fsym were comparable to the control. Dietary fibre increased from 2.8% to 14.3% and 13.6% in Fsym and Frite tortillas, respectively. It was suggested that the incorporation of cross-linked resistant starch from wheat up to 15% increases the dietary fibre of tortillas without negatively affecting the dough handling and end-product quality (Alviola et al., 2010). Emilien et al. (2017) replaced the standard wheat flour with resistant wheat starch up to 40% (equal weight basis) in muffins to determine its effect on plasma insulin. The results indicated that plasma insulin was decreased; however, it did not affect plasma glucose. In another study by Sankhon et al. (2013), wheat flour was replaced by Parika (African locust bean) flour by up to 40% and the digestibility of the bread was investigated. The replacement with the Parika flour increased the crude protein content, ash, crude fibre, and RS in the bread. They suggested that the replacement of wheat flour with Parika flour up to 15% in bread was comparable to control bread making its potential application in bread.

Overall, RS incorporation in food products enhanced the RS content of the product and reduced starch hydrolysis. However, the structure-function relationship of RS in the food system needs to be explored to improve the quality of their application products.

Table 2. 5 *Application of resistant starch in wheat-based products*

Resistant starch source	Application	Effect	Reference
Barley flour (Whole and Pearled)	Bread with 5 to 25% whole and pearled barley flour	Lower postprandial blood glucose of both products; Product acceptability up to 10% whole barley flour bread and 15% pearled barley flour bread	Urooj et al., (1998)
High amylose corn starch	Bread with high amylose (30-70%) corn starch	Plasma glucose and insulin reduced significantly above 50% amylose corn starch	Behall et al., (2002)
Chickpea flour	Pasta with 25% chickpea flour	GI reduced significantly	Goñi, et al., (2003).
Chempedak seed flour (*Artocarpus integer*)	Bread with 30% chempedak seed flour	Significant reduction in the *in vitro* starch hydrolysis; increase in resistant starch content (2 folds)	Zabidi et al., (2009)
Unripe banana flour	Cookies with 15-50% unripe banana flour	Resistant starch content increased and predicted GI decreased with increased unripe banana flour	Agama-Acevedo et al., (2012)

2.5.3 Inclusion of Hydrocolloids

Hydrocolloids, also known as food gums, are generally used as either thickening or gelling agents and improve the stability and texture of foods. Hydrocolloids are widely used in the bakery industry to make gluten-free recipes due to their ability to mimic the rheological properties of gluten (Culetu et al., 2021). Moreover, several hydrocolloids reduce starch digestibility and provide health benefits due to their high water solubility and inhibitory effects against digestive enzymes (Hyun-Jung et al., 2007).

Montemurro et al. (2021) formulated hydrocolloids (psyllium, flaxseed, and chia flours) as structuring agents, along with rice, maize, quinoa, and chestnut flour. The novel gluten-free bread was characterised by high protein content (8.9% of dry matter), more than 75% *in vitro* protein digestibility, low sugar (1.0 % of dry matter) and *in vitro* predicted GI (85±3.1) (Montemurro et al., 2021).

Cereal flours generally contain 0.5-4% non-starch polysaccharides (NSP) contributed by the cell wall material. These NSPs are considered dietary fibres and mainly consist of cell wall-associated AX, β-glucans, and arabinogalactan. The majority of NSPs in wheat flour are insoluble AX (Englyst et al., 1989). Soluble fibres are typically known to have the ability to reduce glycemia, lower plasma cholesterol, and other health benefits (Lewis & Heaton, 1999). Water-soluble β-glucans studied in diabetic patients have been shown to improve blood glucose regulation (Fardet, 2010; Topping, 2007). It has been suggested that the health benefits of β-glucans are due to their solubility in water and their ability to form viscous solutions (Wolever et al., 2010). In a study by Kasprzak et al. (2012), the starch digestibility of breads supplemented with isolated wheat AX and β-glucan from oats was studied in pigs. The starch digestibility in AX bread and white bread was similar, whereas it was reduced significantly in β-glucan supplemented breads. The lower starch digestion was suggested to be due to the β-glucan property of enhancing the viscosity of the solution and reducing the accessibility of

digestive enzymes by limiting the water availability for starch hydration. Zeng et al. (2024) discovered that the gelatinisation and retrogradation of starch in a whole-wheat bread can be restricted by adding yeast β-glucan and proteins, resulting in a lower GI.

Manzoor et al. (2020) have summarised various research studies exploring natural polysaccharides from plants, seaweeds, and mushrooms as an alternative to anti-diabetic drugs. These polysaccharides help manage Type 2 diabetes by promoting glycogen synthesis, inhibiting glucose production, delaying glucose absorption, and improving insulin sensitivity. Examples like oat β-glucan and galactomannan show promise in controlling blood glucose levels and supporting gut health (Manzoor et al., 2020).

Furthermore, starch digestibility has been reported to decrease in cooked white rice made with arabic gum (0.3%) and locust bean gum (0.3%) (Hyun-Jung et al., 2007). Also, guar gum has been reported to reduce the digestibility of potato starch (Gularte & Rosell, 2011) and cooked potatoes (Bordoloi et al., 2012). However, xanthan gum significantly increased the starch hydrolysis in corn and potato starches (Gularte & Rosell, 2011).

Crude malva nut gum application in bread with a dosage of 0-10% resulted in reduced GI of the breads (Phimolsiripol et al., 2017). In a recent study by Liu et al. (2018), the effect of different hydrocolloids (hydroxypropylmethylcellulose, carboxymethylcellulose, xanthan gum, and apple pectin) addition in gluten-free potato flour-based steamed bread was evaluated. The addition of hydrocolloids decreased the RDS content of the bread and reduced starch hydrolysis. In another study, extracted malva nut gum was found to inhibit amylase activity in a solid matrix and reduce the starch hydrolysis of the bread (Srichamroen, 2014). Konjac glucomannan suppresses the retrogradation of amylose due to its high hydrophilicity. This phenomenon restricts the water molecules to rearrange the amylose, thereby keeping the amylose content high (Wang et al., 2022).

Limited research has been carried out for food hydrocolloids of bacterial origin to modulate glucose response. Alshammari et al. (2021) have suggested a positive impact on diabetes and metabolic disorders by incorporating bacterial-origin food hydrocolloids such as xanthan gum, pullulan, and dextran by managing postprandial blood glucose levels. Another study by Osilesi et al. (1985) found that consuming muffins with 12 g of xanthan gum for six weeks significantly reduced fasting and post-load glucose levels in both healthy and borderline Type 2 diabetic subjects. In the diabetic group, fasting glucose decreased by 38% and post-load glucose by 31% compared to the control (Osilesi et al., 1985).

Overall, the impact of hydrocolloids on starch hydrolysis varies depending on the starch origin and food type and needs more research in wheat-based foods to reduce starch hydrolysis and improve product quality.

2.6 Conclusion and research gap

Factors such as particle size, state of starch, protein content, surface protein, lipids, and bran components of wheat grain impact starch digestibility. The microstructure of wheat in the form of grain and flour has been studied extensively; however, limited reports were available to understand the structural changes in the grain during gastrointestinal starch digestion. Therefore, it is important to enhance the understanding of the role of the microstructure of wheat grain in starch digestibility.

Previous studies revealed that the starch-protein interactions play a significant role in the functional properties of wheat flour and dough, and the absence of lipids does not alter the rheological properties of dough (Edwards et al., 2003; Khatkar, 2005; Wang et al., 2014). Starch was considered an inert filler material, but the importance of starch in dough development was proved by Edwards et al. (2002). Studies have shown that starches from different sources do not interact with gluten in the same manner as the interactions in the dough from natural wheat

flour. This leads us to consider the role of starch-associated surface proteins in starch-gluten interactions.

Moreover, the fractionation and reconstitution of wheat starch and gluten to form a dough do not mimic the starch-gluten interaction of a wheat flour dough. The interaction of starch and gluten network was governed by water-soluble components of wheat flour; however, their role has not been studied much. Filling this knowledge gap will help to further explore the functionality of starch-gluten networks.

Extensive research has been going on to reduce the starch digestibility of wheat-based products, including the use of whole grains, flours from millets, legumes, resistant starches, and hydrocolloids. However, the area of research involving the improvement of the functionality of slowly digestible foods needs more attention. An improved understanding of the role of microstructure and functionality of wheat flour components would open more opportunities for slowly digestible wheat-based foods with better functionality.

Chapter 3

How does the structural integrity of wheat grains impact the in vitro starch hydrolysis?

Chapter 3 Impact of Microstructural Changes Induced by Mechanical Processing and Thermal Treatment on *In Vitro* Gastro-Small Intestinal Starch Digestion of Wheat Grains

Abstract

It is important to investigate the changes in the microstructure of wheat grains during processing to understand their influence on physico-chemical properties and starch hydrolysis (SH) kinetics during gastro-small intestinal digestion *in vitro*. Three differently processed formats (kibbled, cut grains, and flour) along with intact wheat grains of a bread wheat variety were compared. Size reduction from raw intact grains to kibbled grains and flour resulted in an increase in overall SH (%) during simulated digestion and followed the order of flour>kibbled>cut>intact grains. The cooked versions of grain formats followed a similar order with a significant increase in SH observed for cooked kibbled grains and flour to 58.38±0.086% and 92.85±1.21%, respectively, whereas cooked cut grains show a lower hydrolysis of 21.56±0.39%. The expected glycaemic indices (eGI) of both cooked cut and intact grains are categorised as low glycaemic with values of 54.08±0.03 and 41.98±0.04, respectively. Scanning electron micrographs revealed the disappearance of starch granules from the grains' exposed surface areas during sequential digestion phases. The size reduction processes resulted in the disruption of bran and intact endosperm cells, making grain structure susceptible to digestion after cooking. The current findings highlight the potential of utilising differently processed grain formats to develop wheat products with low glycaemic index.

3.1 Introduction

Wheat grains consist of three main parts: peripheral layers (bran), germ, and endosperm. The endosperm (80-85% of the grain) is mainly composed of starch granules surrounded by a

protein matrix present within endospermic cell walls (Rosa-Sibakov et al., 2015). Wheat is a significant ingredient for a variety of foods, and most of the wheat-based products are high-glycaemic in nature (Kumar & Prabhasankar, 2014). However, consumption of high glycaemic foods has been related to chronic diseases such as diabetes, coronary heart disease, obesity, and metabolic syndrome due to a rapid increase in blood glucose levels and secretion of insulin(d'Angelo et al., 2019; Vega-López et al., 2018; Zhang et al., 2020).

The digestibility of starch has been correlated to the microstructure of starchy foods in various studies (Singh et al., 2013b; Tian et al., 2019). When cereals are processed and digested in the gastro-intestinal tract, the natural self-assembled structures are disassembled to different levels such as organ, tissue, cell, cell wall, polymer, and molecule; and their interactions can control the digestibility of starch (Do et al., 2019). The particle size of refined durum wheat flour has a significant impact on *in vitro* starch hydrolysis and postprandial blood glucose levels (Edwards et al., 2015; Mandalari et al., 2018) due to the barrier imposed by endosperm cell walls (Konstantinos Korompokis et al., 2019). These studies involved mainly refined wheat flour (coarse/fine); however, the consumption of whole grains is widely encouraged as part of the national dietary recommendations in many countries (Musa-Veloso et al., 2018).

Whole grains can be intact, ground, cracked, kibbled, or flaked kernels, with bran, starchy endosperm, and germ present in the same relative proportion as they exist in the intact kernel (Lin et al., 2020). Kibbled wheat is gaining popularity worldwide as an ingredient for baked products and as a replacement for rice due to the health benefits of whole grain. The starch digestibility of whole wheat has been reported to be affected by differences in processing techniques, such as popping, steam cooking, and flaking of the wheat kernel (Holm et al., 1985). Bread prepared with fine whole wheat flour resulted in a higher release of glucose when compared with coarse and medium flour (Lin et al., 2020). However, to the best of our

knowledge, the changes in the natural microstructure of whole-bread wheat grains due to processing and how they impact starch digestion behaviour have not been studied in detail. This study evaluated the microstructural characteristics of whole wheat grains processed into kibbled, cut, and flour formats to illustrate the consequences of different size reduction technologies in both cooked and uncooked states. The objective of this study was to highlight the impact of wheat grain microstructure (kibbled, cut, and flour) on starch hydrolysis during *in vitro* gastro-small intestinal digestion. Since chewing during oral digestion could alter the microstructure of the samples, oral digestion, and chewing were avoided in order to retain the microstructure of the grains and to understand its influence during gastro-small intestinal digestion.

3.2 Materials and methods

3.2.1 Materials

New Zealand-grown bread wheat (*Triticum aestivum* var. Reliance) and its whole grain flour were procured from a local farm (Milmore Downs Ltd, New Zealand). The enzymes used for *in vitro* starch digestion, pepsin (porcine gastric mucosa, 800-2500 units/mg protein), pancreatin (hog pancreas, 4×USP), and invertase (Invertase, grade VII from baker's yeast, 401 U/mg solid), were all from Sigma-Aldrich Ltd. (St Louis) and amyloglucosidase (3260 U/ml) from Megazyme International Ireland Ltd. (Wicklow, Ireland). All other chemicals used in the study were of analytical grade.

3.2.2 Sample preparation

Intact grain and flour were used as raw materials. Intact grains were cut into two parts longitudinally along the crease of the grains using a razor blade. Kibbled grains were prepared by milling the intact grains into smaller fractions (similar to commercially available kibbled wheat) using a lab-scale stone mill (Mockmill 100, Wolfgang Mock) in the coarse setting. Any

powdered particles were removed by passing through a 0.500 mm sieve to retain kibbled grains (recovery ≥ 97%).

The intact grains were soaked in Reverse Osmosis (RO) water at room temperature (25±5 °C) as hydration or soaking has been reported to facilitate the cooking (Berg et al., 2012). When the grains were soaked for a smaller period (<15 h), further cooking at 121 °C for 20 min did not cook the grains from the core (the uncooked white centre was observed visually). To ensure full hydration of the grains during subsequent cooking, the intact grains were soaked in water for 24 hours, after which the moisture content was close to 50%. The cooking method from Berg et al. (2012) was adopted with slight modifications. The soaked grains were transferred to a Schott bottle, ten times- their mass of RO water was added, and they were cooked in an autoclave at 121 °C for 20 min and rapidly cooled to 100 °C. The bottles were then further cooled under running tap water to room temperature (25±5 °C). Kibbled grains and flour were cooked similarly, but they did not require pre-soaking to achieve full hydration during cooking. The cooked intact grains were cut into two haves using a razor blade to obtain gelatinised cut grains.

3.2.3 Physico-chemical characteristics of whole wheat

Thousand kernel weight was measured using an electronic balance, and the dimensions of 20 randomly selected intact grains were measured using a Vernier Caliper (Ramya et al., 2010). The kibbled grains were passed through standard sieves to measure the particle size. Flour particle size was measured using a mastersizer (Malvern Mastersizer, Malvern Instruments Limited, UK) in the dry dispersion module (Ma et al., 2020). The moisture content of intact, cut, and kibbled grains and flour was determined by drying in an oven at 104 °C for 3 hours (AOAC 935.29). The protein content was determined by the Kjeldahl method (AOAC 2001.11) using Kjeltec system with 2006 Digestor and 2100 Distilling Unit (Foss Tecator Inc, Höganäs, Sweden). A nitrogen-to-protein conversion factor of 5.83 was used for wheat (Maclean et al.,

2003). The total starch content of the samples was determined using a total starch assay kit (K-TSTA 07/11, Megazyme International, Wicklow, Ireland).

3.2.4 Microstructural characteristics of raw and gelatinised wheat

The microstructure of raw and gelatinised grains and flour was examined by scanning electron microscopy (SEM). The gelatinised intact and kibbled grains and flour were frozen immediately after cooking by submerging them in liquid nitrogen. Gelatinised cut grains were prepared by cutting the cooked intact grains and then frozen similarly. Frozen samples were then freeze-dried. The samples were mounted on the stub with double sticky tape and then sputter-coated with gold (Baltec SCD 050, Balzers, Liechtenstein). The intact dry grains were sectioned using a razor blade before mounting on the stub. The gold-coated samples were observed under SEM (FEI Quanta 200, FEI Electron Optics, Eindhoven, Netherlands) at an accelerating voltage of 20 kV.

3.2.5 *In vitro* starch hydrolysis

The *in vitro* gastro-small intestinal starch hydrolysis was conducted for wheat in eight different forms: raw intact, cut, and kibbled grains, raw flour, gelatinised intact, cut and kibbled grains, and gelatinised flour.

A two-stage gastro-small intestinal *in vitro* digestion model similar to Berg et al. (2012) was used in this study. 170 g of mixture with a starch concentration of 4% was prepared with raw or cooked sample and RO water. This mixture was then transferred to jacketed glass reactors (37±1 °C) and continuously stirred by a magnetic stirrer at 300 rpm. The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to Pharmacopeia (2000). For the gastric phase of digestion, the reactor content's pH was reduced to 2 by the addition of 0.5M and 3M HCl, and then SGF (25 ml, pepsin/starch ratio, 1.765:100, w/w) was added. The pH of the reactor content was maintained at 1.2 during the simulated gastric digestion phase.

After 30 min, the simulated gastric digestion phase was terminated by increasing the pH of reactor content to 6.8 by adding 0.5M and 3 M NaOH. The small intestinal digestion phase was then initiated by adding SIF (22 ml, pancreatin/starch ratio, 1.3:100, w/w, amyloglucosidase/starch ratio, 0.26:1, v/w, and invertase/starch ratio, 1:1,000, w/w) to the reactor content. The pH of the reactor content was maintained at 6.8 throughout 120 min of the simulated small intestinal digestion phase. Aliquots of 0.5 ml were withdrawn from the reactor after 0 (G0), 15 (G15), and 30 min (G30) of simulated gastric digestion phase; and after 0 (I0),5 (I5), 10 (I10), 15 (I15), 30 (I30), 60 (I60), 90 (I90) and 120 min (I120) of simulated small intestinal digestion phase. The glucose concentration of these samples was measured using a d-glucose assay kit (GOPOD Format K-GLUK 07/11, Megazyme International Ireland Ltd., Wicklow, Ireland). Results were expressed as the percentage of starch hydrolysis, according to Tamura et al. (2016b).

The estimated glycaemic index (eGI) was calculated as per equation 3.1 (Goñi et al., 1997):

$$eGI = 39.71 + 0.549HI \tag{3.1}$$

Where HI is the hydrolysis index (HI), and it was calculated by measuring the area under the curve during small intestinal digestion using white bread as the reference ($\frac{Area\ under\ the\ curve\ of\ sample}{Area\ under\ the\ curve\ of\ reference} \times 100$) (Chen et al., 2018).

3.2.6 Microstructure and particle size evaluation of digests

For microstructure evaluation, the grains and flour digests were collected into Eppendorf tubes at G0, G30, and I120. The tubes were then immediately submerged in liquid nitrogen, and the frozen samples were freeze-dried. The freeze-dried samples were mounted on a stub with double sticky tape and sputter-coated with gold in a sputter coater (Baltec SCD 050, Balzers, Liechtenstein). SEM (FEI Quanta 200, FEI Electron Optics, Eindhoven, Netherlands) at an accelerating voltage of 20 kV was used to evaluate the samples.

The flour digests were also collected at G30 and I120 to evaluate particle size distribution changes at the end of the gastric and small intestinal digestion phases. The particle size distribution was determined with a laser diffraction particle size analyser (Malvern Mastersizer, Malvern Instruments Limited, UK) immediately after sample collection.

3.2.7 Statistical analysis

Results were expressed as mean±standard deviation for triplicate observations unless specified otherwise. Furthermore, analysis of variance (ANOVA) and Tukey's test were used to determine the significance of differences ($p<0.05$) using Minitab Statistical software version 13 (Minitab Inc., State College, PA).

3.3 Results and discussion

3.3.1 Physicochemical characteristics of wheat grain

The thousand kernel weight of the grains was found to be 32.07±0.22 g. The intact grains had length, width, and thickness of 5.59±0.19 mm, 2.96±0.34 mm, 2.56±0.24 mm, respectively (average of 20 grains). The kibbled grains were able to pass through a standard 3.35 mm sieve (more than 90% kibbled grains) and retained over a 0.5 mm sieve. The particle size of flour was found to be 133.09±0.92 µm (average of 3 samples). The total starch content and protein content were similar for different raw grain forms, such as intact, cut, and kibbled grain, and flour (Table 3.1).

Table 3. 1 *Composition of the wheat grain forms*

Sample	Moisture content (%) (Mean±SD)	Total starch (%dwb) (Mean±SD)	Protein content (%dwb) (Mean±SD)
Intact/ cut grain	11.25±0.01[b]	72.75±0.13[a]	11.53±0.02[a]
Kibbled grain	11.26±0.02[b]	72.63±0.21[a]	11.54±0.01[a]
Flour	11.74±0.02[a]	72.97±0.20[a]	11.57±0.01[a]

Different superscripts in the same column indicate significant differences (n=3, p<0.05).

3.3.2 Microstructural characteristics of raw and gelatinised wheat

When the native intact grains were observed under the SEM, the endosperm cells were present under the bran layer, organised radially from the outer layers towards the centre. Within the endosperm cells, small spherical and large lenticular starch granules were observed to be compactly packed (Figure 3.1-a). The starch granules were surrounded by a protein matrix and enclosed within the endosperm cell walls. The observed structure of the grain resembled the wheat microstructure seen by Heneen and Brismar (1987). The cut grain structure was similar to intact grain; kibbled grain also resembled intact grain microstructure except for the loss of many starch granules due to the action of the stone mill. The microstructure of flour differed from the intact grain, being more amorphous. Milling disrupted the intact grain, and the flour had components of wheat grain in a fragmented form (Figure 3.1-g). The starch granules were evident individually and in the form of clusters (as present in endosperm cells), along with the endosperm cell wall fractions, fragmented protein matrix, and bran.

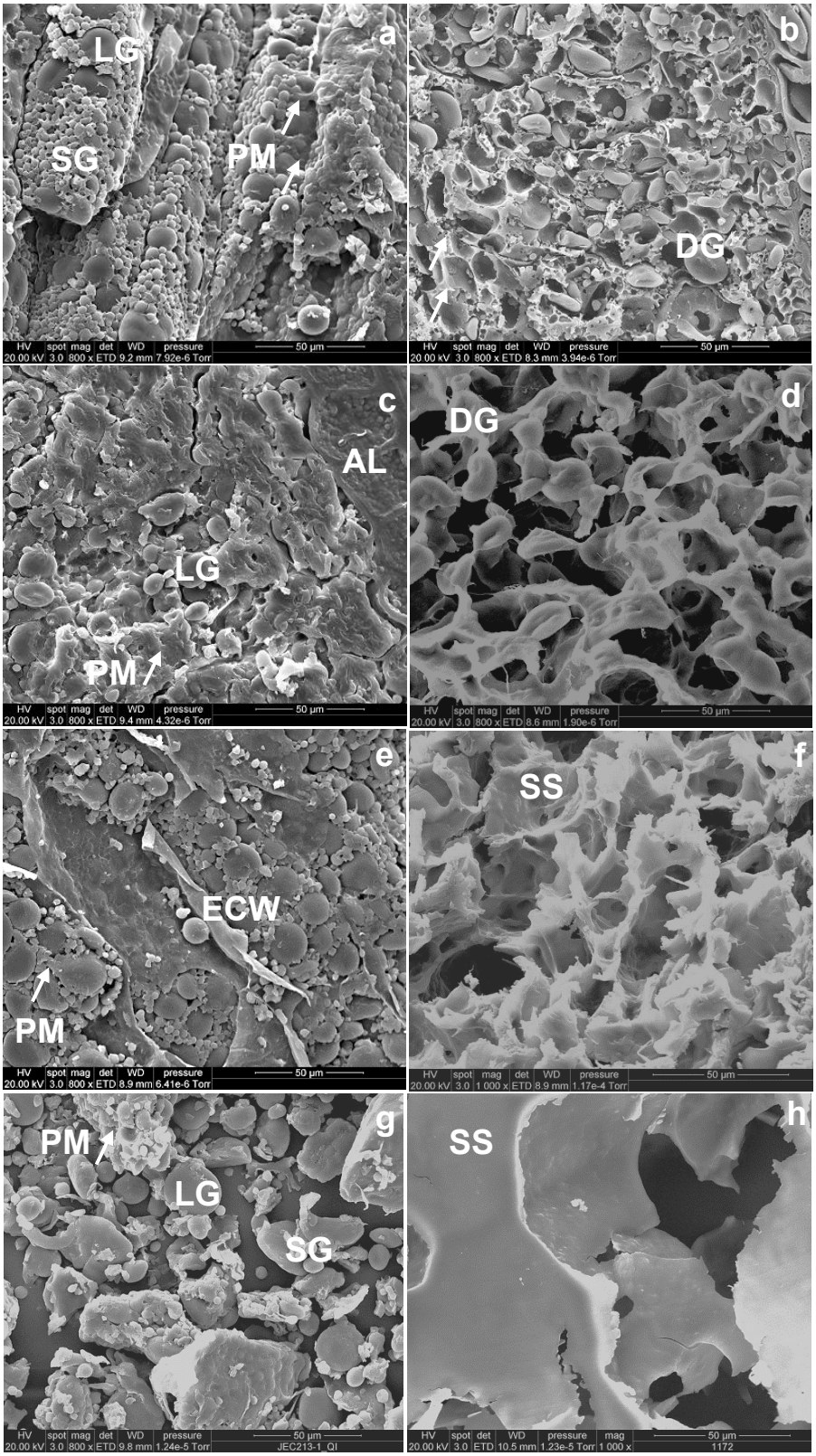

Figure 3. 1 *SEM micrograph of raw a) intact grain, b) cut grain, c) kibbled grain, d) flour; and gelatinised e) intact grain, f) cut grain, g) kibbled grain, h) flour. (Scale: 50µm). SG-small starch granule, LG-large starch granule, AL-aleurone layer, ECW-endosperm cell wall, PM-protein matrix, DG-distorted starch granule, SS-solubilised starch*

When the intact grains were soaked and cooked at 121 °C for 20 min, the microstructure of the grain altered (Figure 3.1-b). Unlike native grain, starch granules were distorted in the gelatinised grain due to amylose leaching, as gelatinization causes starch granules to imbibe water, swell and distort and subsequently release soluble starch (mainly amylose leaching from the granules) (Goesaert et al., 2005). However, the starch granules did not solubilise completely in the gelatinised intact grains due to limited swelling and leaching of amylose, which could be due to space constraints imposed by the surrounding bran layer since grains did not rupture during cooking. Similar results were observed for gelatinised cut grains, where distorted starch granules were evident. Srikaeo et al. (2006) reported that cooking wheat grains at 120 °C for 20 min did not disrupt starch granule structure; however, when the grains were cooked for prolonged periods, the granule structures disintegrated and disappeared. In this study, similar results were observed when kibbled grains and flour were cooked at 121 °C for 20 min. The starch granules disappeared and were solubilised in exposed areas of gelatinised kibbled grains and flour as there was little or no restriction by the bran layer which had been ruptured by milling.

3.3.3 Effect of wheat grain processing (size reduction) on starch hydrolysis

The size of the whole grains was progressively reduced from intact to cut to kibbled grains and flour. Cut grains were half the width of intact grains; kibbling caused a reduction in length, width, and thickness; and the flour had the smallest size among all the four wheat forms. When these grains were compared for the extent of starch hydrolysis at the end of simulated gastro-small intestinal digestion (I120), it was found to be in the order of flour>kibbled grains>cut grains>intact grains in both the raw and gelatinised state (Figure 3.2).

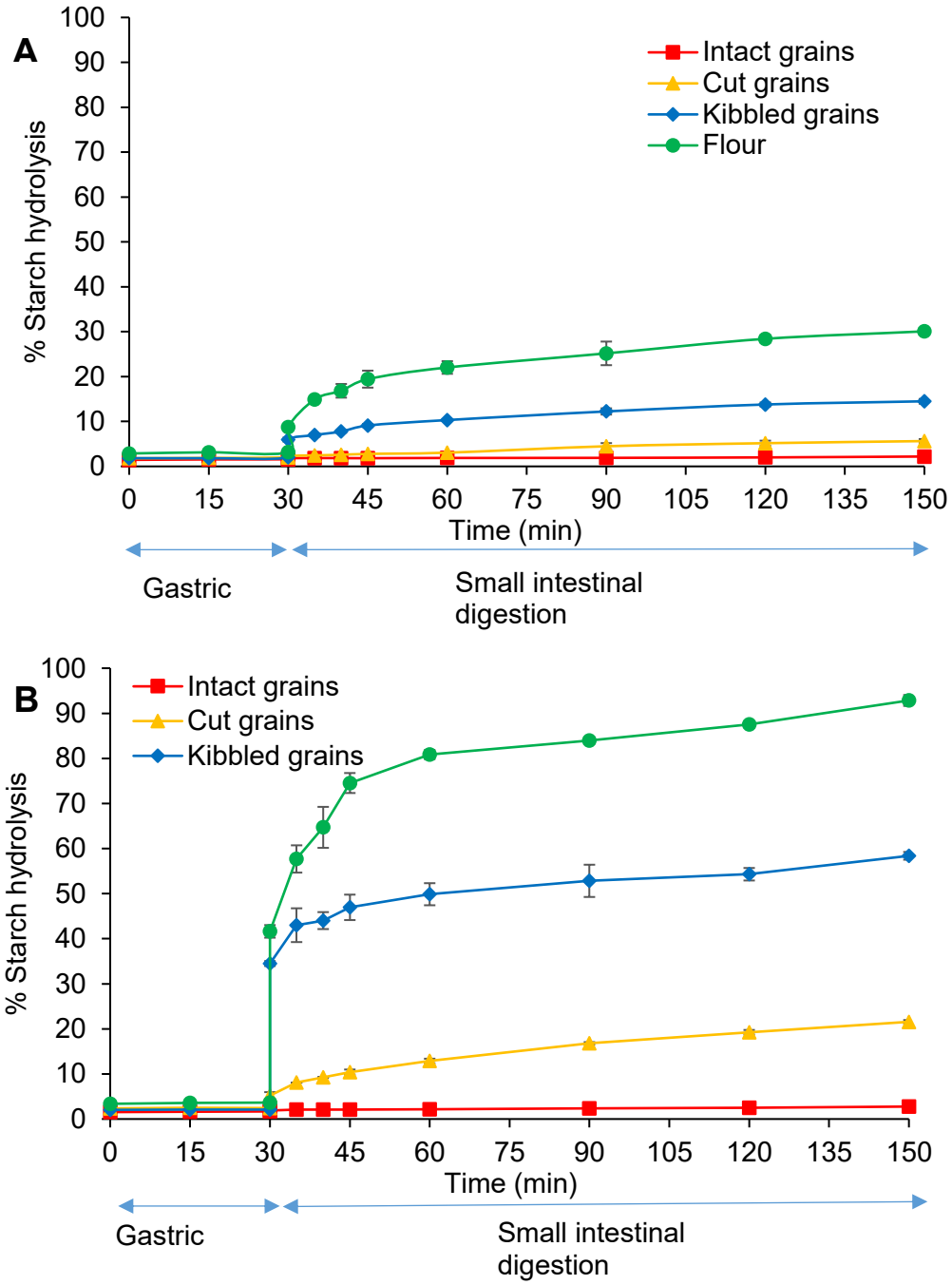

Figure 3. 2 *Effect of size reduction of the grain on starch hydrolysis during in vitro gastro-small intestinal digestion in A) raw and B) gelatinised state. Error bars represent standard deviation (n=3).*

In the raw state, all the components of the grain were enclosed within the bran layer for the intact grains, whereas the cut grains exposed the endosperm contents from one side of the grain. Opening up the endosperm by cutting the grains into two halves enhanced the starch hydrolysis

to 5.63±0.45% from 2.19±0.06% for intact grains at the end of 120 min of small intestinal digestion. Kibbling of wheat grains increased the starch hydrolysis to 14.5±0.53%, whereas milling into flour increased it to 30±0.73%. Similarly, for the gelatinised forms, the starch hydrolysis increased to 21.56±0.39%, 58.38±0.86%, and 92.85±1.21% for cut and kibbled grains and flour, respectively; however, cooked intact grains had only 2.76±0.32% starch hydrolysis. A similar pattern of starch hydrolysis was also observed for the grains of biscuit wheat variety (*Triticum aestivum* var. Ignite) in the form of whole, kibbled, and flour forms (Figure A 3.1 in Appendix).

The raw grain structures except flour were found to have a low eGI according to the classification of Foster-Powell et al. (2002a). Reducing the native intact grain into flour increased the eGI from 41.61±0.05 to 62.63±1.59, causing it to fall in the medium GI category (Table 3.2). In the gelatinised state, intact and cut grains were low glycaemic, whereas eGI increased to 90.11±2.56 and 119.15±0.79 for kibbled grains and flour, respectively, making them high glycaemic.

The kibbled grains had a greater exposed surface area (without the bran encapsulation) than the cut grains, whereas flour revealed the endosperm content completely. Although, the protein matrix and the intact endosperm cell wall have been reported to reduce starch hydrolysis (Jenkins et al., 1987; Konstantinos Korompokis et al., 2019), the size reduction process exposed the endosperm content and fragmented the intact endosperm cell wall and the protein matrix. Since all the grain forms evaluated in this study were ‘whole’ in terms of composition, the bran present in either intact or fragmented form could have impeded the rate of starch hydrolysis if the cellulose present in the bran had inhibited the activity of α-amylase (Dhital et al., 2015).

Table 3. 2 *Total starch hydrolysis (SH), Hydrolysis index (HI) and estimated glycaemic index (eGI) of wheat grain in different forms*

Sample	% SH (Mean±SD)	HI (Mean±SD)	eGI (Mean±SD)	Glycaemic category
Raw intact grains	2.19±0.06^{g}	3.47±0.09^{f}	41.61±0.05^{f}	Low
Raw cut grains	5.63±0.45^{f}	7.08±0.61^{f}	43.6±0.34^{f}	Low
Raw kibbled grains	14.5±0.53^{e}	20.06±0.66^{e}	50.72±0.36^{e}	Low
Raw flour	30.08±0.73^{c}	41.75±2.90^{c}	62.63±1.59^{c}	Medium
Cooked intact grains	2.76±0.32^{g}	4.14±0.08^{f}	41.98±0.04^{f}	Low
Cooked-cut grains	21.56±0.39^{d}	26.17±0.05^{b}	54.08±0.03^{d}	Low
Kibbled-cooked grains	58.38±0.86^{b}	91.79±4.66^{d}	90.11±2.56^{b}	High
Cooked flour	92.85±1.21^{a}	144.70±1.44^{a}	119.15±0.79^{a}	High

Different superscripts in the same column indicate significant differences (n=3, p<0.05).

Similarly, Mandalari et al. (2018) reported for durum wheat that reducing the particle size of wheat endosperm in porridge enhanced *in vitro* starch digestibility. Edwards et al. (2015) have reported that consumption of porridge meal containing coarse particles of durum wheat endosperm caused 33% lower postprandial blood glucose than fine particles. The results of the current study demonstrate that only minimal processing such as cutting and kibbling would lead to the low glycaemic impact of whole wheat grains; further size reduction would upsurge their glycaemic impact.

3.3.4 Effect of cooking and microstructural changes on *in vitro* starch hydrolysis

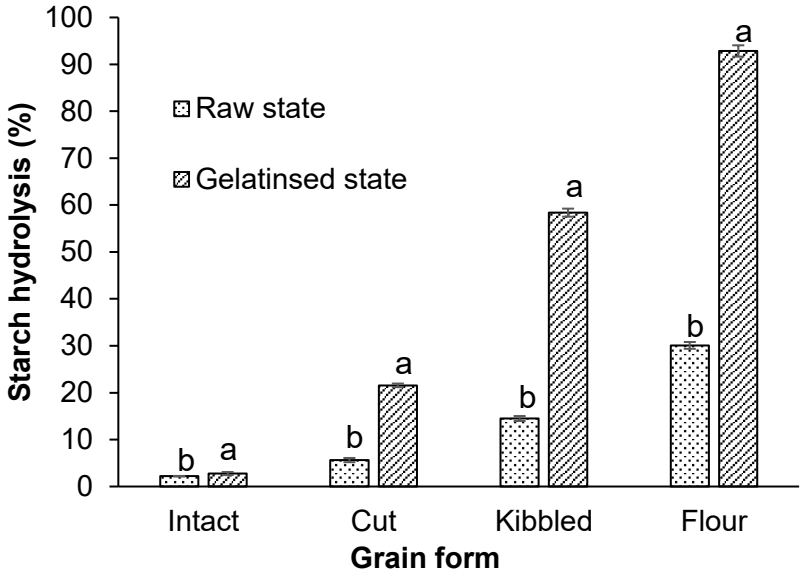

Figure 3. 3 *In vitro gastro-small intestinal starch hydrolysis of raw and gelatinised grain forms by the end of 120 min of small intestinal digestion. Different letters over bars for the same grain form indicate significant differences (n=3, $p < 0.05$).*

A comparison of *in vitro* gastro-small intestinal digestion of raw and gelatinised grains in different forms revealed that cooking increased the extent of starch hydrolysis at 120 min intestinal digestion (I120). The degree of starch hydrolysis was significantly higher for gelatinised cut grains, kibbled grains, and flour than their raw counterparts (Figure 3.4). Native starch granules are digested much slower than gelatinised starch by amylolytic enzymes (Blazek & Copeland, 2010). It was reported that only 10-15% of the native wheat starch was hydrolysed when incubated for 60 min in the presence of α-amylase, whereas gelatinisation enhanced the hydrolysis by 3 folds (Blazek & Copeland, 2010). However, in the present study, the starch hydrolysis of intact grains in the raw and gelatinised state was not significantly different.

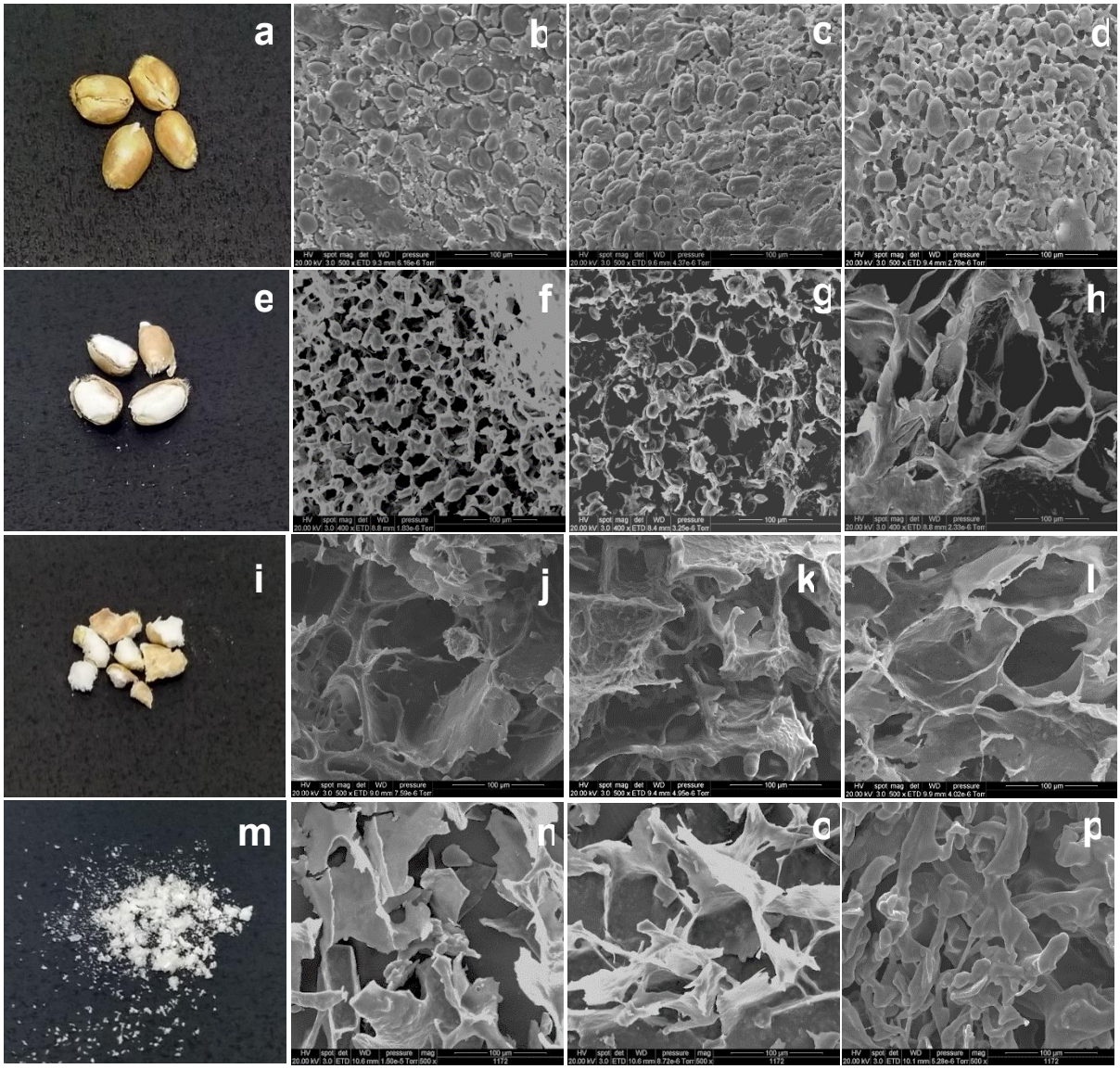

Figure 3. 4 *Freeze-dried grain cooked grain samples (a-intact, e-cut, i-kibbled, and m-flour) and SEM micrographs of their digests collected after G0, G30, and I120 digestion phases: intact grain sectioned after freeze-drying (b-d), cut grain (f-h), kibbled grain (j-l) and flour (n-p), respectively. (scale:100 µm). G0-before gastric digestion, G30-after 30 min of gastric digestion, and I120-after 120 min of small intestinal digestion phase.*

To further understand the effects of grain processing on starch digestion, the microstructure of the digests of gelatinised grain forms was observed under SEM. The intact grain microstructure was found to be quite similar throughout the digestion (Figure 3.4 b-d). Here, the bran layer

could have acted as a barrier to the digestive enzymes and possibly limited amylose leaching into the digestion liquid. In contrast, the structure of cut and kibbled grains was more porous in sequential digestion phases with the absence of any starch-protein matrix in the outer layers (exposed surface of endosperm). In gelatinised cut grains, the bran layer was adherent to the grains' dorsal side, but at the cut surface, the gelatinized starch granules were exposed. The micrographs of gelatinised cut grains during the digestion phases (Figure 3.4 f-h) showed that the starch granules and the matrix around them disappeared by the end of I120, leaving behind the endosperm cell walls and resulting in increased hydrolysis.

The gelatinised kibbled grains' micrographs during different digestion phases (Figure 3.4 j-l) showed that the gelatinized starch-protein matrix was absent from the exposed surface by the end of the small intestinal digestion phase which could have leached in the digestive solution and hydrolysed subsequently.

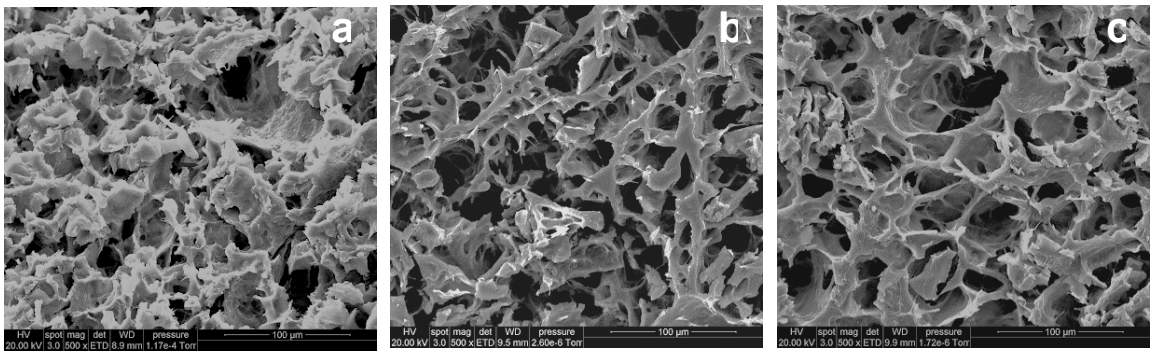

Figure 3. 5 *SEM micrographs of sections of gelatinised kibbled grain digests collected after G0 (a), G30 (b), and I120 (c) digestion phases. (scale:100 µm). G0-before gastric digestion, G30-after 30 min of gastric digestion, and I120-after 120 min of small intestinal digestion phase.*

When the same kibbled grains were sectioned at more depth, solubilised starch-protein matrix was still present (Figure 3.5). However, it became porous in the later phases indicating that the starch was being hydrolysed progressively. The kibbled grains had a larger particle size than flour and therefore contained a significant amount of intact endosperm cells. Previous studies

on refined endosperm have revealed that the intact endosperm cell walls act as a barrier to digestive enzymes; however, cooking can alter the permeability (Konstantinos Korompokis et al., 2019). A study by Berg et al. (2012) has reported the significance of microstructure in the starch digestibility of legumes where endosperm cell walls act as barriers to the digestive enzymes. However, the endosperm cell walls in wheat grains appear to be more fragile than the legumes and become permeable to amylase by cooking.

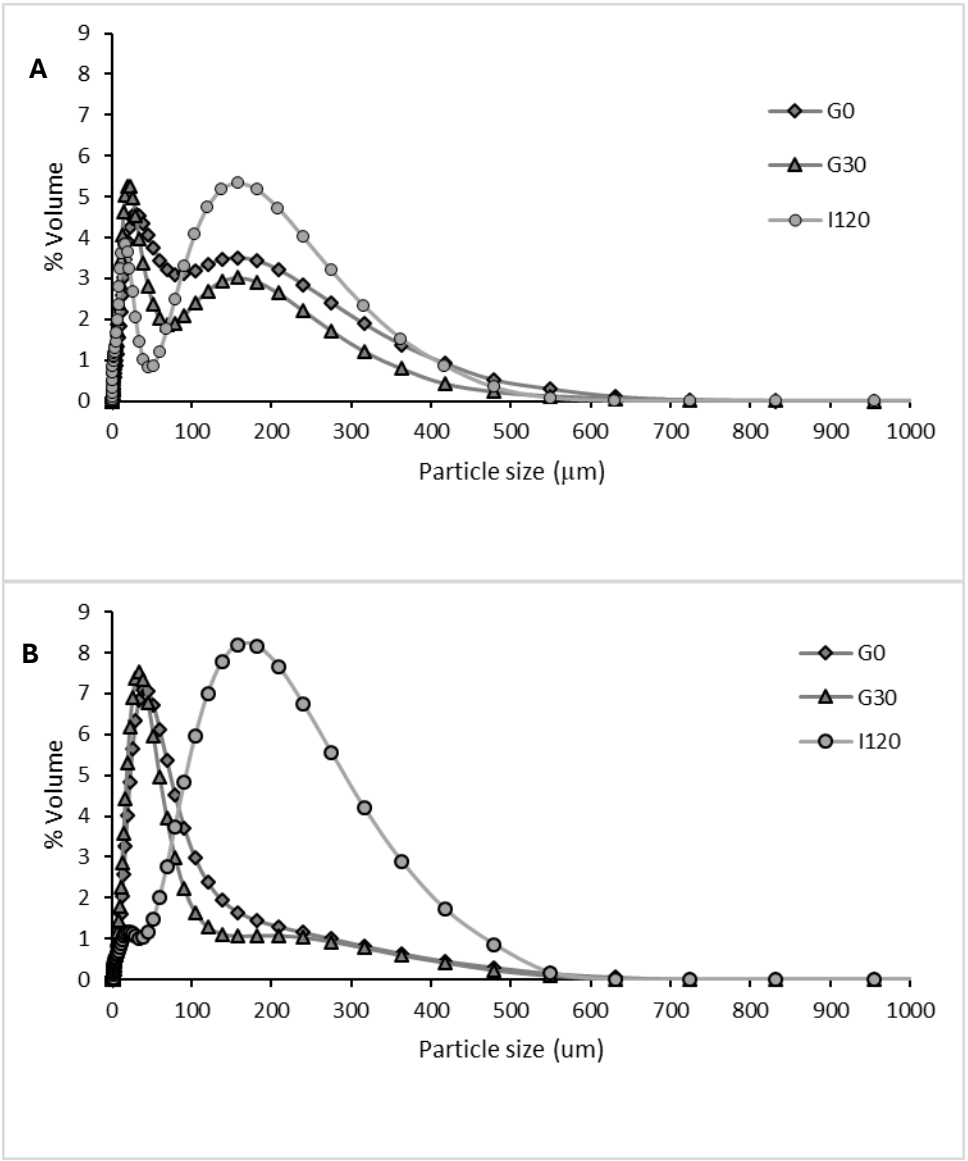

Figure 3. 6 *Particle size distribution of flour digesta sample collected after G0, G30, and I120 A) raw flour and B) gelatinised flour. G0-before gastric digestion, G30-after 30 min of gastric digestion, and I120-after 120 min of small intestinal digestion phase.*

In gelatinised flour, the starch was solubilised during cooking, and no granules were observed under SEM. Gelatinised flour (freeze-dried) appeared flaky (Figure 3.4-n), and it was similar at the end of gastric digestion (Figure 3.4-o). The microstructure of gelatinised flour appeared lumpy by the end of intestinal digestion (Figure 3.4-p). This could be due to the aggregation of remaining gluten proteins after gastric digestion as the pH of SIF was close to the isoelectric point of gluten (Majzoobi & Abedi, 2014). To better understand the digestion of flour, the particle size distribution of raw and gelatinised flour was evaluated as described in section 3.2.6. The particle size distribution curves of raw and gelatinised flour showed that the curves' peak has shifted towards larger particle size with progression in the digestion phase for raw and gelatinised flour (Figure 3.6). The more significant shift in the peak was at the end of the intestinal digestion phase compared to the peak at gastric digestion. In the case of raw flour, the hydrolysis of some protein adherent to starch granules and a small degree of starch hydrolysis could have resulted in the fall of the peak of bigger particles and increased the number of smaller particles after gastric digestion. As a high amount of starch was unhydrolysed, the availability of water and time may have caused swelling of starch granules leading to an increase in overall particle size at the end of small intestinal digestion. However, in the case of gelatinised flour, cooking caused the starch to solubilise, leading to a high number of smaller particles. Agglomeration of unhydrolysed gluten protein in SIF (Majzoobi & Abedi, 2014) might have resulted in an increase in overall particle size at the end of small intestinal digestion. Similar results were observed through microscopy of the flour digests (Figure 3.4-p). Similar observations were also illustrated by the microstructure of digests from gelatinised grains of a biscuit wheat variety in the form of whole, kibbled and flour forms (Figure A 3.2 in Appendix).

Furthermore, the eGI of gelatinised flour was found to be nearly double than its native counterpart (Table 3.2). Proteins in wheat are known to act as a barrier for amylases and cause a reduction in GI (Jenkins et al., 1987). Moreover, α-amylase inhibitors present in native wheat grains are known to inhibit pancreatic α-amylase (Oneda et al., 2004; Singh et al., 2010), and cooking can cause deactivation of the heat-sensitive inhibitors, leading to enhanced starch hydrolysis. These factors together may contribute to the significant alteration in the glycaemic impact of different grain forms as a result of cooking.

3.4 Conclusion

This research makes evident how the starch hydrolysis of wheat grains is governed by the natural microstructure of the grain and the state of starch. It was observed that the entrapment of starch within the protective bran layer helps in lowering of starch hydrolysis by preventing the interaction of digestive enzymes with the starch. The results of *in vitro* starch hydrolysis and the microstructure of the grains suggested that the extent to which starch components are hydrolysed, is dependent on the intactness of the natural microstructure and state of starch. The presence of an intact bran layer, endosperm cell wall, and protein matrix may protect the interaction of digestive enzymes with starch; however, processing may destroy the natural intact microstructure of the grains. Size reduction and cooking used in combination could lead to almost complete digestion (92.85±1.21%) from nearly no digestion (2.19±0.06%). This study did not include chewing (oral digestion phase) to preserve the difference in the microstructures to demonstrate the impact of microstructure during gastro-small intestinal digestion. However, the oral digestion phase could impact the microstructure and starch hydrolysis, which may be considered in future studies. The findings of this investigation explicate the conception of lowering the extent of starch hydrolysis by using whole grains. This study provides a fundamental understanding of microstructural changes that occur in the bread wheat grains during their processing and simulated gastro-small intestinal digestion; and their

impact on *in vitro* starch hydrolysis and eGI. The results of this study include cut and kibbled grains, as well as flour and intact grains, which would be advantageous for the understanding and development of food structures with slow digestion properties.

This study provides a fundamental understanding of microstructural changes that occur in the bread wheat grains during their size reduction (kibbled, cut and milled grains) and simulated gastro-small intestinal digestion; and their impact on *in vitro* starch digestion. In our understanding, this aspect of wheat grains has not been studied earlier by any other researchers. The results of the study are novel and highlight the potential of using differently processed grain formats to develop low glycaemic wheat products.

Chapter 4

Does microstructural modification of whole wheat by hydrothermal treatment and cold storage alter the starch hydrolysis and flour properties?

Chapter 4 Modifying the Microstructure and Starch Digestibility of Whole Wheat: Role of Hydrothermal Treatment and Low-Temperature Storage

Abstract

Based on the understanding of whole grain microstructure in the previous chapter, as well as to explore the possibility of reducing starch hydrolysis by starch retrogradation, this study evaluated the effect of hydrothermal treatment of whole wheat grains at 100 °C followed by cold-storage (4 °C/7 days) on the resulting grains, flakes, and flour characteristics. The extent of starch hydrolysis after oral-gastro-small intestinal digestion *in vitro* was significantly lower *($p<0.05$)* in intact grains, flakes, and flours from the cold-stored grains, 35.73±0.34%, 49.92±0.18% and, 89.04±1.51%, than their non-cold-stored counterparts, 44.86±0.24%, 58.73±0.90% and, 95.96±0.43%, respectively. Scanning electron micrographs, pasting properties, water retention capacities and relative crystallinity of the resulting flours revealed an enhanced degree of gelatinisation with the treatment temperature, however, cold-storage of treated grains resulted in a change in these properties due to the retrogradation of the starch. This study indicates that hydrothermal pre-treatment of grains followed by low-temperature storage for prolonged periods might help to reduce starch digestibility of wheat grains and their resulting products, and could be an effective strategy in developing reduced glycaemic impact grain products.

4.1 Introduction

The consumption of whole grains as a component of foods is increasing due to their perceived nutritional and health benefits. Wheat is one of the most commonly consumed whole grains, and increasingly, breads, other bakery goods, and breakfast cereals made from wheat have

come to contain a proportion of whole grains. In whole grains, the starch is in the form of granules embedded in a highly dispersed protein matrix within the non-living endosperm cells (Srikaeo et al., 2006).

Starch digestibility is a significant parameter to estimate the glycaemic index and serves as the basis for new product development due to the association of high glycaemic products with rapid blood glucose levels and insulin secretion (Bello-Pérez et al., 2021). Processing whole grains into flour or kibbled grains or cooking the grains in the presence of water causes chemical and structural changes that increase their digestibility. For example, the rate of digestion of wheat starch increases by up to 10 times when cooked or processed into other forms, such as kibbled or flour formats (Abhilasha et al., 2021; Elbalshy et al., 2021; K. Korompokis et al., 2019). Heating starch in excess water results in granule swelling due to the disruption of hydrogen bonds in amorphous regions followed by disruption of swollen granules to leach out the amylose (Correa & Ferrero, 2015). The treatment of wheat starch between 35 and 85 °C changed their relative crystallinity from 20% to 0% with visible morphological changes in the starch granules during gelatinisation (Ratnayake & Jackson, 2007).

Dry heat treatment of wheat flour at temperatures between 50 and 200 °C increased the stability of the pseudo-crystalline structure of the amylopectin starch molecules, increasing the gelatinisation temperature (González et al., 2021). Also, dry heat treatment of flour above 100 °C resulted in breads with a reduced rapidly digestible starch content of up to 22%, which was 53% for the control bread (González et al., 2021). Furthermore, superheated steam cooking of whole wheat grains has been reported to reduce the peak hot paste viscosity in their RVA profiles (Srikaeo et al., 2005). The superheated steam treatment of wheat flour caused the deformation of starch granules, formed aggregates of starch, gluten proteins, and/ or lipids, and increased its slowly digestible starch content from 21.5% up to 36%, decreasing the rate of hydrolysis (Ma, Xu, et al., 2021). Hydrothermal treatments such as heat-moisture treatment,

annealing, and shearing change the crystalline and functional properties of isolated starches and whole-grain flours of cereals and pulses, enhancing their application in a broader range of products with improved functional and digestible properties (Chávez-Murillo et al., 2021; Chavez-Murillo et al., 2019; Espinosa-Ramírez et al., 2021).

Retrogradation occurs when the disaggregated amylose and amylopectin in gelatinised starch reassociate to form more ordered structures (Wang et al., 2015). The low-temperature storage of gelatinised starch causes the disrupted pseudo-crystalline regions of the amylopectin molecules to realign to form more ordered structures (Wang et al., 2015). Retrogradation has been reported to reduce the rapidly digestible starch and increase the slowly digestible and resistant starch content (Chen et al., 2018; Park et al., 2009; Zhou & Lim, 2012). Nevertheless, few studies have reported the effect of low-temperature storage of hydrothermally treated wheat grains and flour on their microstructure and digestibility. For example, Alsaffar (2010) reported a 6.7% reduction in the digestibility of starch in cooked wheat subjected to storage at 22 °C for 48 hours compared to freshly cooked wheat. It was also found that the flour of fully cooked wheat grains (56% moisture) possessed a slightly higher retrogradation enthalpy than the wheat flour-water system (49% moisture) during the storage period (Alsaffar, 2010). However, the effects of hydrothermal treatment of whole grains at different temperatures and their low-temperature storage on the digestibility of other grain formats have not been studied. This study evaluated the effect of hydrothermal treatments followed by low-temperature storage of whole wheat grains on their microstructure and the rates of in-vitro starch hydrolysis and estimated glycaemic index (eGI) of the whole grains, and flakes and flour derived from them. Furthermore, this study also evaluated the microstructural, pasting, and crystalline structure of flours from these treatments to understand their functionality for their further food application.

4.2 Materials and methods

4.2.1 Materials

Bread wheat (*Triticum aestivum* var. Saracen) grains with a protein content of 13.6%, moisture content of 11.2%, and total starch content of 62.31% were used for this work. For *in vitro* starch digestion, α-amylase (*Aspergillus oryzae*, 1.5 U/mg), pepsin (porcine gastric mucosa, 800–2500 U/mg protein), pancreatin (hog pancreas, 4 × USP), and invertase (Invertase, grade VII from baker's yeast, 401 U/mg solid), were procured from Sigma–Aldrich Ltd. (St Louis, USA) and amyloglucosidase (3260 U/ml) was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). All other chemicals were of analytical grade.

4.2.2 Hydrothermal treatment of grains

The whole wheat grains, along with three times their mass of water, were added to Duran bottles and immersed in a water bath at 100 °C until the visual disappearance of a white core of the grains (cross-sections of 5 grain samples were tested each time between glass slides). The grain-water mixtures were stirred intermittently for uniform heat and water distribution. Similarly, whole wheat grains were also treated at 60, 70, and 80°C. The visual disappearance of a white core of the grains took between 40 min and 16 h and was assumed to represent equilibrium moisture content. Additionally, grains were also treated at 25 and 50 °C for hydration up to their equilibrium moisture content; however, the visible white core was still present for these temperatures even after 24 h. The excess water was drained from the bottles, and their properties were measured or stored at 4 °C for seven days before further analysis.

4.2.3 Preparation of whole grain formats

Grains hydrothermally treated at 100 °C, and grains hydrothermally treated at 100 °C and then cold-stored at 4 °C for seven days were chosen to prepare flakes since cooking of grains before flaking generally involves high temperatures (steaming). Whole grains hydrothermally treated

at 100 °C, and their cold-stored counterparts were flattened using a rolling pin and dried in a fluidised bed dryer at 40 °C to the initial grain moisture content. Flakes from hydrothermally treated grains and their cold-stored counterparts are referred to as 100F and 100SF, respectively.

After all treatments, grains were dried in a convection oven at 40 °C to the initial grain moisture content. Next, dried grains (hydrothermally treated grains (G) and hydrothermally treated and cold-stored grains (SG)) were milled using a lab-scale stone mill (Mockmill 100, Wolfgang Mock) set to its finest setting to obtain flour. These flours are referred to as 25FL, 50FL, 60FL, 70FL, 80FL, and 100FL from non-cold-stored grains, and 25SFL, 50SFL, 60SFL, 70SFL, 80SFL, and 100SFL from their cold-stored counterparts.

4.2.4 Microstructural characteristics of grain, flakes, and flour

The grain, flake, and flour samples were evaluated using scanning electron microscopy (FEI Quanta 200, FEI Electron Optics, Eindhoven, Netherlands). The samples were placed onto aluminium stubs and sputter-coated with gold (Baltec SCD 050, Balzers, Liechtenstein). The coated samples were observed at an accelerating voltage of 20kV.

4.2.5 Solvent retention capacities of flour

The solvent retention capacities (SRC) of flours were evaluated using the method by Duyvejonck et al. (2011). Briefly, 5 g flour samples were added to 50 ml centrifuge tubes followed by 25 ml water-based solvent. The solvents used were water, 5% sodium carbonate (w/w), 5% lactic acid (w/w), and 50% sucrose (w/w) to evaluate water retention capacity (WSRC), sodium carbonate retention capacity (SCSRC), lactic acid retention capacity (LASRC) and sucrose retention capacity (SuSRC), respectively. The tubes were then horizontally shaken at 150 rpm for 20 min at room temperature (25±5 °C) and centrifuged at

1000 × g for 10 min. The tubes were drained for 15 min, and the sediments were weighed. SRC values of flours were calculated using the following formula on a 14% moisture basis:

$$\text{SRC (\%)} = \left(\frac{Weight\ of\ sediment}{\text{weight of sample}} - 1\right) \times \left(\frac{86}{100 - sample\ moisture}\right) \quad (4.1)$$

4.2.6 Rapid Visco-Analyser viscometric properties of flour

The pasting profile of treated flours was evaluated by Rapid Visco-Analyser (RVA, Newport Scientific, Sydney, Australia) using the Standard method (STD 1) (Ma, Sang, et al., 2021). Flour and water suspensions were prepared using 3.5 g flour (14% moisture basis), and 25 g distilled water. The suspensions were equilibrated at 50 °C for 1 min, heated to 95 °C (12.16 °C/min) and held for 2.5 min, then cooled to 50 °C (12.16 °C/min), and held at 50 °C for 2 min.

4.2.7 X-ray diffraction of flour

The powder X-ray diffraction (XRD) pattern and relative crystallinity of flour samples were evaluated using an X-ray diffractometer (Rigaku MiniFlex PXRD) operating at 15 mA and 40 kV. The sample powders were tightly packed in sample holders and scanned over a range of 5-35° 2θ angle at the rate of 2°/min. The relative crystallinity of the samples was calculated using the equation 4.2 (Chen et al., 2018):

$$Relative\ crystallinity = \frac{Ac}{Ac + Aa} \times 100 \quad (4.2)$$

Where Ac refers to the area of the crystalline peak and Aa is the area of the amorphous peak.

4.2.8 *In vitro* oral-gastro-small intestinal starch digestion of whole grains, flakes, and flour

In vitro oral-gastro-small intestinal starch digestion of whole grains (100G and 100SG), flakes (100F and 100SF), and flours (50FL, 50SFL, 60FL, 60SFL, 100FL, and 100SFL), was performed according to the method of Chen et al. (2020). Simulated salivary fluid (SSF) with α-amylase, simulated gastric fluid (SGF) with pepsin, and simulated intestinal fluid (SIF) with

pancreatin, invertase, and amyloglucosidase were prepared fresh before use. For the oral digestion phase, samples were mixed with SSF in a 1:1 ratio and crushed 20 times using a mortar and pestle to mimic chewing. The bolus was then transferred to the glass reactor and topped up with water to make a 4% starch suspension. Immediately, the pH of reactor content was reduced to 2 using 3 M and 0.5 M HCl followed by the addition of SGF to initiate the gastric digestion phase. The pH of the reactor content was maintained at 1.2 throughout the gastric digestion phase. After 30 min of the gastric digestion phase, the gastric enzymes were deactivated by increasing the pH of reactor content to 6.8 using 3 M and 0.5 M NaOH. Next, the small-intestinal digestion phase was initiated by adding SIF to the same reactor content, and the pH was maintained at 6.8 throughout 120 min of the small-intestinal digestion phase. An aliquot of 0.5 ml was taken at different digestion phases (2 min of oral digestion (O2), 0,15 and 30 min of gastric digestion (G0, G15, and G30), and 0, 5, 10, 15, 30, 60, 90 and 120 min of small-intestinal digestion (I0, I5, I10, I15, I30, I60, I90, and I120)). The aliquots were evaluated for glucose release using GOPOD reagent, and the results were expressed as % starch hydrolysis using equation 4.3 (Tamura et al., 2016a):

$$\%SH = 0.9 \times \frac{Gp}{Si} \tag{4.3}$$

where %SH is the percentage of starch hydrolysis, Gp is glucose produced, and Si is the initial amount of starch.

The estimated glycaemic index (eGI) of the whole grain formats was calculated using equation 4.4 (Goñi et al., 1997):

$$eGI = 39.71 + 0.549HI \tag{4.4}$$

where HI is the hydrolysis index, calculated as the area under the curve during simulated small intestinal digestion using white bread as a reference.

4.2.9 Statistical analysis

Results were reported as mean±standard deviation for triplicate measurements. The data were analysed using one-way analysis of variance (ANOVA) and Tukey's test for the significance of differences in the means using Minitab19.2 software.

4.3 Results and discussion

4.3.1 Effect of hydrothermal treatment and low-temperature storage on the microstructure of whole wheat formats

The endosperm of whole wheat grains consists of starch granules embedded in the compact protein matrix (Figure 4.1-a). Hydrothermal treatment of the grains at 100 °C gelatinised the starch and resulted in distorted starch granules and a denatured protein matrix (Figure 4.1-b). These structural changes are similar to those observed in our previous study on whole wheat grains (Abhilasha et al., 2021). Moreover, the microstructure of the flakes was different from the treated grain in terms of the matrix present around the distorted starch granules (Figure 4.1-c,d). A more continuous matrix was present around the starch granules of flakes from cooked then cold-stored grains than around starch in the whole grains. This could result from the rolling of the grains, leading to the deep embedding of the distorted starch granules in the leached polysaccharide-protein matrix. Furthermore, the micrographs of flour from grains treated at 100 °C and their cold-stored counterparts revealed the clusters of starch and protein rather than a continuous structure (Figure 4.1-e, f), but with no distinguishable difference between treated (100 FL) and cold-stored (100 SFL) samples. However, SEM micrographs of the intact grains and flakes from cold-stored grains presented a slightly more porous structure of the matrix around the starch granules (Figure 4.1-b, d) than their cold-stored counterparts (Figure 4.1-a, c), although storage protein in the whole wheat grains and flakes led to a compact structure.

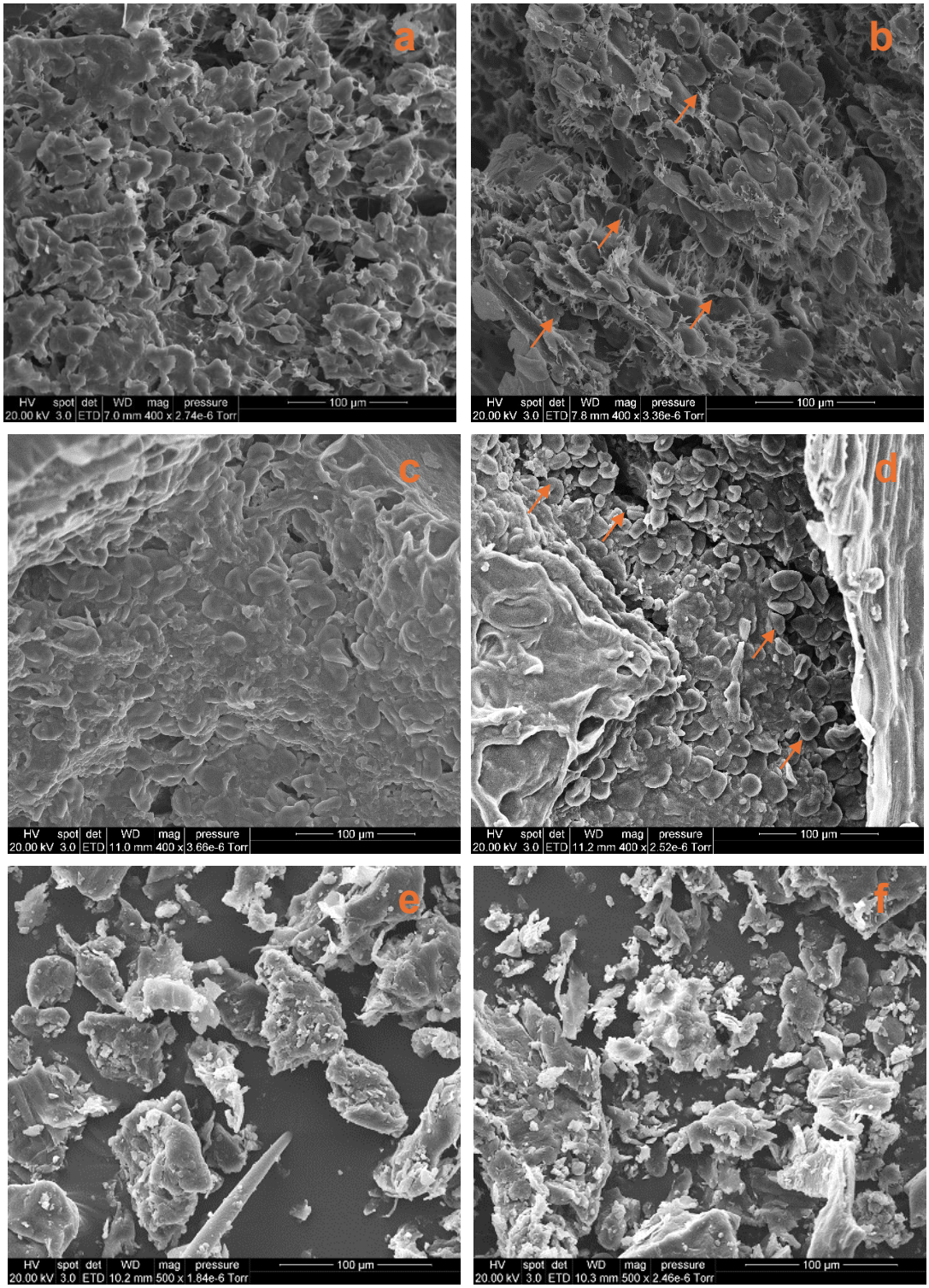

Figure 4. 1 *SEM micrographs of whole grain, flakes, and flour from hydrothermally treated grains at 100 °C (a, c, e) and their low-temperature stored counterparts (b, d, f). (scale: 100µm). Arrows show the porous structure of the grain formats.*

Earlier studies on starch retrogradation have also reported the higher porosity or lacunarity of retrograded starch than gelatinised starch (Utrilla-Coello et al., 2013; Wu et al., 2012). However, when protein was also present with the starch, their retrograded structure became more compact than the starch alone (Zhang et al., 2019). The interaction of starch and protein affects the retrogradation, and protein could act as a filler and lead to smaller pore sizes in starch-protein mixtures (Wang et al., 2020).

4.3.2 Microstructure of flour from hydrothermally treated grains at different temperatures and their cold-stored counterparts

The microstructure of flour includes fractions of starch granules and protein matrix either as individual granules or in the form of a cluster present in the whole grain. The flour from grains treated at 25 °C (Figure 4.2-b) and 50 °C (Figure 4.2-c) resulted in more discrete starch granules and fewer clusters compared to control (Figure 4.2-a) or other treated samples. In addition, the starch granules appeared similar to the native starch granules present in the control flour. This showed that the starch was not gelatinised at the lower temperature treatments. In contrast, discrete and slightly distorted starch granules were present in the flour from grains treated at 60 °C, which was due to a small degree of gelatinisation as starch gelatinisation onsets above temperatures of 55 °C (Yoo & Jane, 2002). When the treatment temperature was raised further, the flour contained more distorted starch granules, primarily present in the starch-protein matrix cluster, representing the increased degree of starch gelatinisation at higher treatment temperatures. However, no distinguishable effect of cold storage was observed in the micrographs of flours from grains hydrothermally pre-treated at any temperature. The lack of observable difference between these flour microstructures was due to the destruction of the natural microstructure that had been present in the intact grain structures (Figure 4.1).

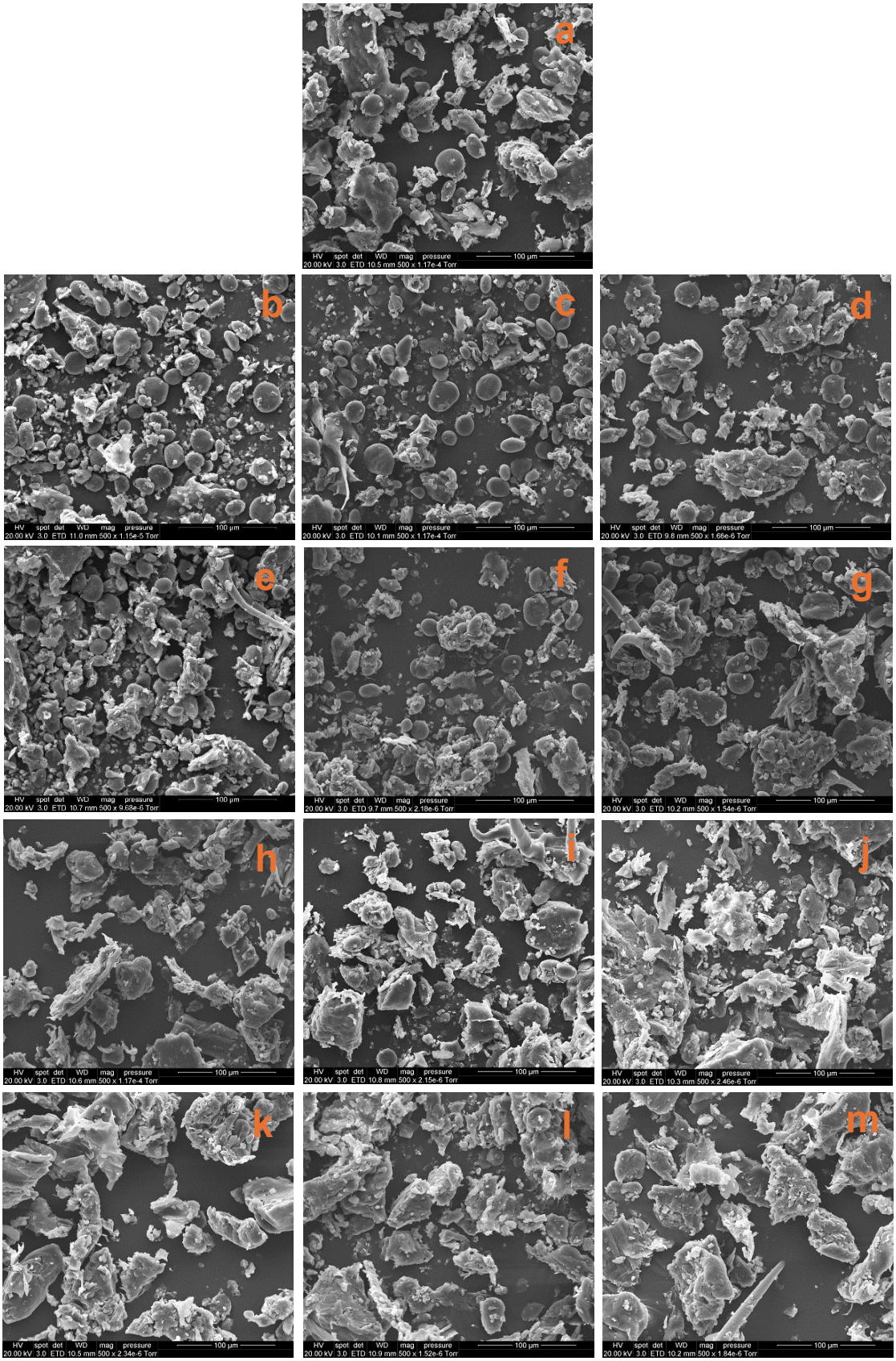

Figure 4. 2 *SEM micrographs of control whole wheat flour (a) and flours from hydrothermally treated grains at 25 (b), 50 (c), 60 (d), 70 (h), 80 (i), 100 (j) and from their low-temperature stored counterparts, 25 (e), 50 (f), 60 (g), 70 (k), 80 (l), 100 °C (m). (Scale100µm).*

4.3.3 Solvent retention capacity of flours from hydrothermally treated and cold-stored grains

Table 4. 1 *Solvent retention capacities of flours from control, hydrothermally treated grains, and their low-temperature stored counterparts*

Sample	WSRC (%) (Mean±SD)	LASRC (%) (Mean±SD)	SCSRC (%) (Mean±SD)	SuSRC (%) (Mean±SD)
Control	68.75±0.69^{g}	90.85±1.52^{j}	108.03±0.88^{h}	102.9±0.36^{h}
25 FL	70.04±0.05^{g}	91.16±0.32^{j}	105.93±1.57^{h}	109.15±0.36^{g}
25 SFL	69.69±0.44^{g}	94.53±0.74^{i}	106.2±1.11^{h}	109.41±1.41^{g}
50 FL	73.47±0.28^{f}	94.08±0.52^{i}	115.35±1.56^{g}	112.15±1.09^{gf}
50 SFL	73.83±0.52^{f}	97.55±0.63^{h}	118.93±1.13^{g}	112.99±0.62^{f}
60 FL	91.4±0.65^{e}	110.89±0.7^{g}	128.02±1.45^{f}	133.25±0.73^{e}
60 SFL	91.12±0.8^{e}	109.69±0.67^{g}	129.31±1.1^{f}	130.82±0.68^{e}
70 FL	107.94±0.48^{d}	129.56±0.57^{f}	147.79±0.25^{e}	161.35±0.71^{d}
70 SFL	107.83±0.29^{d}	133.55±0.74^{e}	158.29±1.04^{d}	162.22±0.74^{d}
80 FL	132.67±2.01^{c}	161.62±0.87^{c}	187.28±2.05^{c}	182.87±1.55^{c}
80 SFL	130.94±0.03^{c}	156.91±0.86^{d}	185.63±0.44^{c}	181.6±1.3^{c}
100 FL	176.84±1.29^{a}	207.88±0.71^{b}	248.61±1.99^{a}	221.85±1.61^{a}
100 SFL	156.1±2.07^{b}	188.7±0.86^{a}	217.9±0.65^{b}	205.18±1.56^{b}

WSRC, water retention capacity; LASRC, lactic acid retention capacity; SCSRC, sodium carbonate retention capacity; SuSRC, sucrose retention capacity. Different superscripts in the same column indicate a significant difference (n=3, p<0.05). FL-flour from hydrothermally treated grains, SFL-flour from hydrothermally treated and cold-stored grains.

The solvent retention capacity of the flours from hydrothermally treated and cold-stored grains generally increased with an increase in the treatment temperature, as shown in Table 4.1. WSRC of flour has been associated with the water-holding capabilities of all flour components, such as protein, starch, and other non-starch polysaccharides (Duyvejonck et al., 2011). The

WSRCs of control flour and flours from grains treated at 25 °C were not significantly different in this study. For other treatment temperatures, WSRC increased with an increase in the treatment temperature. This is attributed to the degree of gelatinisation of starch, reductions in the pseudo-crystalline structure, allowing enhanced interaction between the polar groups of carbohydrates and water, and increased exposure of hydrophilic sites of proteins (Espinosa-Ramírez et al., 2021; Ma, Xu, et al., 2021). The WSRC of flours from treated and cold-stored grains was slightly lower than the treated grains; however, this difference was not statistically significant except for the 100 °C treatment *($p<0.05$)*. At 100 °C, the WSRC was 11% lower in the cold-stored sample than treated samples, suggesting that the retrograded starch had slightly reduced water absorption capacity.

The SCSRC of flour has been associated with the damaged starch present in the flour sample (Duyvejonck et al., 2011). Similar to WSRC, SCSRC of the flours also increased with the increase in the treatment temperature, which could result from higher damaged starch content and increased gelatinisation of starch. SCSRC of cold-stored samples was similar to the treated samples at 25, 50, 60, and 80 °C, indicating that the swelling behaviour of damaged or gelatinised starch was not affected due to low-temperature storage. However, its value decreased by 12.5% at 100 °C for cold-stored samples compared to treated samples, indicating that the treated grains resulted in less damaged starch while milling and changed the swelling behaviour of damaged starch at this temperature. This could also result from the retrograded starch's lower water absorption capacity, decreasing overall SCSRC.

The LASRC has been correlated to the gluten quality and the loaf volumes for wheat flour (Wessels et al., 2020). The acidic environment of 5% lactic acid solution exaggerates the swelling of gluten proteins and, therefore, indicates the quality of gluten present in the flour (Kweon et al., 2011). Dry heat treatment of wheat flour has been reported to decrease the swelling ability of gluten due to its cross-linking with other flour components (Van Steertegem

et al., 2013). However, in the present study, the LASRC of flour samples increased with the whole grains' treatment temperature. The increased LASRC could be due to the enhanced water absorption capacity of the gelatinised starch, and it did not represent the swelling ability of gluten. Also, LASRC increased slightly for the cold-stored samples compared to their non-stored counterparts until 70 °C. However, there was a decrease in the LASRC of cold-stored samples compared to their non-stored counterparts at 80 and 100 °C. Therefore, the LASRC results in this study contradicted the gluten quality since gluten denatures with the increased treatment temperatures (Schofield et al., 1983).

Furthermore, sucrose retention capacity (SuSRC) was similar for hydrothermally treated samples and their cold-stored counterparts at each treatment temperature except 100 °C, where it decreased for the cold-stored sample. Since SuSRC is associated with the arabinoxylans of the flour (Duyvejonck et al., 2011), it remained similar for treated samples and their cold-stored counterparts. However, the increase in SuSRC with increased treatment temperature is likely to be a result of the increasing level of gelatinisation.

Overall, even though SRC has been used as an indicator of raw or dry heat-treated wheat flour quality for baking, it is not a good indicator of flour quality from hydrothermally treated grains.

4.3.4 Effect of hydrothermal treatment and low-temperature storage of grains on RVA viscometric properties of flour

The pasting profile of the flours from treated grains has been shown in Figure 4.3. The peak hot paste and final viscosity of pastes made using flours from hydrothermally treated grains generally decreased with an increase in the treatment temperature. Similar results were obtained by Espinosa-Ramírez et al. (2021), where the hot paste viscosity and final viscosities were significantly reduced by the hydrothermal treatment of cereals and legume flours through extrusion. Therefore, it is clear that the degree of starch gelatinisation increased with treatment temperature between 60 and 100 °C. It appears that little gelatinisation occurs between 25 and

50 °C, and as a result, all gelatinisation occurred during pasting in RVA. Peak viscosity has been found to be decreased with an increase in the degree of gelatinisation in cereals (Puspitowati & Driscoll, 2007).

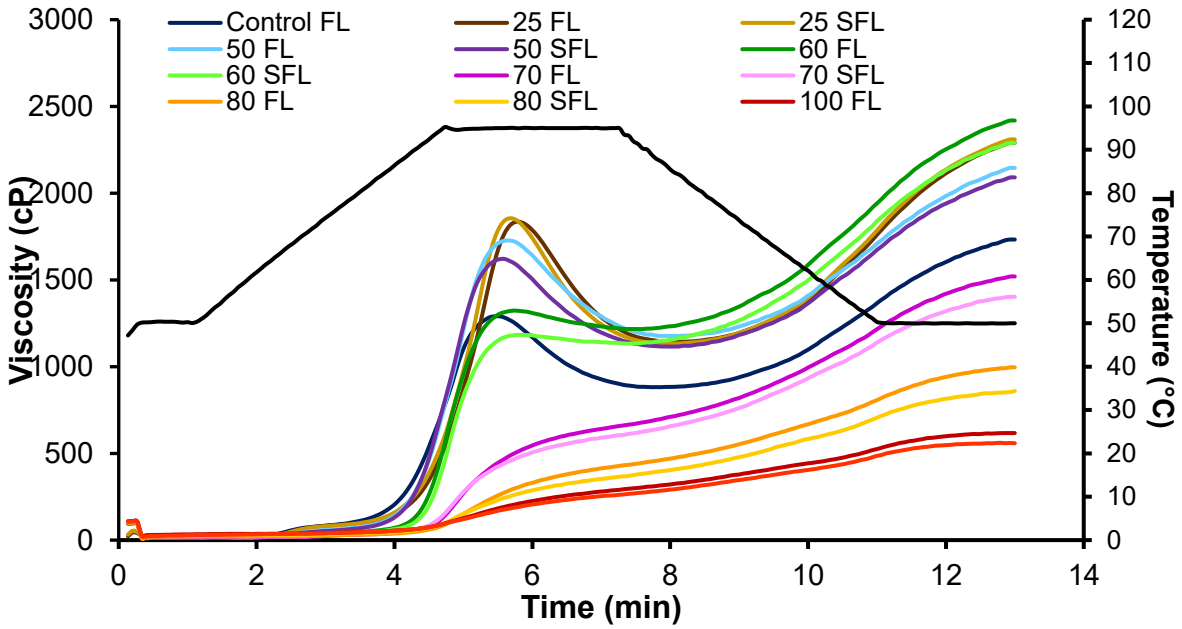

Figure 4. 3 *Pasting profile of flours from control, hydrothermally treated grains at different temperatures (FL), and their low-temperature stored counterparts (SFL).*

However, the peak hot paste viscosity and final viscosity of flours from cold-stored grains were generally lower than their hydrothermally treated counterparts (Table 4.2). The recrystallization of leached amylose hinders the swelling of starch and reduces the pasting ability of the starch (Zhou & Lim, 2012). Also, the retrograded starch may not absorb as much water as the gelatinised starch (Chen et al., 2018), which is also evident from the lower water retention capacity of these flours (Section 4.3.3), resulting in the lower hot paste and final viscosities of pastes made using the cold-stored samples.

Table 4. 2 *RVA pasting parameters of flours from control, hydrothermally treated grains, and their low-temperature stored counterparts*

Sample	PV (cP) (Mean±SD)	TV (cP) (Mean±SD)	FV (cP) (Mean±SD)	BD (cP) (Mean±SD)	SB (cP) (Mean±SD)	Pasting temperature (°C) (Mean±SD)
Control	1295±11 ^{e}	881±6 ^{e}	1733±28 ^{e}	413±6 ^{e}	851±22 ^{d}	85.6±0 ^{e}
25 FL	1836±6 ^{a}	1143±6 ^{c}	2291±11 ^{b}	693±0 ^{b}	1148±5 ^{b}	88.3±0.4 ^{d}
25 SFL	1855±17 ^{a}	1132±16 ^{c,d}	2310±12 ^{b}	724±1 ^{a}	1178±4 ^{a,b}	88.1±0.1 ^{d}
50 FL	1728±14 ^{b}	1176±3 ^{b}	2146±2 ^{c}	552±12 ^{c}	970±1 ^{c}	88±0 ^{d}
50 SFL	1621±12 ^{c}	1116±4 ^{d}	2091±4 ^{d}	505±9 ^{d}	975±7 ^{c}	88±0 ^{d}
60 FL	1323±2 ^{d}	1216±2 ^{a}	2419±10 ^{a}	107±1 ^{f}	1202±12 ^{a}	90.5±0 ^{c}
60 SFL	1183±2 ^{f}	-	2290±23 ^{b}	-	-	91.1±0.4 ^{c}
70 FL	654±4 ^{g}	-	1520±1 ^{f}	-	-	94.7±0.9 ^{a}
70 SFL	602±3 ^{h}	-	1403±7 ^{g}	-	-	93.7±0 ^{b}
80 FL	424±7 ^{i}	-	996±12 ^{h}	-	-	-
80 SFL	362±1 ^{j}	-	860±0 ^{i}	-	-	-
100 FL	288±1 ^{k}	-	617±0 ^{j}	-	-	-
100 SFL	259±1 ^{l}	-	558±6 ^{k}	-	-	-

PV, peak viscosity; TV, trough viscosity; FV, final viscosity; BD, breakdown viscosity; SB, setback viscosity. Different superscripts in the same column indicate a significant difference (n=3, p<0.05). FL-flour from hydrothermally treated grains, SFL-flour from hydrothermally treated and cold-stored grains.

The pasting temperature for both the cold-stored and treated samples was increased with an increase in treatment temperature. The amylose leached out of the swollen starch granules could protect the starch granules during the pasting process (Zhou & Lim, 2012). Furthermore, increased amylose content in a starch mixture could also lead to a higher pasting temperature (Juhász & Salgó, 2008). Therefore, the increased pasting temperature in this study is likely due to the leaching of amylose in the treated samples.

4.3.5 Effect of hydrothermal treatment and low-temperature storage of grains on the crystalline structure of the resulting flour

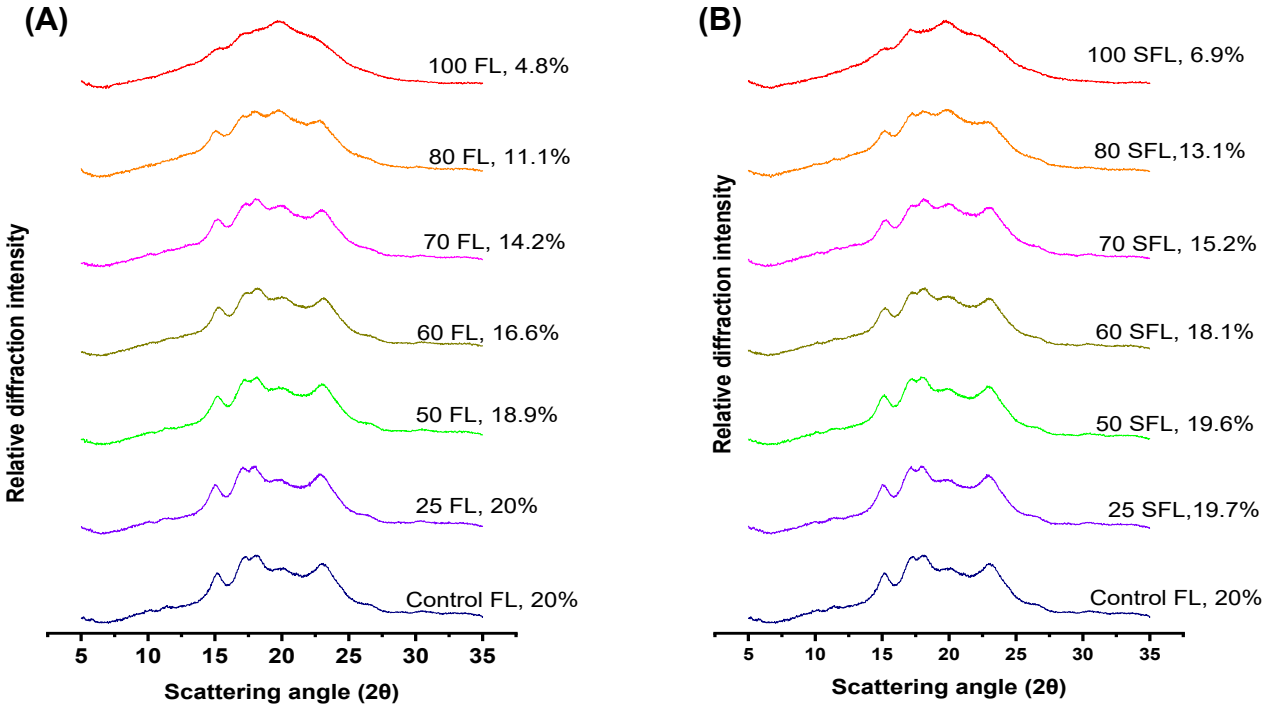

Figure 4. 4 *X-ray diffraction pattern and relative crystallinity of flours from (A) hydrothermally treated grains at different temperatures (FL) and (B) their low-temperature stored counterparts (SFL).*

The relative crystallinity and X-ray diffractograms of the flours from hydrothermally treated grains and their cold-stored counterparts have been shown in Figure 4.4. The XRD pattern of the native whole wheat flour had a typical A-type pattern with peaks around the 2θ angles of

15, 17.3, 18.1, 23.3, and 26.7° (Leblanc et al., 2008; Ma, Xu, et al., 2021). The flours from hydrothermally treated grains followed a similar pattern; however, these peaks flattened, and the relative crystallinity decreased as the treatment temperature increased from 60 to 100 °C (Figure 4.4-A). Moreover, a peak at 2θ angle of 20° emerged as the treatment temperature was enhanced, presenting a typical V-type pattern (Figure 4.4-A). The interaction between the amylose and the naturally present phospholipids and fatty acids in the whole grain could result in V-amylose complexes (Chen et al., 2015). A peak at 20° has also been recorded in earlier studies on wheat starches involving heat-moisture treatments (Chen et al., 2015; Ismailoglu & Basman, 2016). Moreover, the higher relative crystallinity of samples treated at lower temperatures indicates lower levels of gelatinisation.

The XRD patterns of the flours from the cold-stored grains were similar to their non-stored counterparts. The presence of the amylose-lipid complexes formed during the hydrothermal treatment or storage has been reported to interfere with the retrogradation of the starch since they leave less amylose for the recrystallisation opportunity (Wang et al., 2015). However, the relative crystallinity of the stored samples increased for treatment temperatures above 50 °C, compared to their non-stored counterparts, indicating the retrogradation of starch during low-temperature storage of the treated grains. Also, the intensity of peaks at diffraction angles of 17° was significantly higher for 100 SFL than 100 FL, demonstrating the recrystallisation of the gelatinised starch (Niu et al., 2018). Therefore, the hydrothermally treated grains were gelatinised grains with different degrees of gelatinisation, and their cold-stored counterparts were retrograded grains. However, the relative crystallinity of flours from retrograded grains was much lower than control, which could be attributed to the loss in the gained crystallinity during the drying process of the grains.

4.3.6 *In vitro* starch hydrolysis of whole wheat grains in different formats from hydrothermally treated and cold-stored grains

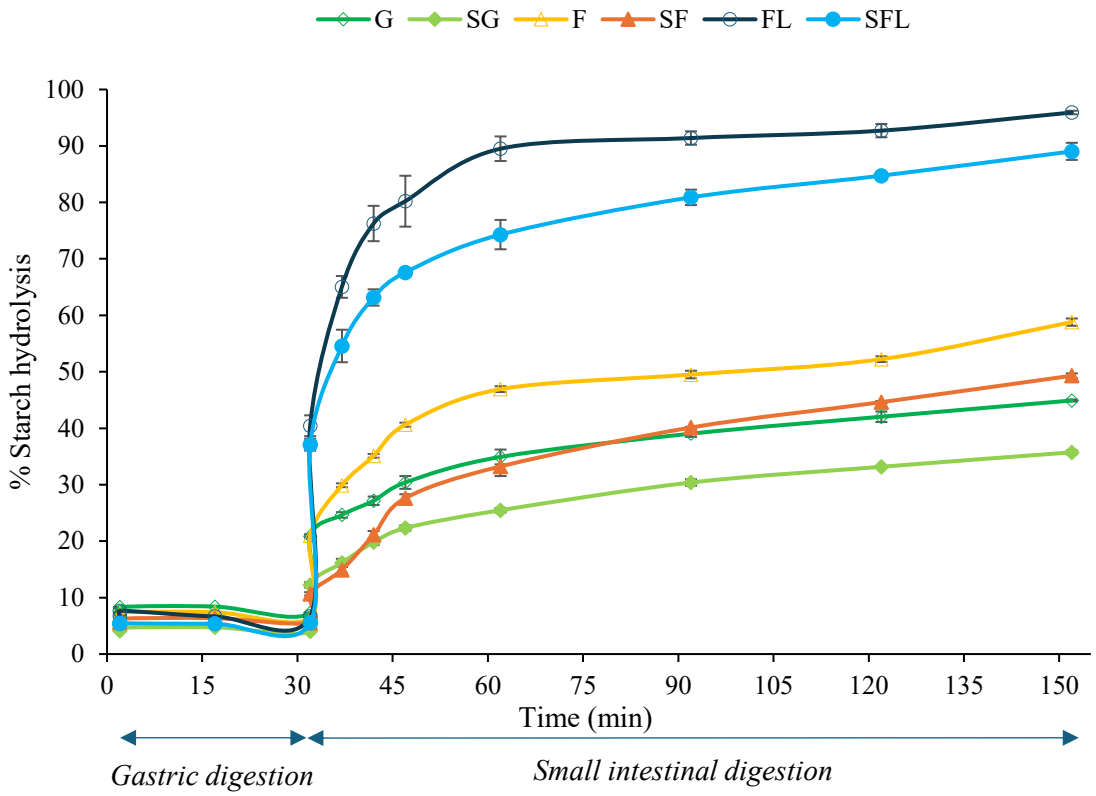

Figure 4. 5 *In vitro starch hydrolysis of intact grains, flakes, and flour from hydrothermally treated grains at 100 °C (G, F, FL) and their low-temperature stored counterparts (SG, SF, SFL). Error bars represent standard deviation (n=3).*

The *in vitro* oral-gastro small-intestinal starch hydrolysis of wheat grain cooked at 100 °C and their cold-stored counterparts processed into different formats, such as intact grains, flakes, and flour, has been shown in Figure 4.5. Nearly 4-7% of the starch was digested from these grain formats during the 2 min of oral digestion phase. As expected, the extent of their starch hydrolysis did not change much during the gastric phase, but it increased during the small intestinal phase. At the end of 120 min of small intestinal digestion, the amount of starch hydrolysed was significantly lower *($p<0.05$)* for the intact grain format (44.92±0.18%) than

either flake (58.8±0.64%) or flour (95.93±0.31%). Compared with the flour samples, the extent of starch hydrolysis for the intact grains and flakes was about 47% and 61% of the flour, respectively. The impact of microstructure on starch hydrolysis has also been observed in previous studies where the intact microstructure of whole grains limits the enzymatic action on the starch (K. Korompokis et al., 2019). Similarly, in the flakes, the layer of bran and the flattened matrix of protein and starch still succeed in limiting the starch hydrolysis, but the open microstructure of flour allows easy access to the digestion enzymes evident from the SEM micrographs and starch hydrolysis results.

On the other hand, the cold-stored counterparts of hydrothermally treated grain formats showed a significant difference between the extent of starch hydrolysis at the end of 120 min of the small intestinal digestion phase as well as their eGI (Table 4.2). The extent of starch hydrolysis was generally lower for all the incubation times during the simulated oral-gastro-small intestinal digestion of these grain formats (Figure 4.6). This is attributed to the retrogradation of starch during the low-temperature storage as evident through water absorption capacity and X-ray diffraction patterns. Earlier studies on retrograded starches have also reported a reduction in the overall starch hydrolysis due to retrogradation (Alsaffar, 2011; Chen et al., 2018). However, soluble fibres interfere with the retrogradation of starch in whole grains (Srichuwong et al., 2017).

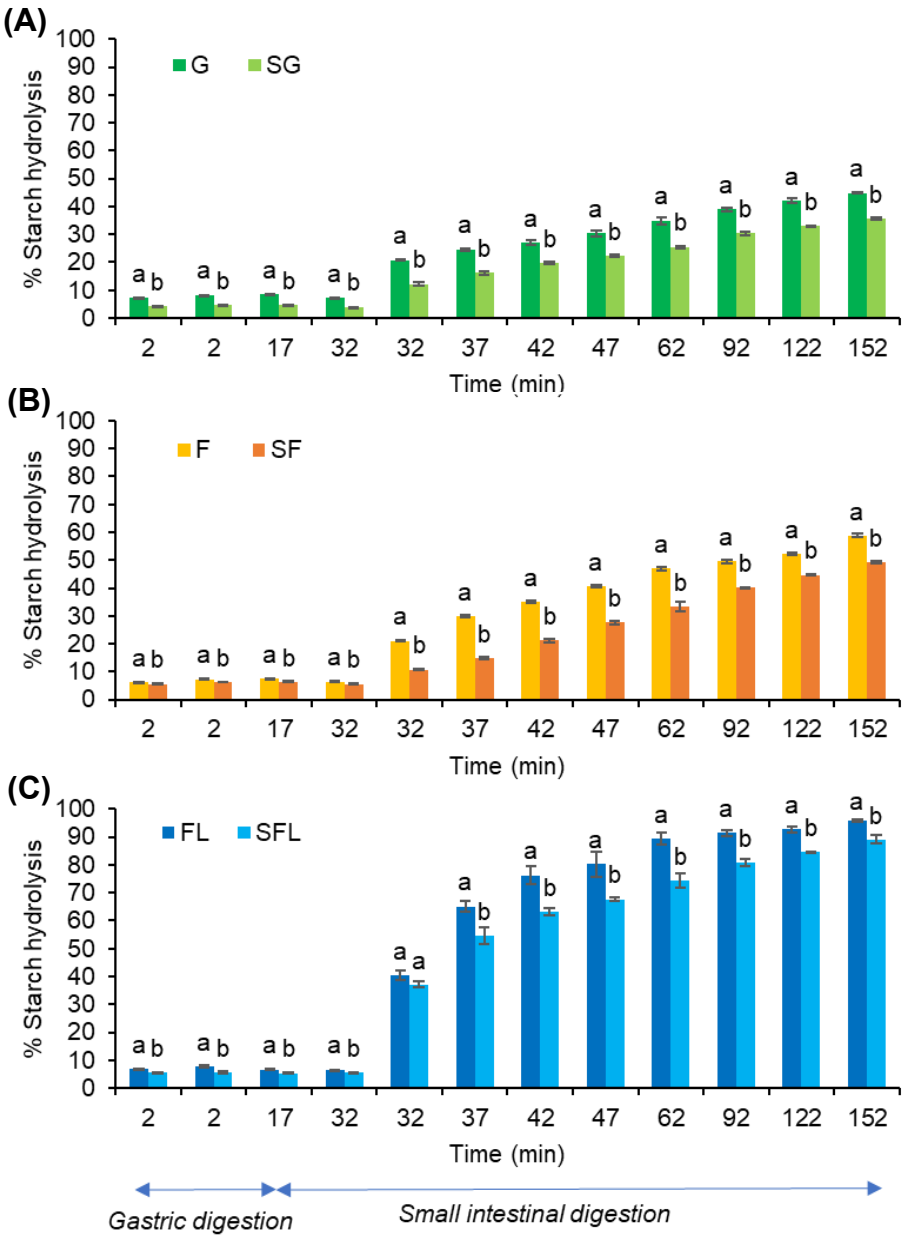

Figure 4. 6 *In vitro starch hydrolysis of grain formats from hydrothermally treated grains at 100 °C and their low-temperature stored counterparts. (A) Intact grains, (B) Flakes, and (C) Flour. Error bars represent standard deviation, and different letters over the bars indicate significant differences between the treatments at each stage of simulated digestion (n=3, p<0.05).*

In addition, the presence of wheat proteins also plays a role in the retrogradation of wheat starch, where glutenin could retard the retrogradation (Guo et al., 2016). The extent of starch

hydrolysis in the present study for the retrograded grain formats was 80%, 84%, and 93% of their non-retrograded counterparts for the intact grains, flakes, and flour, respectively. Even though the raw material to prepare different formats were the same retrograded whole grains, the difference in their further processing, i.e., flaking and drying or drying and milling, resulted in the difference in their total starch hydrolysis. The treatments in this study significantly reduced the eGI *($p<0.05$)* for all the formats for their cold-stored counterparts (Table 4.2). The reduced eGI of these formats could be helpful for people with diabetes and overweight and obese individuals.

Table 4. 2 *Total starch hydrolysis (SH), hydrolysis index (HI), and estimated glycaemic index (eGI) of whole wheat grain formats (grains, flakes, and flour) from hydrothermally treated grains and their cold-stored counterparts*

Sample	**% SH (Mean±SD)**	**HI (Mean±SD)**	**eGI (Mean±SD)**
G	44.92±0.18^{e}	41.78±0.84^{d}	62.65±0.46^{d}
SG	35.73±0.34^{f}	31.98±0.38^{e}	57.27±0.21^{e}
F	58.79±0.64^{c}	53.53±0.49^{c}	69.1±0.27^{c}
SF	49.31±0.41^{d}	41.84±0.2^{d}	62.68±0.11^{d}
100FL	95.93±0.31^{a}	98.14±1.52^{a}	93.59±0.84^{a}
100SFL	89.04±1.51^{b}	86.52±1.17^{b}	87.21±0.65^{b}

SH-the extent of starch hydrolysis at the end of simulated small intestinal digestion, HI-hydrolysis index, eGI-estimated glycaemic index. G, F, FL- intact grains, flakes, and flour from hydrothermally treated grains at 100 °C and SG, SF, SFL-their low-temperature stored counterparts, respectively. Different superscripts in the same column indicate a significant difference ($n=3$, $p < 0.05$).

4.3.7 *In vitro* starch hydrolysis of flour from hydrothermally treated and cold-stored grains

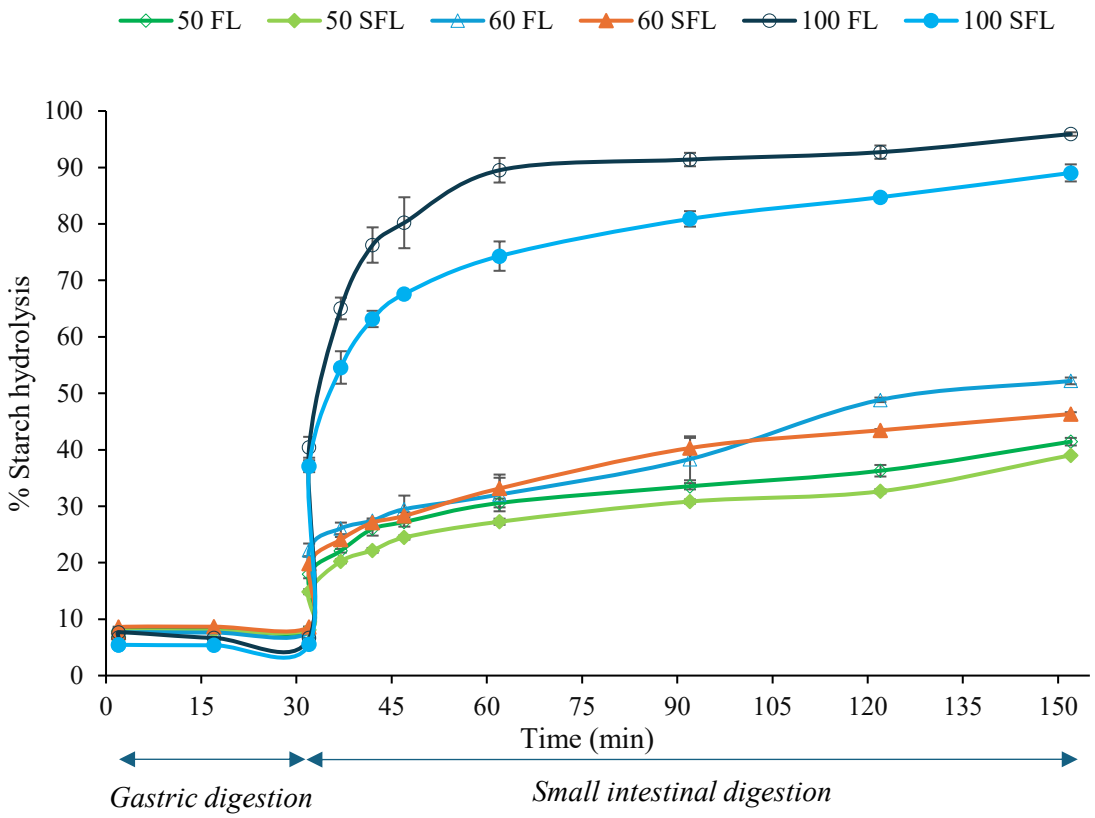

Figure 4. *7 In vitro starch hydrolysis of flours from hydrothermally treated grains at 50 °C, 60 °C, 100 °C (50 FL, 60 FL, 100 FL) and their low-temperature stored counterparts (50 SFL, 60 SFL, 100 SFL). Error bars represent standard deviation (n=3).*

The *in vitro* starch hydrolysis curves for flours from grains treated at 50, 60, and 100 °C and their cold-stored counterparts have been shown in Figure 4.7. After 120 min of small intestinal digestion, the extent of starch hydrolysis was 40.25±0.45% for 50 FL, and it increased by 1.3 times for 60 FL and by 2.4 times for 100 FL compared to 50 FL. This increase in starch hydrolysis is attributed to the enhanced degree of gelatinisation with increased treatment temperature, as evident from X-ray diffraction patterns, relative crystallinity, pasting profile, and water absorption capacity of these flours.

On the other hand, the extent of starch hydrolysis of the flour from grains treated at 100 °C followed by cold-storage was lower than their non-cold-stored counterparts during most of the simulated oral-gastro-small intestinal digestion (Figure 4.8).

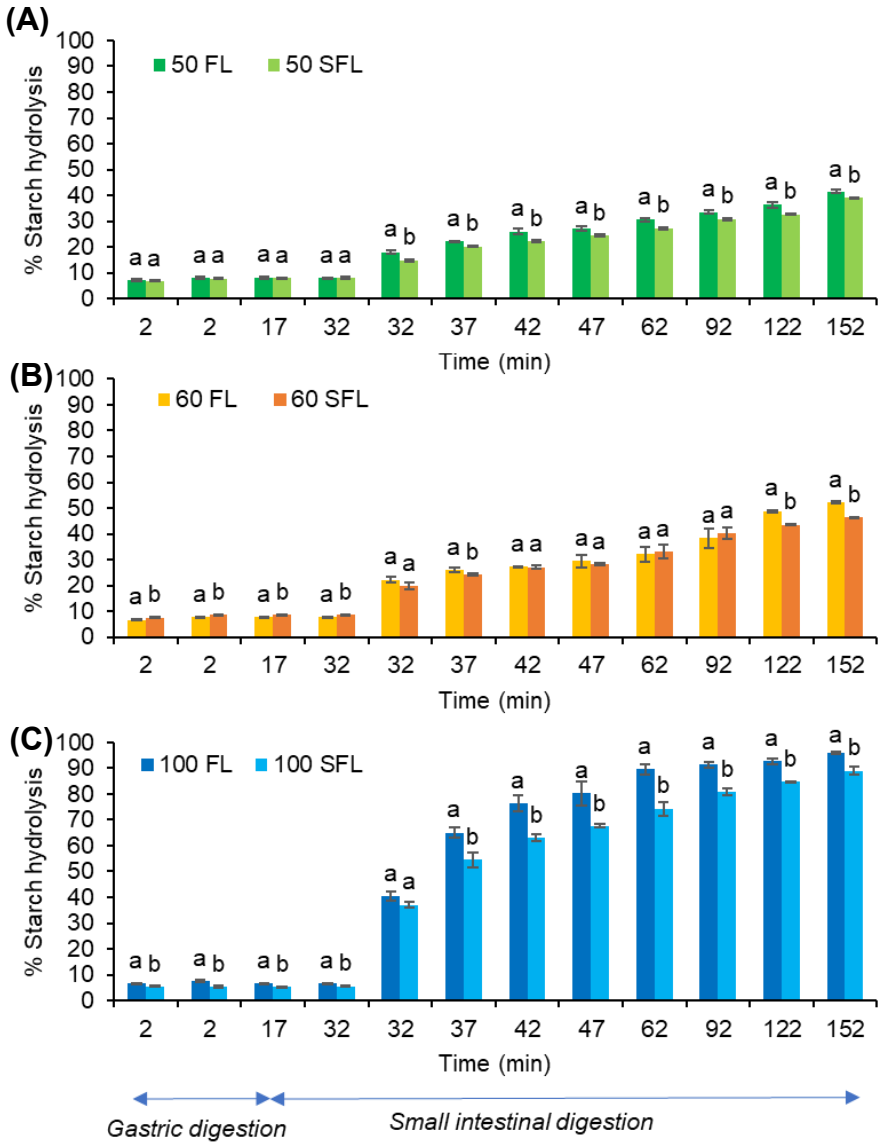

Figure 4. 8 *In vitro starch hydrolysis of flours from hydrothermally treated grains at different temperatures and their low-temperature stored counterparts. (A) 50 °C, (B) 60 °C, and (C) 100 °C. Error bars represent standard deviation, and different letters over the bars indicate significant differences between the treatments at each stage of simulated digestion (n=3, p<0.05).*

The flours from the cold-stored grains generally had lower overall starch hydrolysis compared to the non-stored counterparts. The overall starch hydrolysis of the flours from cold-stored grains was about 90% and 93% of their non-stored counterparts for treatments at 60 and 100 °C, respectively; however, the difference was not significant at 50 °C *($p<0.05$).*

The eGI of the flour from cold-stored grains was significantly reduced *($p<0.05$)* for treatment temperature of 100 °C; however, it was not significantly different at lower temperature treatments (Table 4.3). These results show that the hydrothermal treatment followed by cold storage resulted in significant retrogradation of starch only above temperatures of 60 °C. However, the reheating involved during the drying could impact the reordered starch structures. Chen et al. (2018) have also reported enhanced overall starch hydrolysis of tuber starch due to disruption of the reordered structures after reheating the retrograded starch.

Table 4. 3 *Total starch hydrolysis (SH), hydrolysis index (HI), and estimated glycaemic index (eGI) of flours from hydrothermally treated grains at different temperatures and their cold-stored counterparts*

Sample	**% SH (Mean±SD)**	**HI (Mean±SD)**	**eGI (Mean±SD)**
50 FL	52.2±0.59^{e}	43.92±1.99^{d}	63.82±1.09^{d}
50 SFL	47.14±0.57^{e}	42.44±1.41^{d}	63.01±0.78^{d}
60FL	40.25±0.45^{c}	36.56±0.65^{c}	59.78±0.36^{c}
60SFL	39.03±0.17^{d}	33.34±0.36^{c}	58.02±0.2^{c}
100FL	95.93±0.31^{a}	98.14±1.52^{a}	93.59±0.84^{a}
100SFL	89.04±1.51^{b}	86.52±1.17^{b}	87.21±0.65^{b}

SH-the extent of starch hydrolysis at the end of simulated small intestinal digestion, HI-hydrolysis index, eGI-estimated glycaemic index.. Different superscripts in the same column indicate a significant difference (n=3, $p<0.05$).

4.4 Conclusion

The effects of hydrothermal treatment and low-temperature storage of whole wheat grains on their *in vitro* starch digestibility in different formats and resulting flour properties were investigated in this study. The effect of retrogradation was visible through the SEM micrographs of whole grain in its intact and flake format. Processing the treated whole grains into different formats, such as flakes or flour, significantly enhanced their extent of starch hydrolysis. But then, the low-temperature storage reduced their starch hydrolysis by up to 9% in different formats such as dried whole grain, flakes, and flour, thereby reducing their eGI.

Moreover, hydrothermal treatment of whole wheat grains at temperatures below the starch gelatinisation temperature and up to 100 °C resulted in flours with modified pasting and digestion properties. The treatments above 60 °C led to distorted starch granules and leached starch-denatured protein matrix fragments in the flour, resulting in enhanced water absorption capacities. The relative crystallinity and pasting viscosities of the flours decreased with an increase in the treatment temperature of the grains, and their starch hydrolysis and eGI were increased. But then again, low-temperature storage of the hydrothermally treated grains generally reduced the water absorption, pasting viscosities, and the extent of starch hydrolysis of flour, which could be attributed to the retrogradation of the starch. However, the results obtained for SRCs in this study were not representative of the flour quality. Therefore, it can be said that the SRC method may not be used to predict the flour quality for the hydrothermally treated grain flour.

Overall, this study provides a fundamental understanding of the changes in microstructure and its impact on starch hydrolysis and eGI due to hydrothermal treatment and low-temperature storage of whole wheat grains. These treatments on whole wheat grains provide an opportunity to use whole wheat in new products with broad functional and digestible properties. However, the presence of soluble fibres, proteins, and amylose-lipid complex formed during the

hydrothermal treatment of the whole grains could interfere with the retrogradation of the starch, and additional processing might further interfere with their effect in reducing the overall starch hydrolysis. Moreover, in the preliminary trials, flours from hydrothermally treated and low-temperature stored grains were evaluated for dough functionality; however, doughs of inferior quality were obtained (Figure A 4.1 in Appendix).

Chapter 5

a) *How do soluble components from wheat modify the structural, physicochemical properties, and in vitro digestion behaviour of resistant starch?*

b) *How does the addition of hydrocolloids modify resistant starch's structural, physicochemical properties, and in vitro digestion behaviour?*

Chapter 5 Modification and Characterisation of High Amylose Corn Starch using Wheat Solubles and Hydrocolloids: Physico-chemical, Microstructural and *In Vitro* Starch Hydrolysis

Abstract

Resistant starch has gained a lot of attention due to its potential health benefits, which include improved digestive health and reduced glycaemic response. This chapter intends to modify the resistant starch-high-amylose corn starch (HACS) through different treatments with the aim of reducing starch digestibility and improving the functionality of wheat-based food products. Two approaches were employed to modify HACS: one involving wheat solubles (soluble extracts from wheat flour and vital gluten) (water solubles, salt-assisted water-solubles, and acid-solubles) and the other utilising hydrocolloids (guar gum, xanthan gum, locust bean gum, and carboxymethyl cellulose). The modification with wheat solubles resulted in modified surface morphology with clusters of starches and wheat solubles, as well as wheat solubles on individual starch granule surfaces. The modification of HACS with solubles from wheat resulted in increased protein content up to 4.03±0.03%; moreover, their water-holding capacity increased up to 1.54 times. Their relative crystallinity was reduced; however, the starch hydrolysis rate decreased for most cooked modified starches. Moreover, the modification with hydrocolloids resulted in numerous starch-hydrocolloid clusters and improved their pasting profile. The peak viscosity value was increased from 132±1 cP up to 511±1 cP. Furthermore, *in vitro* digestion studies revealed a slower starch hydrolysis profile until 90 min of simulated digestion for most of the hydrocolloids used, carboxymethyl cellulose being the least effective in slowing the starch hydrolysis rate. The *in vitro* starch hydrolysis of cooked HACS reduced from 52.1±1.37% to 48.65±1% due to modification with locust bean gum.

5.1 Introduction

Wheat starch in flour is digested rapidly after cooking, which is undesirable for people who are conscious of their blood sugar. Increasing dietary concerns and the health implications of high-GI foods have prompted interest in alternative ingredients that could be digested slowly but also have applications in wheat-based products.

Resistant starches present a promising option due to their potential health benefits, including improved digestive health and reduced glycemic response (Walsh et al., 2022). High amylose corn starch (HACS) is known for its resistance to digestion (RS2), which makes it a suitable candidate to be used as a raw material for creating food structures with slowly digestible properties. However, when used in wheat flour-based products, resistant starches, such as HACS, result in inferior quality products(Arp et al., 2021a; Ozturk, Koksel, & Ng, 2009). For instance, addition of HACS (>30%) to Chinese steamed bread has been reported to cause a discontinuous gluten network, increased chewiness and hardness and reduced specific volume of the product (Gu et al., 2023). The objective of this chapter was to modify a resistant starch-high-amylose corn starch, with the aim of reducing the digestibility and modifying the functionality of wheat-based food products.

Wheat flour components play an important role in the digestion and functionality of the flour system. The proteins in wheat can restrict starch gelatinisation, impact retrogradation, and help reduce their digestibility (Abhilasha et al., 2021, 2022; Bhattarai et al., 2016; Jenkins et al., 1987; Yao et al., 2020). Also, arabinoxylans from wheat behave as soluble dietary fibre and help reduce the digestibility of food (Lu et al., 2000). Moreover, the components of wheat flour have been shown to play an important role in the functionality of flour as well (Goesaert et al., 2005). A recent study by Rezette et al. (2025) has reported that soluble starch, water-extractable arabinoxylans and proteins of wheat flour significantly impact the water absorption in wheat flour, which is a crucial parameter for wheat-based product development. Therefore, it is

important to investigate the use of wheat flour components to modify the functional and digestible properties of high-amylose corn starch (HACS).

In preliminary trials, it was observed that when an isolated starch such as wheat starch, corn starch, or high-amylose corn starch is mixed with vital gluten, it makes a dough of very inferior quality (Figure A 5.1 in Appendix). A few preliminary trials were performed to modify the surface of corn starch by adhering vital gluten to its surface via dough mixing and freeze-drying (Figure A 5.2 in Appendix). Moreover, the surface of HACS was attempted to modify with an acid-soluble (dilute HCl) extract from vital gluten and oven drying, which resulted in improved functionality in the wheat flour system (Figure A 5.3 in Appendix).

It was hypothesised that the water-soluble extract from wheat flour, which contains water-soluble protein albumin and polysaccharides (Pauly & Delcour, 2018), could modify HACS to improve the functionality and starch digestibility when used in a wheat flour-based system. Similarly, salt-assisted (NaCl) water-soluble extract rich in gliadin (Ukai et al., 2008) would help modify HACS for better functionality and starch digestibility. Moreover, acid-soluble (dilute HCl) extract from wheat flour and vital gluten (Macritchie, 1985) might help modify HACS being rich in gluten protein components. Therefore, this chapter aims to utilise water-soluble and salt-assisted water-soluble extracts from wheat flour and acid-soluble extracts from wheat flour and vital gluten to modify HACS. A high-temperature and short-time processing spray drying has been used for the treatment of the starch.

Furthermore, hydrocolloids have been used in gluten-free formulations to achieve a texture similar to wheat-based products. Hydrocolloids have also been studied to improve the digestion properties of the starch systems. Dry heat treatment of starch-hydrocolloids at high-temperature and long times have been used to modify water binding capacity, peak viscosity, and starch digestibility of the starch (Bae & Lee, 2018; Chandanasree et al., 2016; Lim et al., 2006). Previous studies have explored the use of heat processing, such as jet cooking and extrusion,

instead of simple aqueous mixing of starch and hydrocolloids to provide new functionality to starch (Fanta et al., 1999). A recent study combined rice starch with hydrophilic colloids such as xanthan gum and locust bean gum and gave heat-moisture treatment to reduce the starch digestibility (Y. Zhang et al., 2023). However, the role of hydrocolloids in modifying HACS to improve their functional and digestible properties has not been studied much. Therefore, this chapter also involves developing the modified starches using HACS and selected hydrocolloids by spray drying and characterising their functional properties and starch digestibility.

5.2 Materials and methods

5.2.1 Materials

Standard wheat flour (Champion) and commercial vital gluten were procured from the local market. High amylose corn starch (HACS)-Hylon VII and guar gum were provided by Ingredion ANZ Pty Ltd, New Zealand. Xanthan gum and locust bean gum were from Danisco Australia Pty Ltd, and sodium carboxymethylcellulose was from CP Kelco Oy, Finland. For *in vitro* starch digestion, α-amylase (*Aspergillus oryzae*, 1.5 U/mg), pepsin (porcine gastric mucosa, 800-2500 U/mg protein), pancreatin (hog pancreas, 4 × USP), and invertase (Invertase, grade VII from baker's yeast, 401 U/mg solid), were procured from Sigma-Aldrich Ltd. (St Louis, USA). Amyloglucosidase (3260 U/ml) used for *in vitro* digestion was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland). All other chemicals used in the study were of analytical grade.

5.2.2 Extraction of soluble components of wheat and modification of HACS

Briefly, the solubles from wheat, i.e., water-soluble, salt-assisted water-soluble, and acid-soluble fractions were extracted from wheat flour. An acid-soluble fraction was also extracted from vital gluten. These soluble extracts from wheat flour and vital gluten have been mentioned

as wheat solubles throughout the thesis. HACS was then added to these extract solutions, and the mixture was dried using spray drying.

5.2.2.1 Water-soluble extract from wheat flour

For a water-soluble extract, a method by Mani et al. (1992) and Hill et al. (2008) was followed with slight modifications. The wheat flour was mixed with water in a 1:10 ratio using a high-shear mixer (LMA-5 Laboratory Mixer, Silverson) at 7000rpm for 10 min and then stirred for 2 h at 8 °C using a magnetic stir bar. The slurry was centrifuged at 4000 × *g* for 20 min to separate the water-soluble fraction which was used for further study.

HACS was then added to this extract to prepare a 10% (w/v) mixture, and it was allowed to mix for 15 h at room temperature (25±5 °C) with continuous stirring. This mixture was then dried using a bench-top spray dryer (Mini Spray Dryer B-290 Acid resistant 200 V, BUCHI), with an inlet temperature of 150 °C, outlet temperature of 85-90 °C, and pump setting at 10-14%. This treated starch powder was collected, sealed, and stored at a cool and dry place before further analysis and termed as HWS.

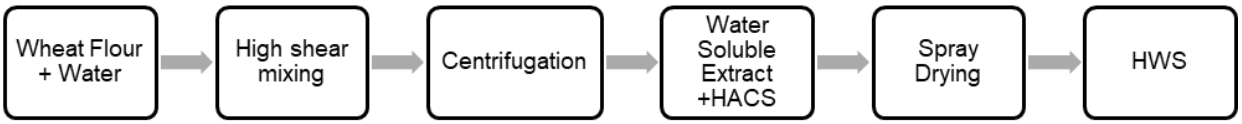

Figure 5. 1 *Schematic diagram of the preparation of HWS (high amylose corn starch-modified with water-soluble wheat flour extract). HACS-high amylose corn starch*

5.2.2.2 Salt-assisted water-soluble extract from wheat flour

The salt-assisted water-soluble fraction was extracted by preparing the dough with salt water and then washing the dough to separate the solubles using a method by Ukai et al. (2008) with some modifications. Briefly, a dough was prepared with 0.51 M NaCl solution (67 ml NaCl solution/100 g flour) by mixing for 20 min using a stand mixer (KSM195, KitchenAid). The

dough was washed sequentially 3 times with distilled water (500 ml/100 g flour) by mixing in the stand mixer for 10 min. The wash liquid was separated from the dough and then centrifuged at 18000 × *g* for 10 min. The supernatants from 3 washes were combined and used for further study.

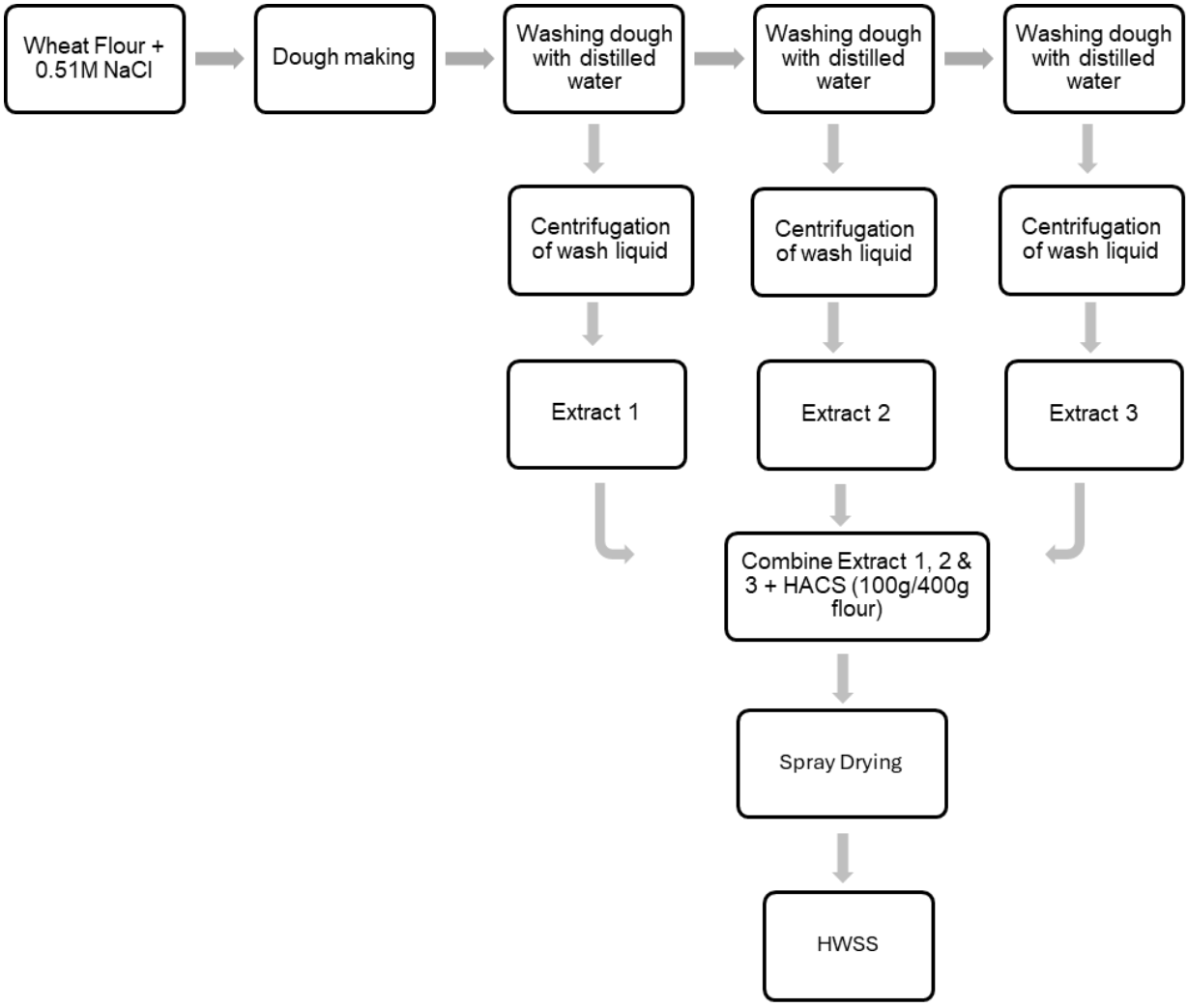

Figure 5. 2 *Schematic diagram of the preparation of HWSS (high amylose corn starch-modified with salt-assisted water-soluble wheat flour extract). HACS-high amylose corn starch*

HACS was then added to this extract to prepare a 10% (w/v) mixture, and it was allowed to mix for 15 h at room temperature (25±5 °C) with continuous stirring. This mixture was then dried using a bench-top spray dryer (Mini Spray Dryer B-290 Acid resistant 200 V, BUCHI),

with an inlet temperature of 150 °C and pump setting at 10-14%. This treated starch powder was collected, sealed, and stored at a cool and dry place before further analysis and termed HWSS.

5.2.2.3 Acid-soluble extract from wheat flour and vital gluten

For the acid-soluble extract from wheat flour, the flour was mixed with 0.625 mM HCl solution at a ratio of 1:3 using a high-shear mixer (LMA-5 Laboratory Mixer, Silverson) at 7000 rpm for 10 min. This mixture was then centrifuged at 4000xg for 15 min, and the supernatant was adjusted for pH 5.8 using food-grade NaOH (0.1 M) (Larsson & Eliasson, 1997; MacRitchie, 1987; Murase et al., 2001).

Similarly, the acid-soluble extract was obtained from vital gluten using the same procedure with vital gluten powder to HCl solution ratio of 1:19.6 (to maintain the initial protein content similar to wheat flour).

Similar to the earlier treatments, HACS was added to the soluble extract to prepare a 10% (w/v) solution, stirred for 15 h at room temperature (25±5 °C). Spray drying was conducted using a bench-top spray dryer (Mini Spray Dryer B-290 Acid resistant 200 V, BUCHI) at an inlet temperature of 150 °C. The treated starch powder was collected, sealed, and stored at a cool and dry place before further analysis. Treated starches with an acid-soluble extract from wheat flour and an acid-soluble extract from vital gluten were termed as HASF and HASV, respectively.

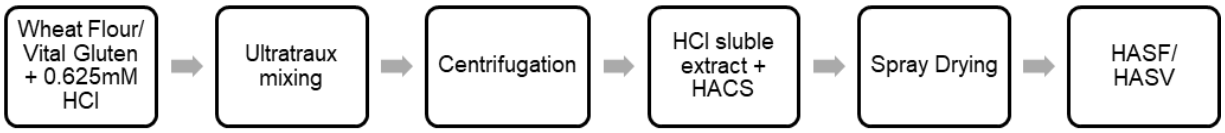

Figure 5. 3 *Schematic diagram of the preparation of HASF and HASV (High amylose corn starch-modified with acid-soluble extracts from wheat flour and vital gluten). HACS-high amylose corn starch*

5.2.3 Modification with hydrocolloids

Solutions of guar gum, xanthan gum, locust bean, and sodium carboxymethylcellulose at a concentration of 0.25% were prepared by solubilising the hydrocolloid powder in water using a high-shear mixer (LMA-5 Laboratory Mixer, Silverson). HACS (100 g) was added to 1000 g of each hydrocolloid solution and stirred for 15 h at room temperature (25±5 °C). The starch-hydrocolloid mixture was then spray dried using a bench-top spray dryer (Mini Spray Dryer B-290 Acid resistant 200 V, BUCHI) at 150 °C inlet temperature. The treated starch powders were collected and stored in airtight pouches at a cool and dry place before further analysis. The HACS treated with guar gum, xanthan gum, locust bean gum, and sodium carboxymethylcellulose were termed HGG, HXG, HLBG, and HCMC, respectively.

Figure 5. 4 *Schematic diagram of the preparation of HGG, HXG, HLBG, and HCMC (high amylose corn starch-modified with guar gum, xanthan gum, locust bean gum, and carboxymethyl cellulose, respectively). HACS-high amylose corn starch*

5.2.4 Dry matter, protein, total starch, and water-holding capacity of modified starches

The treated starch samples were evaluated for moisture and protein content using oven drying at 104 °C for 3 h (AOAC 935.29) and the Keldahl method (AOAC 2001.11), respectively. A total starch assay kit (K-TSTA 07/11, Megazyme International, Wicklow, Ireland) was used to determine the total starch content. Water holding capacity was determined using a method by Ocloo et al. (2014). A suspension of starch sample in water (1:5) was prepared and stirred for 30 min in a shaking water bath at room temperature (25±5 °C), followed by centrifugation at

3500xg for 30 min at 20 °C. The weight of the sediment resulting after decanting the supernatant was measured to calculate the water retained per gram of sample (g water/g solid).

5.2.5 Microstructural characteristics of modified starches

The starch samples were placed onto aluminium stubs and sputter-coated with gold (Baltec SCD 050, Balzers, Liechtenstein). An accelerating voltage of 20 kV was used to observe the coated samples using scanning electron microscopy (FEI Quanta 200, FEI Electron Optics, Eindhoven, Netherlands).

5.2.6 X-ray diffraction (XRD) of modified starches

The starch powders were tightly packed in sample holders and scanned over a range of 4-35° 2θ angle at the rate of 2°/min using an X-ray diffractometer (Rigaku MiniFlex PXRD). Operating conditions of 15 mA and 40 kV were used to evaluate the powder X-ray diffraction (XRD) pattern of modified starch samples. The relative crystallinity of the samples was calculated using equation 5.1 (Chen et al., 2018):

$$Relative\ crystallinity = \frac{Ac}{Ac+Aa} \times 100 \quad (5.1)$$

Ac refers to the area of the crystalline peak, and Aa is the area of the amorphous peak.

5.2.7 RVA pasting properties of modified starches

The modified starch samples were evaluated for the pasting profile by Rapid Visco-Analyser (RVA, Newport Scientific, Sydney, Australia). An extended testing profile was created to observe the pasting properties of high amylose starch following a method by Ozturk, Koksel, Kahraman, et al. (2009) with slight modifications. Starch and water suspensions were prepared using a 4 g sample (14% moisture basis) and 25 g of distilled water. The suspensions were equilibrated at 30 °C for 6 min, heated to 95 °C (13 °C/min), and held for 22 min, then cooled to 40 °C (11 °C/min) and held at 40 °C for 2 min.

5.2.8 *In vitro* oral-gastro-small intestinal starch digestion of modified starch

5.2.8.1 Preparation of cooked sample

The modified starch powders were mixed with water (1:5; w/v) and cooked at 100 °C in a boiling water bath for 20 min. The cooked mixture was immediately cooled to 37 °C and used for further analysis.

5.2.8.2 In vitro starch hydrolysis

In vitro oral-gastro-small intestinal starch digestion of the cooked modified starches was performed according to the method of Chen et al. (2020). Simulated salivary fluid (SSF) with α-amylase, simulated gastric fluid (SGF) with pepsin, and simulated intestinal fluid (SIF) with pancreatin, invertase, and amyloglucosidase were prepared fresh before use. For the oral digestion phase, the cooked starch samples were mixed with SSF in a 1:1 ratio and crushed 20 times using a mortar and pestle to mimic chewing. The bolus was then transferred to the glass reactor and topped up with water to make approximately 4% starch suspension. Immediately, the pH of reactor content was reduced to 2 using 3 M and 0.5 M HCl followed by the addition of SGF to initiate the gastric digestion phase. The pH of the reactor content was maintained at 1.2 throughout the gastric digestion phase. After 30 min of the gastric digestion phase, the gastric enzymes were deactivated by increasing the pH of reactor content to 6.8 using 3 M and 0.5 M NaOH. Next, the small-intestinal digestion phase was initiated by adding SIF to the same reactor content, and the pH was maintained at 6.8 throughout 120 min of the small-intestinal digestion phase. An aliquot of 0.5 ml was taken at different digestion phases (2 min of simulated oral digestion (O2), 0, 15 and 30 min of simulated gastric digestion (G0, G15, and G30), and 0, 5, 10, 15, 30, 60, 90 and 120 min of simulated small-intestinal digestion (I0, I5, I10, I15, I30, I60, I90, and I120)). The aliquots were evaluated for glucose release using GOPOD reagent, and the results were expressed as % starch hydrolysis using equation 5.2 (Tamura et al., 2016a):

$$\% SH = 0.9 \times \frac{Gp}{Si} \quad (5.2)$$

where %SH is the percentage of starch hydrolysis, Gp is glucose produced, and Si is the initial amount of starch.

5.2.9 Statistical analysis

Results were reported as mean±standard deviation for triplicate measurements. The data were analysed using one-way analysis of variance (ANOVA) and Tukey's test for the significance of differences in the means *($p<0.05$)* using Minitab Statistical Software (version 21.3.1).

5.3 Results and discussion

5.3.1 Effect of modifications on the dry matter, protein, total starch, and water holding capacity of HACS

The dry matter, protein content, and total starch of modified starches have been mentioned in Table 5.1. The protein content of the starches varied from 0.59±0.03% to 4.03±0.03% and appeared to depend on the treatment type of modification. The protein content differed significantly among all the starches treated with wheat solubles. The differences in the protein content of differently treated starches show the difference in the protein content extracted from different extraction methods. HWS was found to contain lower protein content and higher starch content than HWSS. HWS is expected to contain mainly the water-soluble albumins from wheat flour (Osborne, 1924) as well as other water-soluble polysaccharides; on the other hand, the water-soluble extraction with the aid of NaCl is expected to be rich in gliadin as reported by Ukai et al. (2008). Moreover, treating HACS with the acid-soluble extract from flour resulted in the highest protein content. This shows that the acid-soluble extract resulted in a higher amount of protein extraction from wheat flour compared to vital gluten, indicating the extraction of other soluble proteins from flour in the acid-soluble extract. The protein in the extracts from flour has been reported to be rich in gliadin fraction of the protein (Murase et al.,

2001); therefore, HASF and HASV could be assumed to be loaded with protein, rich in gliadin-like protein from wheat flour and vital gluten respectively. The total starch content was also found to vary among the differently treated starches, which could be due to the difference in protein content as well as the presence of other soluble wheat polysaccharides (Comino et al., 2014).

Table 5. 1 *Dry matter, protein, and total starch of control HACS and its modified counterparts with wheat solubles and hydrocolloids*

Sample	Dry matter (%) (Mean±SD)	Protein (%dwb) (Mean±SD)	Total starch (%dwb) (Mean±SD)	Water holding capacity (%dwb) (Mean±SD)
HACS	87.35±0.11^{f}	0.66±0.04^{e}	98.56±0.12^{a}	1.25±0.04^{e}
HWS	90.13±0.11^{a,b,c}	1.89±0.07^{c}	89.46±0.13^{e}	1.88±0.04^{a,b}
HWSS	90.26±0.17^{a,b}	2.27±0.04^{b}	88.33±0.39^{f}	1.93±0.03^{a,b}
HASF	90.35±0.17^{a,b}	4.03±0.03^{a}	88.28±0.34^{f}	1.65±0.04^{c}
HASV	90.79±0.2^{a}	1.49±0.08^{d}	98.24±0.45^{a}	1.46±0.03^{d}
HGG	88.3±0.31^{e}	0.64±0.01^{d}	93.68±0.1^{d}	1.96±0.03^{a}
HXG	89.18±0.42^{d}	0.62±0.03^{d}	94.28±0.66^{c,d}	1.86±0.02^{b}
HLBG	89.69±0.32^{b,c,d}	0.64±0.01^{d}	95.6±0.31^{b}	1.57±0.02^{c}
HCMC	89.44±0.46^{c,d}	0.59±0.03^{d}	95.27±0.45^{b,c}	1.32±0.03^{e}

Different superscripts in the same column indicate a significant difference (n=3, p<0.05). HACS-high amylose corn starch, HWS-modified with water-soluble wheat flour extract, HWSS-modified with salt-assisted water-soluble wheat flour extract, HASF-modified with acid-soluble wheat flour extract, HASV-modified acid-soluble vital gluten extract, HGG-high modified with guar gum, HXG-modified with xanthan gum, HLBG-modified with locust bean gum, HCMC-modified with carboxymethyl cellulose.

Furthermore, the variation in the protein content of HACS treated with hydrocolloids was found to be insignificant, as shown in Table 5.1, while the total starch was found to be slightly higher in the HLBG compared to HCMC, HGG, and HXG. As the hydrocolloids contain negligible amounts of proteins, the protein content of resulting modified starches was not affected significantly.

The water-holding capacity of HACS increased after all the treatments. It was found in the order of HGG>HXG>HLBG>HCMC for the starches modified with hydrocolloids and HWSS>HWS>HASF>HASV for the starches modified with wheat solubles. Since the hydrocolloids used in this study possess a high water absorption capacity (Dogan et al., 2011), an increased water absorption capacity was evident after the treatment with hydrocolloids. Moreover, the presence of water-soluble polysaccharides, soluble starches, and protein components from wheat flour also increased the water absorption capacity of the treated starches (Roman-Gutierrez et al., 2002).

5.3.2 Microstructure of modified starches

The treated starches were observed under SEM, and their microstructure was studied. The micrographs were evaluated for the changes in granule morphology and surface characteristics that occurred in the treated starches during modification (Figures 5.5 and 5.6).

5.3.2.1 Effect of modification with wheat solubles

The surface of the treated starches was found to be significantly different from the control HACS. The smooth surface exhibited by the control starch (Figure 5.5-a) has appeared to turn into a rougher surface texture due to the extracted components attached to it. The SEM micrograph of HWS revealed cluster formation of the starch as indicated by arrows in Figure 5.5-b. This cluster formation could be due to the heating and drying of soluble polysaccharides and proteins from water-soluble wheat flour extract, resulting in clusters of starch granules.

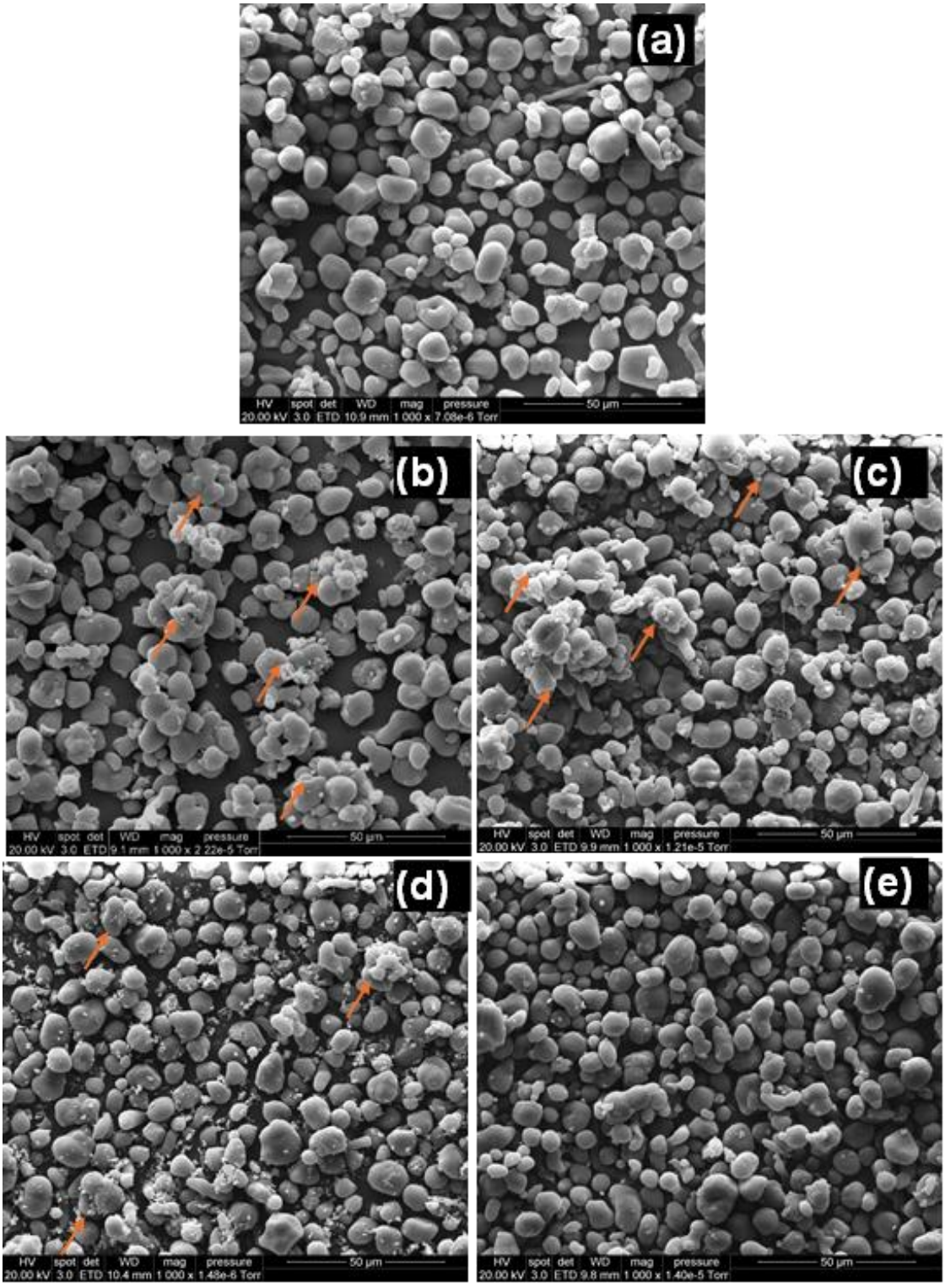

Figure 5. 5 *SEM micrographs of HACS (a), and its modified counterparts with wheat solubles, HWS (b), HWSS (c), HASF (d), and HASV (e). Arrows indicate the cluster formation. (scale: 50µm). HACS-high amylose corn starch, HWS-modified with water-soluble wheat flour extract, HWSS-modified with salt-assisted water-soluble wheat flour extract, HASF-modified with acid-soluble wheat flour extract, HASV-modified with acid-soluble vital gluten extract.*

This phenomenon was also evident in SEM micrographs of HWSS and HASF but least observable in the HASV, as shown in Figures 5.5-c, d, and e. This showed that the presence of water-soluble components such as polysaccharides from wheat flour resulted in cluster formation of starch granules, mimicking the microstructure of wheat flour where clusters of starch granules are present along with individual starch granules.

Surface modification of starches has been reported in the literature where the corn starch granules were completely coated or entrapped within the zein protein using a spray drying technique (Xu & Zhang, 2014); however, the present study shows cluster formation of starch granules. Cluster formation has also been observed in studies by Mao et al. (2023) and Zhu et al. (2020), where starch and protein were treated with dry heat. Other than the cluster formation, the soluble components of wheat were observed to be present on the surface of the starch granules. Similarly, the protein has been found to adhere to the starch granules after dry heat treatment of starch-protein mixtures in previous studies (Mao et al., 2023; Noisuwan et al., 2011; Zhu et al., 2020).

5.3.2.2 Effect of modification with hydrocolloids

Starches treated with hydrocolloids revealed a microstructure similar to HWS. The hydrocolloids aggregated the starches and resulted in numerous clusters of starch-hydrocolloids from all the treatments. Other than the formation of clusters, hydrocolloids were also present on the surfaces of starch granules (Figure 5.6). These microstructures also mimic the wheat flour microstructure due to the presence of starch-hydrocolloid clusters and individual starch granules with hydrocolloids present on their surface. Similar clusters of starch-hydrocolloids have been observed in a previous study where the corn starch was modified with guar gum and xanthan gum using heat-moisture treatment (Zhou et al., 2020). CMC has also been reported to create clusters of starch and hydrocolloids when modified using dry heat treatment (Chandanasree et al., 2016).

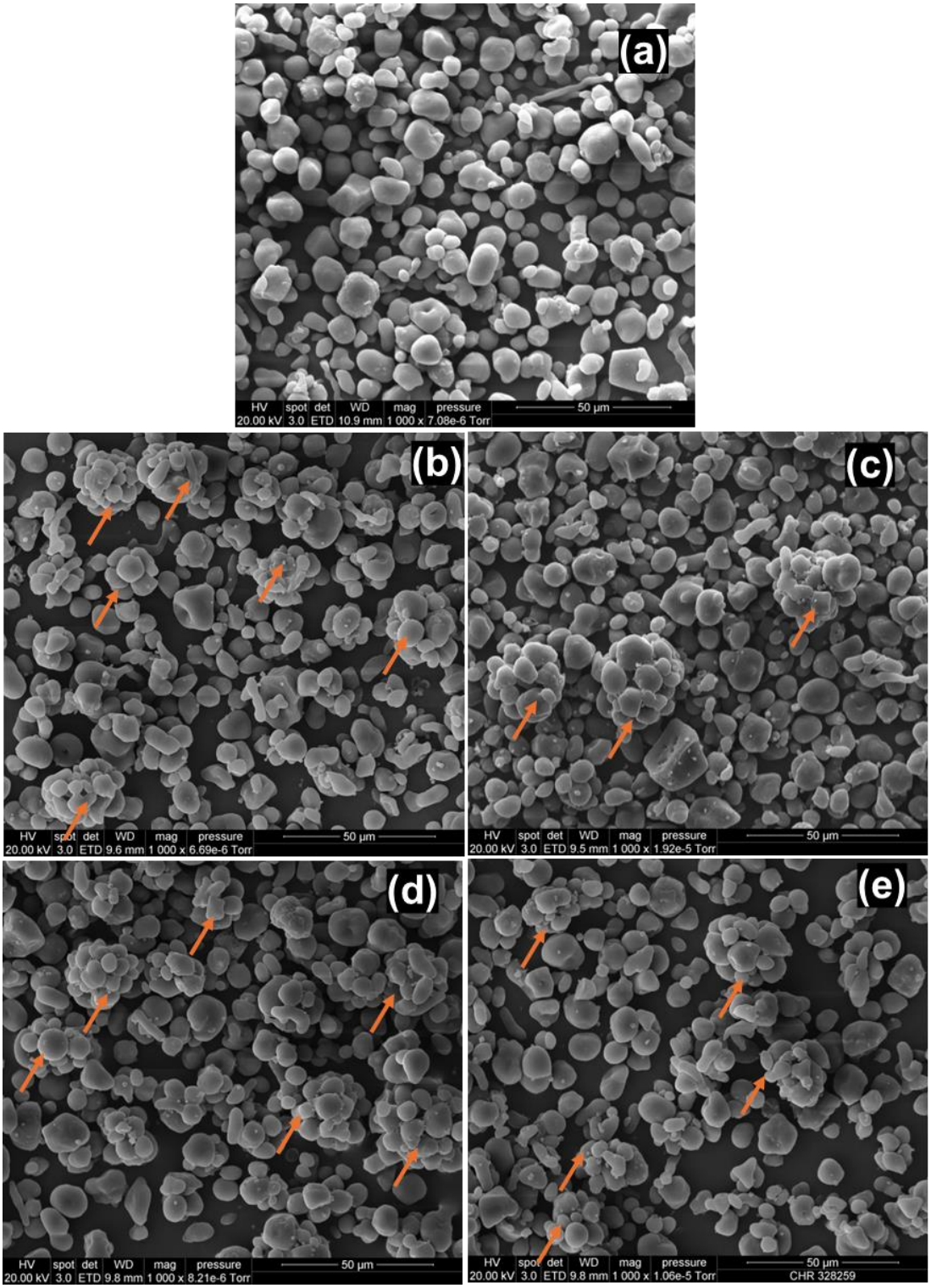

Figure 5. 6 *SEM micrographs of HACS (a), and its modified counterparts with hydrocolloids, HGG (b), HXG (c), HLBG (d), and HCMC (e). Arrows indicate the cluster formation. (scale: 50µm). HACS-high amylose corn starch, HGG-modified with guar gum, HXG-modified with xanthan gum, HLBG-modified with locust bean gum, HCMC-modified with carboxymethyl cellulose.*

5.3.3 Crystalline structure of modified starches

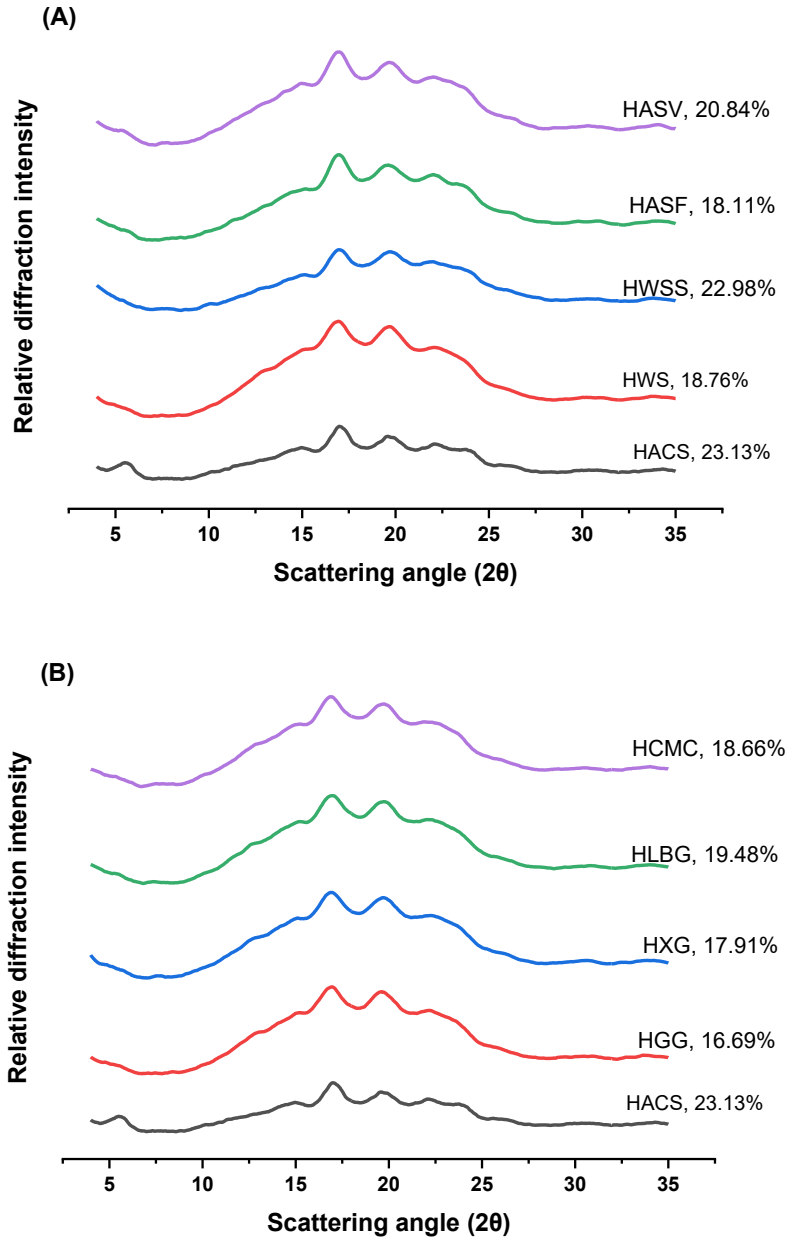

***Figure 5.** 7 X-ray diffraction pattern and relative crystallinity of HACS and its modified counterparts with wheat solubles (A) and hydrocolloids (B). HACS-high amylose corn starch, HWS-modified with water-soluble wheat flour extract, HWSS-modified with salt-assisted water-soluble wheat flour extract, HASF-modified with acid-soluble wheat flour extract, HASV-modified acid-soluble vital gluten extract, HGG-high modified with guar gum, HXG-modified with xanthan gum, HLBG-modified with locust bean gum, HCMC-modified with carboxymethyl cellulose.*

X-ray diffraction revealed the distinctive pattern corresponding to HACS. Distinctive peaks at 2θ angles of 5.5°, 11.3°, 15°, 17°, 19.8°, 22°, and 23.7° were observed in agreement with previous studies (Guo et al., 2024; Tian et al., 2024). It shows that HACS and its modified counterparts exhibited a combination of B-type and V-type crystalline patterns. In general, the crystalline structure of starch was not much affected by the modification processes as a similar pattern of peaks was followed by all the modified starches, as shown in Figure 5.7. However, the peak intensity at 2θ 5.5° was reduced after all the treatments, possibly attributed to spray drying involving high-temperature, short-time treatment. Similar results were obtained by Yang et al. (2016), where hydrothermal treatment of HACS caused the depression of peak at 2θ 5°. The relative crystallinity decreased after the modification, which could be due to the exposure to high temperatures for a short time during spray drying.

5.3.3.1 Effect of modification with wheat solubles

X-ray diffraction pattern of the modified starches with wheat solubles followed a pattern similar to HACS; however, the peak intensity at 2θ 5.5° was reduced after all the treatments. The relative crystallinities of modified starches were lower than HACS. HASF was found to have the lowest relative crystallinity, followed by HWS, HASV, and HWSS. This could be due to the high temperature and high moisture treatment of the starches during spray drying, causing the loss of some of the crystalline regions of the starch (Zhou et al., 2020). In addition, the interaction of starch with water-soluble polysaccharides and proteins from wheat flour could impact the relative crystallinity of the modified starches. The addition of protein and fibre during thermo-mechanical modification has been reported to reduce the crystallinity of rice starch (Qadir & Wani, 2023). The relative crystallinity of HASV was higher than HASF, indicating that the soluble protein component from vital gluten had less impact on crystallinity as compared to soluble protein and other soluble polysaccharides from wheat flour. Zhao et al. (2021) have reported that the interaction of soluble polysaccharides from soybeans reduced the

relative crystallinity of starch. Moreover, the relative crystallinity of HWSS was close to HACS despite the presence of soluble proteins and polysaccharides derived from wheat flour. This similarity could be due to the small amount of NaCl introduced during the modification process.

5.3.3.2 Effect of modification with hydrocolloids

Modification with hydrocolloids resulted in a similar X-ray diffraction pattern as HACS. However, the peak intensity at 2θ 5.5° was reduced, similar to the modification with wheat solubles, possibly due to the heat treatment during spray drying. The relative crystallinity was also reduced as a result of modification with hydrocolloids. Guar gum resulted in the lowest relative crystallinity among all the hydrocolloids used, whereas HLBG was found to have a relative crystallinity higher than HCMC and HXG. This difference could be due to the interaction of the particular hydrocolloid with the starch during modification. Heat-moisture treatment reduces the relative crystallinity of starch, possibly caused by damage and rearrangement of the double helix in the crystalline region of the starch (Wang, Li, et al., 2017; Zhou et al., 2020). Furthermore, the addition of hydrocolloids could also weaken the crystalline region by interacting with starch (Zhou et al., 2020).

5.3.4 RVA pasting properties of modified starches

RVA was used to evaluate the viscometric properties of the modified starches under controlled heating and cooling cycles. RVA pasting parameters, peak viscosity, and final viscosity of these starches have been shown in Table 5.2.

Table 5. 2 *RVA pasting parameters of control HACS and its modified counterparts with wheat solubles and hydrocolloids*

Sample	PV (cP) (Mean±SD)	FV (cP) (Mean±SD)
HACS	132±1 ^{e}	267±1 ^{e}
HWS	119±2 ^{f}	124±2 ^{h}
HWSS	134±1 ^{e}	138±1 ^{g}
HASF	120±2 ^{f}	120±3 ^{h}
HASV	196±2 ^{d}	278±1 ^{d}
HGG	511±1 ^{a}	747±1 ^{a}
HXG	241±1 ^{c}	299±1 ^{c}
HLBG	344±2 ^{b}	538±3 ^{b}
HCMC	131±2 ^{e}	194±1 ^{f}

Different superscripts in the same column indicate a significant difference (n=3, p<0.05). PV-peak viscosity, FV-final viscosity. HACS-high amylose corn starch, HWS-modified with water-soluble wheat flour extract, HWSS-modified with salt-assisted water-soluble wheat flour extract, HASF-modified with acid-soluble wheat flour extract, HASV-modified acid-soluble vital gluten extract, HGG-high modified with guar gum, HXG-modified with xanthan gum, HLBG-modified with locust bean gum, HCMC-modified with carboxymethyl cellulose.

5.3.4.1 Effect of modification with wheat solubles

The pasting profile of HACS and its modified counterparts with wheat solubles has been shown in Figure 5.8. Compared to HACS, the peak hot paste viscosity (PV) of HASV was found to be higher, HWSS was similar, while PV for HWS and HASF were slightly lower. However, overall, a low value of PV was observed for all the starches. The high amylose content of these starches, being an inhibitor of swelling, resulted in lower peak viscosity values (Lv et al., 2021). This low value of PV could also be due to the presence of amylose-lipid complexes in the HACS (Singh et al., 2014), and it corresponds to the low swelling power of the starches (Obadi

et al., 2023). Similar results for low PV of HACS, attributed to their resistance to disintegration and limited swelling, have been reported by Schirmer et al. (2013).

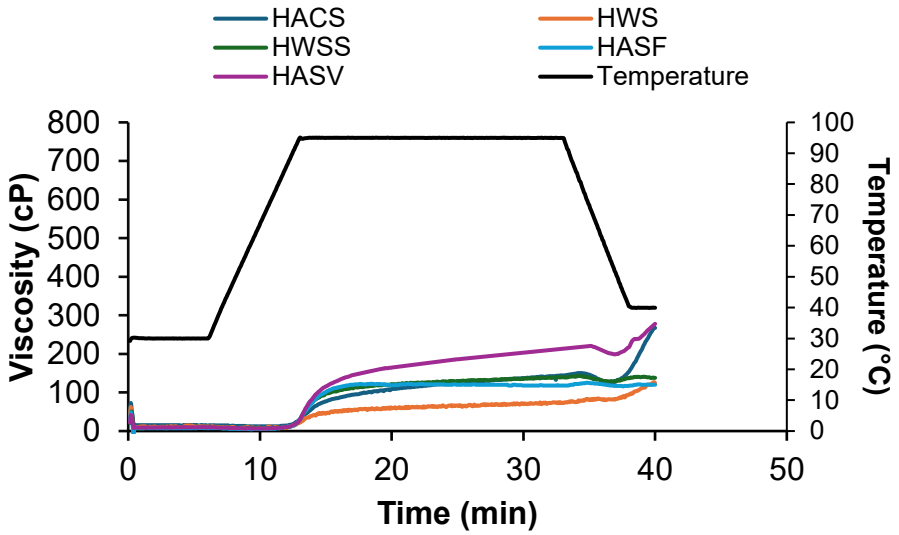

Figure 5. 8 *Pasting profile of HACS, and its modified counterparts treated with wheat solubles. HACS-high amylose corn starch, HWS-modified with water-soluble wheat flour extract, HWSS-modified with salt-assisted water-soluble wheat flour extract, HASF-modified with acid-soluble wheat flour extract, HASV-modified with acid-soluble vital gluten extract.*

The RVA pasting curve demonstrated that the final viscosity of HACS was slightly lower than HASV, while higher than HWS, HWSS, and HASF. The modification of HACS with the wheat solubles could have altered the amylose content of the starch since the total starch content was reduced after modification. Moreover, the proteins from wheat associated with the modified starches could result in a starch-protein interaction in such a way that it impacts the swelling of the starch granules and the gelatinisation of the starch granules. The final viscosity was observed to decrease as the protein content of the modified starches increased. W. Li et al. (2023) have reported that the presence of protein in corn starches increased their heat stability and pasting temperature, impacting their gelatinisation and viscosity profile. The protein components present in the modified starch reduced the interaction of starch and water and

slowed down the swelling of starch, resulting in lower values of peak and final viscosities (Shao et al., 2023). However, the pasting profile of HASV differed from that of other modified starches, which could be attributed to the lower protein content. The absence of any soluble polysaccharides from wheat flour could also be a contributing factor to the viscosity profile of the modified starches HASV (Kong et al., 2020).

A continued increase in the viscosity was observed for all the starches during the heating, holding, and cooling phases; therefore, the pasting parameters, such as trough viscosity, breakdown viscosity, and setback viscosity, were not recorded. Also, the pasting temperature of any of the starch samples was not detected in the RVA measurements in this study, suggesting that temperatures up to 95 °C were insufficient to cause the complete gelatinization of high amylose corn starch. The high pasting temperature of these starches is indicative of their higher resistance to swelling (H. Li et al., 2019).

5.3.4.2 Effect of modification with hydrocolloids

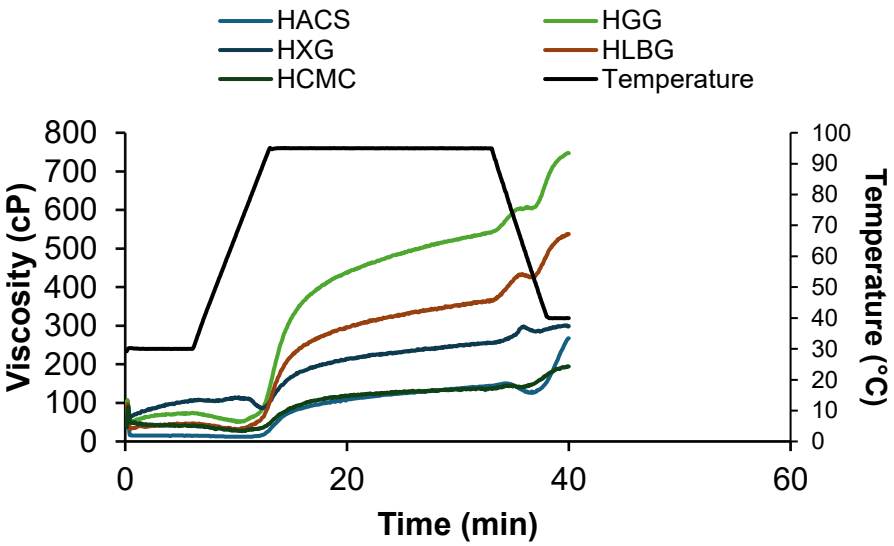

Figure 5. 9 *Pasting profile of HACS and starches treated with hydrocolloids. HACS- unmodified high amylose corn starch, HGG-modified with guar gum, HXG-modified with xanthan gum, HLBG-modified with locust bean gum, HCMC-modified with carboxymethyl cellulose.*

The pasting profiles of starches modified with hydrocolloids were found to be significantly different than HACS, as is shown in Figure 5.9. The PV increased for all the starches modified with hydrocolloids in this study and was found to be highest for HGG, followed by HXG, HLBG, and HCMC. This increase in PV could be due to the high water absorption capacity of hydrocolloids associated with the starches, as PV is correlated with the swelling power or its ability to bind with free water (X. Zhang et al., 2023). The increase in peak viscosity has also been reported in the literature with the addition of hydrocolloids. Locust bean gum has been reported to enhance the peak viscosity of rice starch (Correa et al., 2013), and κ-carrageenan increased the peak viscosity of hydroxypropylated cassava starch (Mi et al., 2021). The increase in the peak viscosity on adding hydrocolloids could be associated with the interaction of starch and hydrocolloids, such as hydrogen bonding (X. Zhang et al., 2023).

Similar to the starches modified with wheat solubles, the pasting temperature was not detected, indicating an insufficient temperature range for complete gelatinisation of the starches in this study due to the high amylose content of these starches. The final viscosity of modified starches was found to be highest for HGG, followed by HLBG, HXG, and HCMC. Increased viscosity for the modified starches might be correlated with a higher water absorption capacity of hydrocolloids (Santamaria et al., 2023).

5.3.5 *In vitro* starch hydrolysis of modified starches

The results of *in vitro* oral-fastro-small intestinal digestion of profiles of the starches cooked at 100 °C for 20 min indicated that the modifications of HACS significantly influenced the starch hydrolysis, as shown in Figures 5.10 and 5.11.

5.3.5.1 Effect of modification with wheat solubles

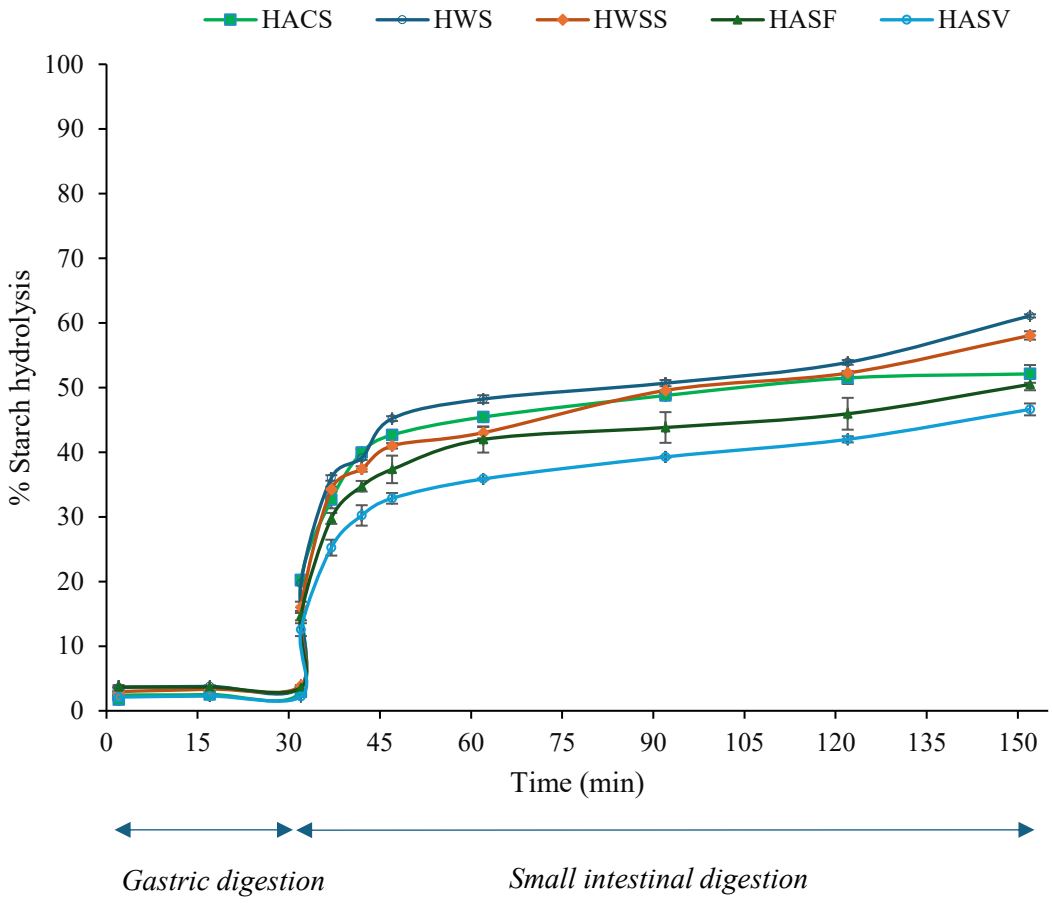

Figure 5. 10 *In vitro starch hydrolysis of HACS and its modified counterparts with wheat solubles. Error bars represent standard deviation. HACS-high amylose corn starch, HWS-modified with water-soluble wheat flour extract, HWSS-modified with salt-assisted water-soluble wheat flour extract, HASF-modified with acid-soluble wheat flour extract, HASV-modified with acid-soluble vital gluten extract.*

The majority of studies available in the literature have evaluated the digestibility of high amylose starches in their native state. In this study, the modified HACSs with solubles from wheat using high heat treatment for a short time during spray drying were cooked as mentioned in section 5.2.7.1 before their *in vitro* starch hydrolysis. Compared to HACS, the overall starch hydrolysis was found to be reduced for HASV, and it was not significantly different from

HASF. This reduced overall starch hydrolysis could be correlated to the microstructure observed by SEM, where the acid-soluble protein from wheat flour and vital gluten modified the surface, making it less available for digestive enzymes. These results indicate that gliadin could inhibit digestive enzymes since the acid-soluble extracts are rich in gliadin-like protein (Murase et al., 2001). Also, Wu and Warren (2023) have reported that dilute HCl soluble extract from wheat flour was found to contain proteinaceous α-amylase inhibitors, which helped in lowering the starch hydrolysis of wheat starch. In addition, the lesser impact on relative crystallinity was also a contributing factor to the lower starch hydrolysis of HASV compared to HACS. The slower rate of digestion in HASV could also be attributed to its RVA pasting profile; the increased viscosity of the starch could hinder the digestive enzymes, leading to slower digestion. The addition of protein has been reported to reduce starch hydrolysis (Wu et al., 2024); however, in the case of HASF, the overall starch hydrolysis was found to be higher than HASV despite the higher protein content of HASF. This could be attributed to the lower relative crystallinity as well as the viscosity profile of HASF compared to HASV. Moreover, soluble starch from wheat flour could have resulted in enhanced starch hydrolysis.

On the other hand, the starch hydrolysis increased for HWS and HWSS compared to HACS. Despite the higher protein content of HWS and HWSS compared to HASV, the rate of starch hydrolysis was found to be higher. This could be due to the solubles extracted from the flour, which resulted in the lower viscosity of these starches, as shown in section 5.3.3. Even though clusters of starch and solubles from wheat flour were observed through SEM, they were found to have less of an effect in reducing the rate of starch hydrolysis. HWS was found to have lower relative crystallinity as well as a weak pasting profile, which could be accounted for the higher starch hydrolysis of HWS. Along with possessing relative crystallinity closer to HACS, the HWSS also contains gliadin-like protein from wheat flour (Ukai et al., 2008); however, the

concentration of protein present in HWSS was not efficient in reducing the starch hydrolysis of HWSS.

5.3.5.2 Effect of modification with hydrocolloids

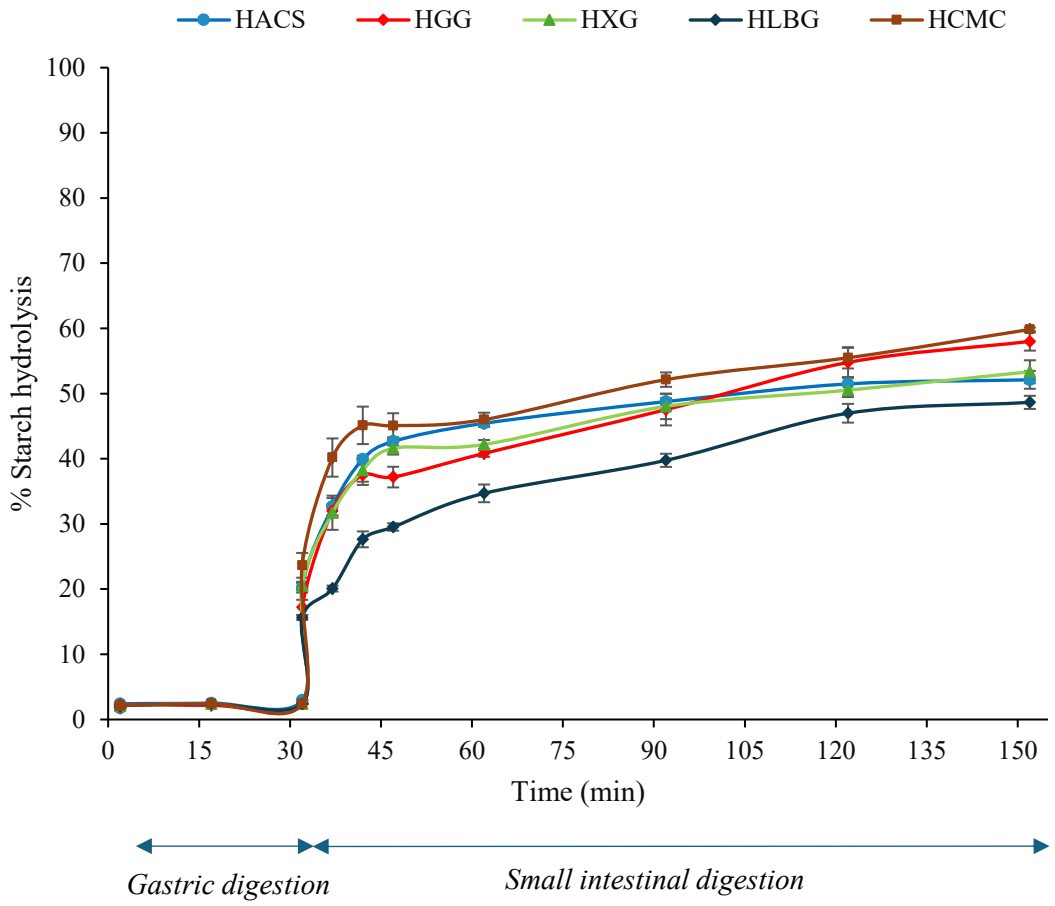

Figure 5. 11 *In vitro starch hydrolysis of HACS and its modified counterparts with hydrocolloids. Error bars represent standard deviation. HACS-high amylose corn starch, HGG-modified with guar gum, HXG-modified with xanthan gum, HLBG-modified with locust bean gum, HCMC-modified with carboxymethyl cellulose.*

The starch hydrolysis profile of HACS and its hydrocolloid-modified counterparts has been shown in Figure 5.11. All other modified starches, except for HCMC, revealed a slower starch hydrolysis profile until 90 min of simulated digestion. However, the total starch hydrolysed

after 120 min of small intestinal digestion was found to be increased for HCMC and HGG. HLBG was found to maintain a lower extent of overall starch hydrolysis than HACS throughout the simulated digestion. In previous studies, locust bean gum has been reported to reduce the starch hydrolysis rate of pea starch (Santamaria et al., 2023) and high amylose rice starch (Jung et al., 2017). This could be attributed to its high viscosity and ability to form large clusters of starch-hydrocolloids, as evident from sections 5.3.2 and 5.3.3. This slower rate of digestion indicates a stronger interaction of HACS and locust bean gum as a result of modification. The initial rate of starch hydrolysis was slower for HGG as well, indicating the role of the high viscosity profile of HGG as shown in section 5.3.3; however, the interaction of HACS and GG was insufficient to maintain the slower digestion rate. Xanthan gum has been reported to reduce the starch hydrolysis. Moreover, HXG maintained its starch hydrolysis rate close to HACS, indicating that the xanthan gum-HACS interaction did not negatively impact the starch hydrolysis. Guar gum and xanthan gum have been reported to reduce the overall starch hydrolysis of corn starch modified with heat-moisture treatment (Zhou et al., 2020). However, the starch hydrolysis was increased in the case of HCMC. This could be attributed to the lower water holding capacity and weak viscosity profile, as evidenced by the results of this study. These results indicate the poor interaction of CMC with HACS as a result of modification.

5.4 Conclusion

This chapter investigated the possibility of utilising soluble components of wheat to modify a resistant starch source, HACS. The role of water-soluble wheat flour extract, salt-assisted water-soluble wheat flour extract, acid-soluble wheat flour extract, and acid-soluble vital gluten extracts was explored to modify HACS. The resulting starches revealed modified physicochemical, microstructural, pasting, and *in vitro* starch hydrolysis characteristics. The modification with solubles from wheat flour resulted in clusters of starch and solubles, reduced

the relative crystallinity, and helped in reducing the rate of starch hydrolysis in the case of HASV, HASF, and HWSS. Modifications of HACS with acid-soluble extracts from wheat flour and vital gluten were found to be more effective in reducing the starch hydrolysis of the cooked (100 °C for 20 min) starch. Salt-assisted water-soluble extracts played a role in reducing the rate of starch hydrolysis until 90 min of simulated digestion; however, total starch hydrolysis was increased.

Similarly, HACS was also modified using hydrocolloids to understand their role in microstructure modification as well as in starch hydrolysis. The modification resulted in starch-hydrocolloid cluster formation and improved their pasting profile. Interaction of locust bean gum with HACS was found to be effective in modifying microstructure and reducing starch hydrolysis. Moreover, guar gum and xanthan gum also played a role in structure modification and slowing down the starch hydrolysis rate of the modified starches.

The results obtained in this chapter create an opportunity for these modified starches to be used in a wheat-based system to improve their digestibility and modify their functional properties.

Chapter 6

How do modified resistant starches behave in a wheat flour system?

a)Would the water-soluble and acid-soluble components from wheat, when associated with resistant starch, help improve its functionality and reduce starch digestibility in a wheat flour system?

b)Would modification with hydrocolloids make them a better ingredient to be used in a wheat flour system?

Chapter 6 Functionality of Resistant Starch Modified with Wheat Solubles and Added Hydrocolloids in a Wheat Flour System with Partial Replacement: Pasting Properties, Dough Characteristics and *In vitro* Starch Hydrolysis of Flatbread (Chapatti)

Abstract

This chapter aimed to formulate a wheat flour system by partial replacement of wheat flour with the modified starches with wheat solubles (soluble extracts from wheat flour and vital gluten) and hydrocolloids developed in the previous chapter and investigate their functionality and impact on starch digestibility. Flatbread (chapatti) was selected as a model food product. Modified starches with wheat solubles improved the dough network as observed through confocal laser scanning microscopy. Also, rheological characteristics such as G' and G" improved with modification with wheat solubles compared to unmodified high amylose corn starch. The starch digestibility was reduced for all the chapattis containing wheat solubles modified starches except with water-soluble wheat flour extract compared to unmodified starch. Furthermore, the interaction of the hydrocolloid-modified starches with vital gluten and wheat flour components resulted in improved viscosity of the functional flour. The microstructure of the functional flour dough indicated that the hydrocolloid-modified starches improved the starch-protein matrix and gluten network. The modified starch with hydrocolloids showed dough rheology closer to wheat flour when the water level in the dough was increased. Moreover, the *in vitro* digestion study revealed that the overall starch hydrolysis of chapattis from most functional flour formulations with hydrocolloid-modified starches was significantly lower than the wheat flour chapatti.

6.1 Introduction

Wheat flour is a major ingredient in many popular and staple foods such as bread, flatbread, noodles, pasta, etc. However, the property of wheat starch to be digested readily makes wheat-based products a choice to consider when it comes to consumers' consciousness of their blood sugar levels. There comes a challenge to develop similar products but with a slower digestion rate. Numerous studies have attempted to replace part of wheat flour with other ingredients such as legume flour or resistant starches in wheat-based products (Arp et al., 2021b; Bajka et al., 2021; Utrilla-Coello et al., 2007). Due to their slower digestion property, resistant starches could be used in wheat-based products to reduce their digestibility; however, they have been reported to lack the functional properties of wheat flour, which limits their application in food products with at-par quality (Arp et al., 2018; Barros et al., 2018).

Therefore, in the previous chapter (Chapter 5), one of the commonly known resistant starches high amylose corn starch (HACS), was loaded with some of the isolated wheat grain components such as water-solubles from wheat flour, salt-assisted water-solubles from wheat flour, and acid-solubles from wheat flour and wheat protein gluten. It was hypothesised that the water-soluble extract from wheat flour containing water-soluble protein albumin and polysaccharides (Pauly & Delcour, 2018), and salt-assisted (NaCl) water-soluble extract rich in gliadin (Ukai et al., 2008) could modify HACS to improve the functionality and starch digestibility when used in a wheat flour-based system. Similarly, acid-soluble (dilute HCl) extract from wheat flour and vital gluten (Macritchie, 1985), being rich in gluten protein components, would help modify HACS for better functionality and starch digestibility. In Chapter 5, HACS were also modified with hydrocolloids, which are commonly used in wheat-based as well as gluten-free products to improve their texture and processing. In wheat flour matrices, hydrocolloids could improve water absorption, pasting behaviour, dough rheology as well as digestibility by interacting with both starch and gluten (J. Li, M. P. Yadav, et al., 2019).

The hydrocolloids used in this study included guar gum, improving water-holding capacity and specific volume (Encina-Zelada et al., 2019; Sasaki, 2018); xanthan gum, improving water-holding capacity and elasticity of dough and adding volume to product (Jafari et al., 2018; Tebben & Li, 2019); locust bean gum, enhancing dough stability during mixing (Blibech et al., 2015); and carboxymethyl cellulose, improving specific volume of bread (Harsono et al., 2021). Hydrocolloids are typically used in the dough preparation step for food product preparation; however, this study modified the matrix structure at a foundation level by integrating hydrocolloids onto resistant starch using spray-drying, offering a novel approach.

In the current chapter, these modified starches have been applied in a wheat flour system by partial replacement of wheat flour to investigate their functionality and impact on the starch digestibility of a resulting food product. Since individual starch and vital gluten interactions result in inferior quality dough, the modified starches were used in a 50% wheat flour system (Figure A 5.3 in Appendix). Chapatti is an unleavened flatbread, and wheat flour is the most commonly used ingredient for the preparation of chapatti. Since chapatti is a staple food in many regions of the world, this chapter leveraged the opportunity to evaluate the dough functionality and *in vitro* starch hydrolysis of chapatti using the modified resistant starches from the previous chapter.

6.2 Materials and methods

6.2.1 Materials

Standard wheat flour (Champion, New Zealand) (moisture content 12.4%, and protein content 12.1%) and commercial vital gluten were procured from the local market. High amylose corn starch (HACS)-Hylon VII was provided by Ingredion ANZ Pty Ltd. Its modified counterparts, HWS-modified with a water-soluble wheat flour extract, HWSS-modified with salt-assisted water-soluble wheat flour extract, HASF-modified with acid-soluble wheat flour extract,

HASV-modified with acid-soluble vital gluten extract, HGG-modified with added guar gum, HXG-modified with added xanthan gum, HLBG-modified with added locust bean gum, and HCMC-modified with added carboxymethyl cellulose, developed in Chapter 5 were used in this study. For *in vitro* starch digestion, α-amylase (*Aspergillus oryzae*, 1.5 U/mg), invertase (Invertase, grade VII from baker's yeast, 401 U/mg solid), pancreatin (hog pancreas, 4 × USP), and pepsin (porcine gastric mucosa, 800-2500 U/mg protein) were procured from Sigma-Aldrich Ltd. (St Louis, USA); and amyloglucosidase (3260 U/ml) was obtained from Megazyme International Ireland Ltd. (Wicklow, Ireland). All other chemicals were of analytical grade.

6.2.2 Formulation of functional flour

The modified starches described in Chapter 5 were mixed with vital gluten to mimic the wheat flour. A mixture of modified starch and vital gluten (85:15) was blended with wheat flour in a ratio of 1:1 to prepare a functional flour formulation. When the modified starches with wheat solubles (soluble extracts from wheat flour and vital gluten) were used, the formulations were termed as HACS FL, HWS FL, HWSS FL, HASF FL, and HASV FL for high amylose corn starch, modified with a water-soluble wheat flour extract, modified with salt-assisted water-soluble wheat flour extract, modified with acid-soluble wheat flour extract and modified with acid-soluble vital gluten extract, respectively. Additionally, the formulations were termed as HGG FL, HXG FL, HLBG FL, and HCMC FL when using starches modified with guar gum, xanthan gum, locust bean gum, and carboxymethyl cellulose, respectively. Formulation with 100% wheat flour was termed as WFL.

6.2.3 Moisture content and water-holding capacities of functional flour

The moisture content of the functional flours was evaluated using oven drying at 104 °C for 3 h (AOAC 935.29). To determine water holding capacity, a suspension of flour in water (1:5)

was prepared and kept in a shaking water bath for 30 min at room temperature (25±5 °C), followed by centrifugation at 3500 × *g* for 30 min at 20 °C. The weight of the sediment resulting after decanting the supernatant was measured to calculate the water retained per gram of sample (g water/g solid) (Ocloo et al., 2014).

6.2.4 RVA pasting properties of functional flour

The functional flours were evaluated for the pasting profile by Rapid Visco-Analyser (RVA, Newport Scientific, Sydney, Australia) using the Standard method (STD 1) (Ma, Sang, et al., 2021). Flour and water suspensions were prepared using a 3.5 g sample (14% moisture basis), and 25 g distilled water. The suspensions were equilibrated at 50 °C for 1 min, heated to 95 °C (12.16 °C/min) and held for 2.5 min, then cooled to 50 °C (12.16 °C/min), and held at 50 °C for 2 min.

6.2.5 Dough rheology

The dough was prepared from the functional flour formulations using a stand mixer (KSM195, KitchenAid) by adding water at room temperature (25±5 °C) and mixing the dough for 5 min. The dough was prepared at three different water levels, 62 ml/100 g flour, 65 ml/100 g flour, and 70 ml/100 g flour to understand the impact of water level on dough functionality. The rheological properties of dough samples were studied by the method of He et al. (2023). The frequency sweep test in a range of 0.1-100 rad/s at 0.01% strain (in the linear viscoelastic region) at 25 °C was performed on the dough samples. A dynamic rheometer (Physica MCR 302, Anton Paar GmbH, Germany) using a parallel plate geometry with a serrated surface probe to prevent slippage was used. After loading, the dough sample was left to rest for 30 min. The excess dough was removed just before the measurement to avoid moisture loss during the resting period. The storage modulus (G'), loss modulus (G"), and loss tangent (tanδ = G"/G'), were recorded to evaluate the rheological characteristics of the dough samples.

6.2.6 Dough microstructure

Doughs were prepared as explained in section 6.2.5 with a water addition of 65 ml/100 g and freeze-dried. Freeze-dried dough samples were sectioned using a razor blade, and fluorescein isothiocyanate (FITC, 0.01%) and rhodamine B (0.001%) were used to stain the starch and protein, respectively in the samples (Cao et al., 2020). The dough microstructure was evaluated using confocal laser scanning microscopy (Zeiss LSM900, Zeiss). The excitation wavelengths were 625 nm and 518 nm for rhodamine B and FITC, respectively.

6.2.7 Preparation of chapatti

Chapattis were prepared using functional flour formulations mentioned in section 6.2.2 following a method by Sachanarula et al. (2022) with some modifications. Dry ingredients were mixed thoroughly and 65 ml water was added at room temperature (25±5 °C), followed by mixing for 5 min in a stand mixer (KSM195, KitchenAid) (Figure A 6.1 in Appendix). The dough was then hand-kneaded for 2 min and allowed to rest for 30 min by covering it with a shrink wrap. Small portions of the rested dough were weighed and then rolled to prepare discs of approximately 2 mm thickness. They were then baked on a temperature-controlled preheated hot plate at 200 °C for 30 s on each side followed by 30 s to puff the chapatti by putting small pressure. The cooked chapattis were cooled on a wire rack to room temperature before further analysis. The loss in weight of chapattis was evaluated as % bake loss by recording the weight of chapattis before baking, and after baking and cooling for 10 min at room temperature (25±5 °C) (Moza & Gujral, 2018).

6.2.8 *In vitro* oral-gastro-small intestinal starch digestion

The i*n vitro* starch digestibility of chapatti samples was assessed using a three-step process simulating oral, gastric, and small intestinal phases as described in section 5.2.8.2.

The estimated glycaemic index (eGI) of the chapatti samples was calculated using equation 6.1 (Goñi et al., 1997):

$$eGI = 39.71 + 0.549HI \quad (6.1)$$

where HI is the hydrolysis index, calculated as the area under the curve during simulated small intestinal digestion using white bread as a reference.

6.2.9 Statistical analysis

Results were reported as mean±standard deviation for triplicate measurements. The data were analysed using one-way analysis of variance (ANOVA) and Tukey's test for the significance of differences in the means using Minitab statistical software (version 21.3.1).

6.3 Results and discussion

6.3.1 Moisture content and water-holding capacity of the functional flour

The moisture content of the functional flours has been shown in Table 6.1. The variation in the moisture content could be attributed to the difference in the moisture content of the modified starches. Moreover, the water-holding capacity of wheat flour and the functional flours with modified starches have been shown in Table 6.1. The amount of water that remains attached to the hydrated flour components after the separation of water using external centrifugation is the water-holding capacity (Hasmadi et al., 2020). Water-holding capacity was found to be increased significantly in all the functional flour formulations. The presence of water-soluble polysaccharides, soluble starches, and protein components from wheat flour in the wheat solubles-modified starches increased their water absorption capacity (Roman-Gutierrez et al., 2002). Therefore, the increase in water-holding capacity of functional flours with wheat soluble-modified starches was due to the high water absorption property of modified starches with wheat solubles. Likewise, the increased water-holding capacity of functional flours containing hydrocolloid-modified starches could be attributed to the high water absorption

property of hydrocolloids and HACS. An increase in water-holding capacity was also observed for the individual-modified starches in the previous chapter, therefore these results are in line with the water-holding capacity of individual-modified starches. The high water absorption capacity of composite flours with resistant starches has also been reported in the literature (Arp et al., 2017; Chandra et al., 2015).

Table 6. 1 *Moisture content and water-holding capacity of functional flours*

Sample	Moisture content (%) (Mean±SD)	Water holding capacity (%dwb) (Mean±SD)
WFL	12.79±0.35 [a]	0.68±0.03 [f]
HACS FL	12.68±0.2 [a]	1.06±0.03 [e]
HWS FL	11.5±0.13 [c]	1.32±0.01 [a]
HWSS FL	11.45±0.21 [c]	1.34±0.03 [a]
HASF FL	11.41±0.2 [c]	1.23±0.03 [b]
HASV FL	11.23±0.26 [c]	1.15±0.03 [c,d]
HGG FL	12.28±0.3 [a,b]	1.36±0.02 [a]
HXG FL	11.91±0.34 [b,c]	1.31±0.01 [a]
HLBG FL	11.69±0.25 [b,c]	1.19±0.03 [b,c]
HCMC FL	11.8±0.21 [b,c]	1.09±0.03 [d,e]

Different superscripts in the same column indicate a significant difference (n=3, $p < 0.05$). WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-solubles wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-solubles wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.

6.3.2 Effect of modified starches on pasting properties of functional flours

6.3.2.1 Effect of modified starches with wheat solubles

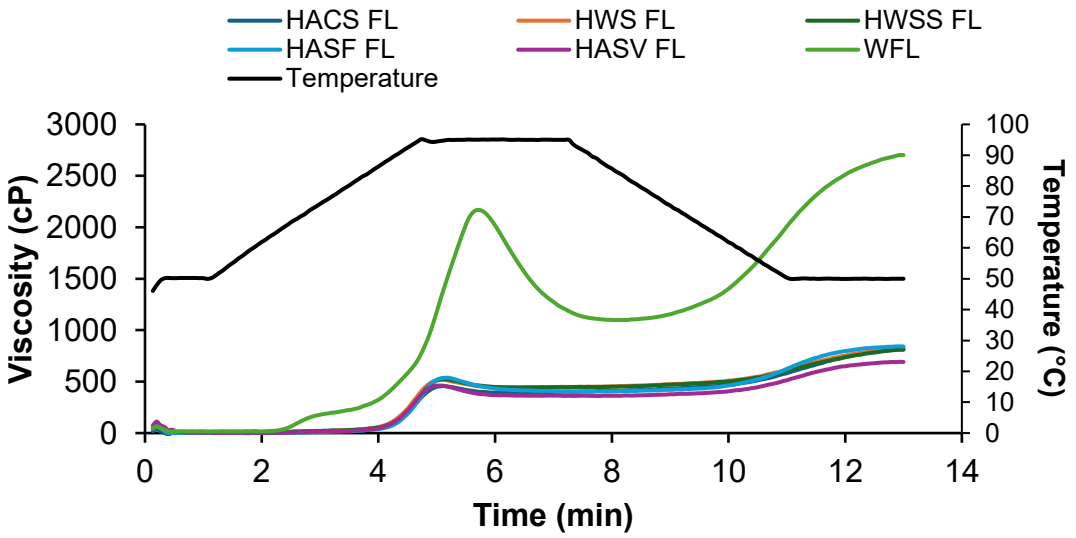

Figure 6. 1 *RVA pasting profile of Wheat flour, and functional flour with HACS, and its modified counterparts with wheat solubles. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-soluble wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-soluble wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.*

As shown in Figure 6.1, the RVA pasting profile of the functional flours containing HACS with wheat solubles revealed a similar pattern to HACS FL, however, the pasting curve was found to be lower than wheat flour (WFL) for all the functional flours. All the parameters from the pasting profile of functional flours, i.e. the peak viscosity, trough viscosity, breakdown viscosity, setback viscosity, and final viscosity were found to be much lower than WFL (Table 6.2). This reveals that the pasting profile is impacted negatively for the functional flour formulation containing 50% wheat flour and 50% biomimetic flour (modified starch+vital gluten).

Table 6. 2 *RVA pasting parameters of functional flours*

Sample	PV (cP) (Mean±SD)	TV (cP) (Mean±SD)	FV (cP) (Mean±SD)	BD (cP) (Mean±SD)	SB (cP) (Mean±SD)	PT (°C) (Mean±SD)
WFL	2168±2 [a]	1098±2 [a]	2702±2 [a]	1070±3 [a]	1604±1 [a]	68.7±0.1 [f]
HACS FL	457±2 [f]	391±2 [e]	815±1 [c]	66±3 [d]	424±3 [c]	90.5±0.1 [d]
HWS FL	525±2 [c]	442±2 [b]	810±3 [d]	82±3 [c]	367±2 [e]	90.5±0.1 [d]
HWSS FL	539±1 [b]	403±1 [d]	842±2 [b]	135±1 [b]	438±1 [b]	90.5±0.1 [d]
HASF FL	500±1 [d]	361±1 [f]	691±2 [e]	139±0 [b]	330±2 [f]	88.8±0 [e]
HASV FL	462±2 [e]	421±2 [c]	814±1 [c,d]	41±3 [e]	393±2 [d]	88.7±0 [e]
HGG FL	296±2 [g]	-	320±2 [i]	-	-	91.3±0 [c]
HXG FL	269±2 [h]	-	472±0 [g]	-	-	93.4±0.5 [a]
HLBG FL	238±2 [i]	-	339±3 [h]	-	-	92.6±0.4 [b]
HCMC FL	178±3 [j]	-	577±2 [f]	-	-	-

Different superscripts in the same column indicate a significant difference (n=3, p<0.05). PV- peak viscosity, TV- trough viscosity, FV- final viscosity, BD- breakdown viscosity, SB- setback viscosity, PT-pasting temperature. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-solubles wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-solubles wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose

Similar results have been reported by Fu et al. (2008) where wheat flour and resistant starch blends resulted in lower values of RVA pasting parameters. The high amylose content of the resulting functional flours containing the modified high amylose starches could be attributed to the lower peak viscosity values as they resist swelling (Lv et al., 2021). Moreover, among the functional flours, the peak viscosity and final viscosity were highest for HASF FL and lowest for HASV FL. On the other hand, the final viscosity was observed to be lower for the individual modified starches than HACS in the previous chapter. The presence of soluble polysaccharides and proteins from wheat flour could have resulted in an interaction that enhanced the viscosity, while the absence of polysaccharides from wheat flour, such as in the case of HASV FL, lowered the viscosity of the resulting flour formulation. Sasaki et al. (2000) have also reported that the addition of NSP from wheat increases the peak viscosity of wheat starch. Additionally, the pasting temperature of the functional flour was increased due to the incorporation of modified HACS containing HACS as well as proteins and polysaccharides. Again, the increase in pasting temperature could be due to the presence of high amylose corn starches and indicates their higher resistance to swelling (H. Li et al., 2019).

6.3.2.2 Effect of modified starches with hydrocolloids

The functional flours with hydrocolloid-modified starches were found to have a pasting profile similar to or lower than the HACS FL. Similar to the previous section, the pasting profile for all the functional flours was weaker than wheat flour. The peak viscosity of HGG FL was slightly higher than HACS FL, while it was much lower for all other formulations. HGG FL showed a final viscosity similar to HASC FL, but it decreased for all other functional flour formulations. Based on the RVA pasting profile of individual modified starches from the previous chapter, the viscosity of the functional flour system was hypothesised to increase; however, contrasting results have been obtained. The interaction of hydrocolloid-modified starches with wheat flour components impacted the viscosity profile negatively.

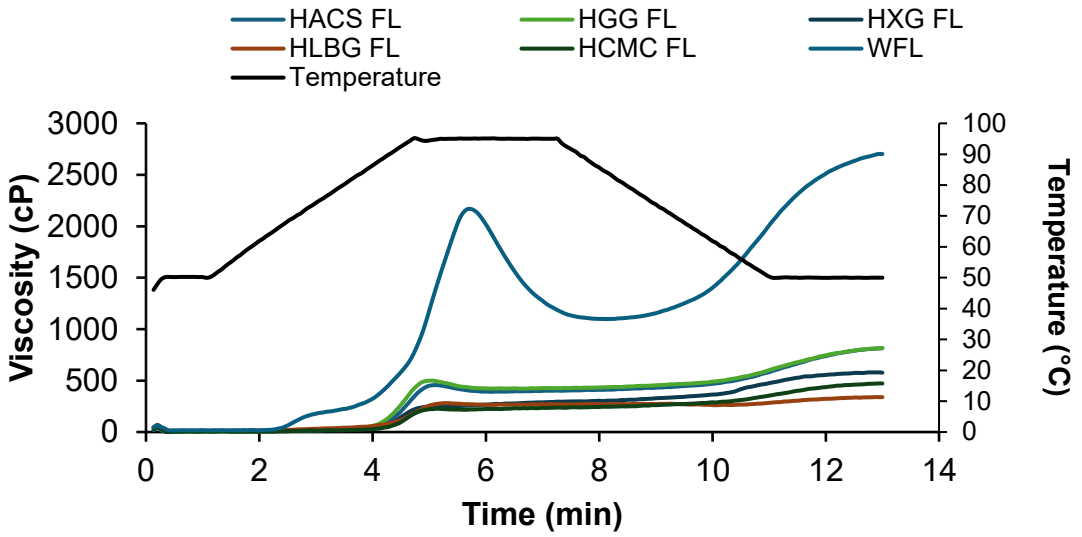

Figure 6. 2 *Pasting profile of wheat flour and functional flour with HACS and its modified counterparts with hydrocolloids. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.*

Moreover, the decrease in the final viscosity could be due to the lower amount of starch available due to the addition of hydrocolloids (Alamri et al., 2013). Anioninc hydrocolloids such as xanthan gum have been reported to lower the peak viscosity and final viscosity of wheat flour-hydrocolloid blends at concentrations of 0.5-2 g/100 g flour in a study by Hammed et al. (2016). The decrease in viscosity could be attributed to the repelling forces between the anionic hydrocolloids and negative charge groups of flour polymers (Shi & BeMiller, 2002). However, a few studies have also reported increased peak viscosity of wheat flour-xanthan gum mixtures. Moreover, guar gum and locust bean gum have also been reported to enhance the pasting viscosities of wheat flour in contrast to the results obtained in this study (Hammed et al., 2016; Rojas et al., 1999).

Similar to the functional flours with modified starches with wheat solubles, the pasting temperature for functional flours with hydrocolloid-modified starches was also found to be increased. This increase could be attributed to the presence of hydrocolloids in the formulations (Shahzad et al., 2019).

6.3.3 Effect of modified starches on rheology of functional flour dough

6.3.3.1 Effect of modified starches with wheat solubles

Frequency sweep tests were performed on the dough to understand their rheological behaviour. The storage modulus behaviour of dough from functional flour with HACS and its modified counterparts with wheat solubles at different water levels, 62, 65, and 70 ml/100 g flour has been shown in Figure 6.3. The storage modulus (G') was found to be strongly dependent on the frequency and increased with an increase in frequency. Also, the G' was higher for the doughs from functional flours with modified starches compared to wheat flour dough. The increased G' indicates stiffer and less elastic dough. The dough from HASF FL and HWSS FL were observed to behave closer to wheat flour dough. When the water level was increased to 70 ml/100 g, the HWSS FL dough behaved similarly to wheat flour dough showing improved dough rheology due to interaction between wheat flour and salt-assisted water-soluble components from wheat flour loaded on HACS. The other functional flour formulations had higher G', revealing a more solid-like behaviour of these doughs. Among the functional flours, HASV FL and HWS FL were found to behave closer to HACS FL at all water levels. Furthermore, G' for all the functional flour doughs decreased as the water level was increased as evident from Figure 6.3. This could be due to the higher water demand of the modified starch compared to wheat flour, and when the water was increased, their dough started to behave closer to wheat flour dough.

Furthermore, the loss modulus pattern, which represents the viscous component of the dough has been shown for the functional flours in Figure 6.4. The loss modulus (G") of all the doughs

was higher than wheat at the water level of 62 ml/100 g flour. When the water level was increased, the G" of the functional flours decreased. At water levels 65 and 70 ml/100 g, HASF FL dough overlapped the G" curve for wheat flour dough, revealing the improved interactions of wheat flour and acid-soluble components loaded on HACS.

Overall, when the loss tangent (tanδ) was compared (Figure 6.5), its value was found to be lower than 1 for all the samples, showing that the G' was higher than the G" for all the dough samples. These results indicate that the elastic behaviour of the dough was more prominent than the viscous behaviour. Similar results have been obtained in the literature for composite flours with germinated bean flour (Atudorei et al., 2021). Similar to the G' and G", the tanδ values of HASF FL dough were closest to wheat flour dough followed by HWSS FL at water levels of 62 and 65 ml/100 g. Moreover, when water addition was increased to 70 ml/100 g, HASF FL and HWSS FL behaved in a similar manner.

These results indicate solubles from wheat flour extracted through salt-assisted water-soluble and acid-soluble have a positive interaction with wheat flour in dough with better visco-elastic properties compared to the incorporation of HACS in wheat flour. Ryan and Brewer (2007) have suggested the role of the endogenous protein of the wheat starch granules in the adsorption of the exogenous protein onto the starch granules. Therefore, the wheat proteins from the soluble extracts present on the surface of modified starches could be attributed to their improved interaction with wheat flour proteins as evidenced by their rheological behaviour.

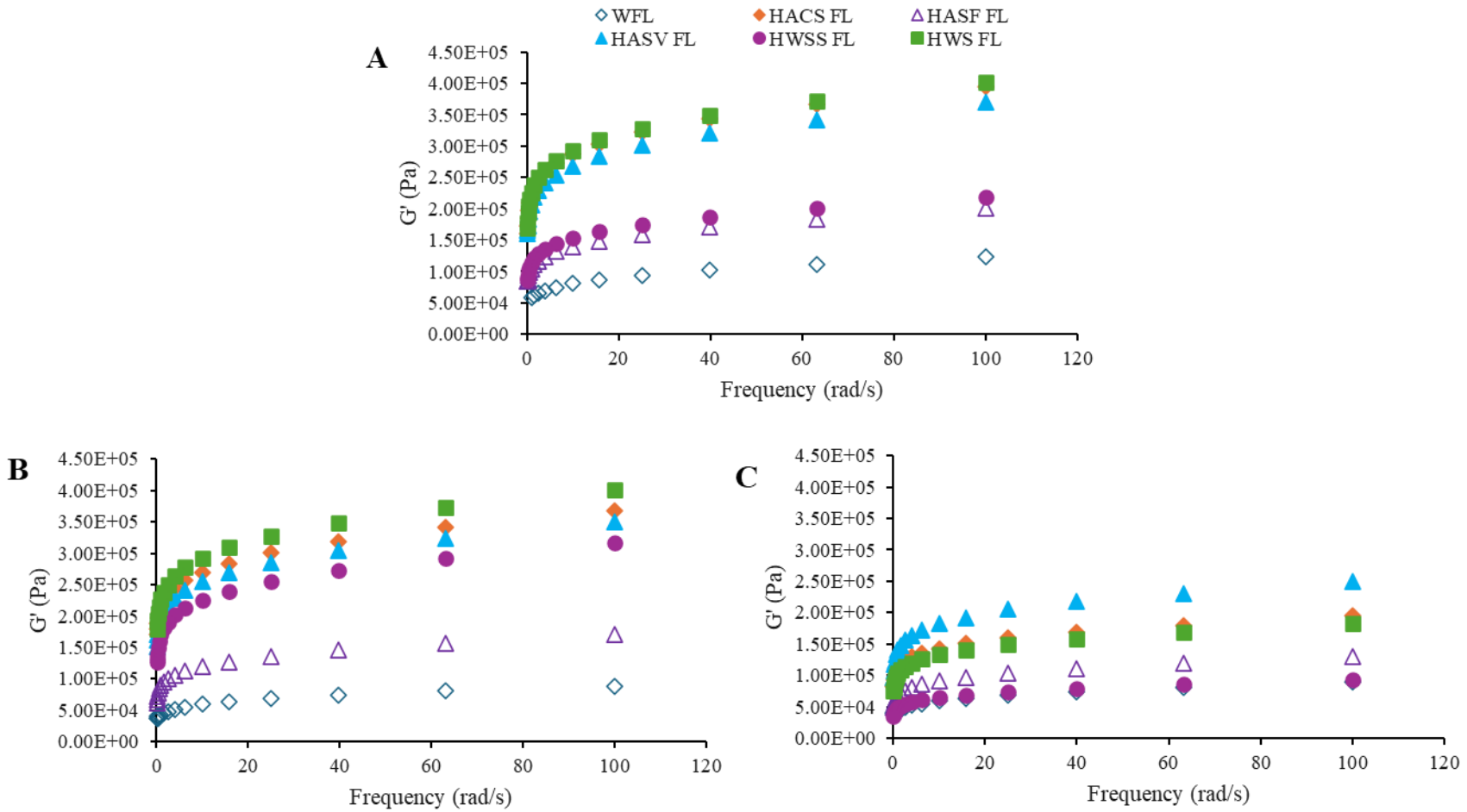

Figure 6. 3 *Storage modulus of dough from functional flour with HACS and its modified counterparts with wheat solubles at different water levels (A) 62 ml/100 g, (B) 65 ml/100 g, and (C) 70 ml/ 100g. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-solubles wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-solubles wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.*

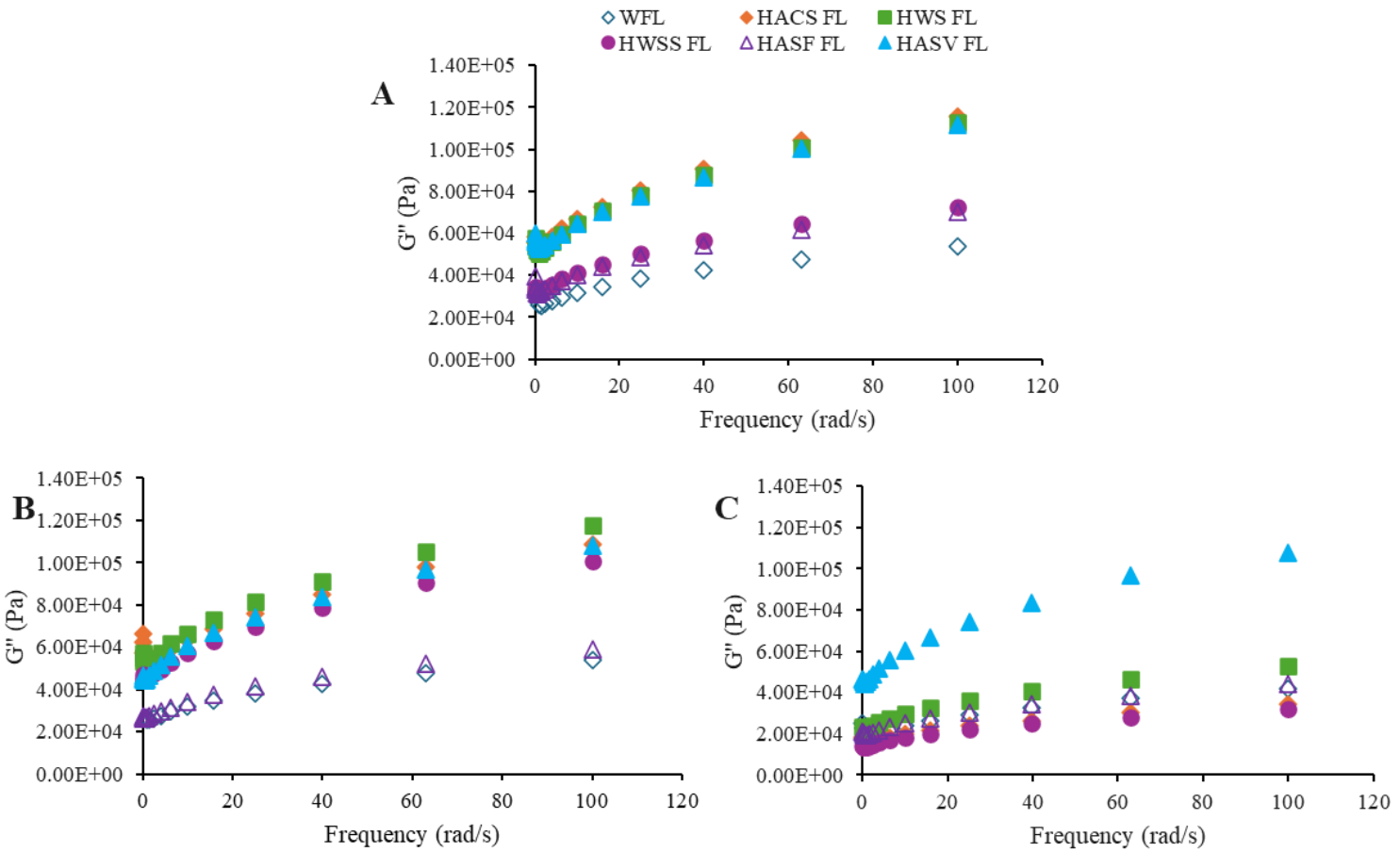

Figure 6. 4 *Loss modulus of dough from functional flour with HACS and its modified counterparts with wheat solubles at different water levels (A) 62 ml/100 g, (B) 65 ml/100 g and (C) 70 ml/100 g. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-soluble wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-soluble wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.*

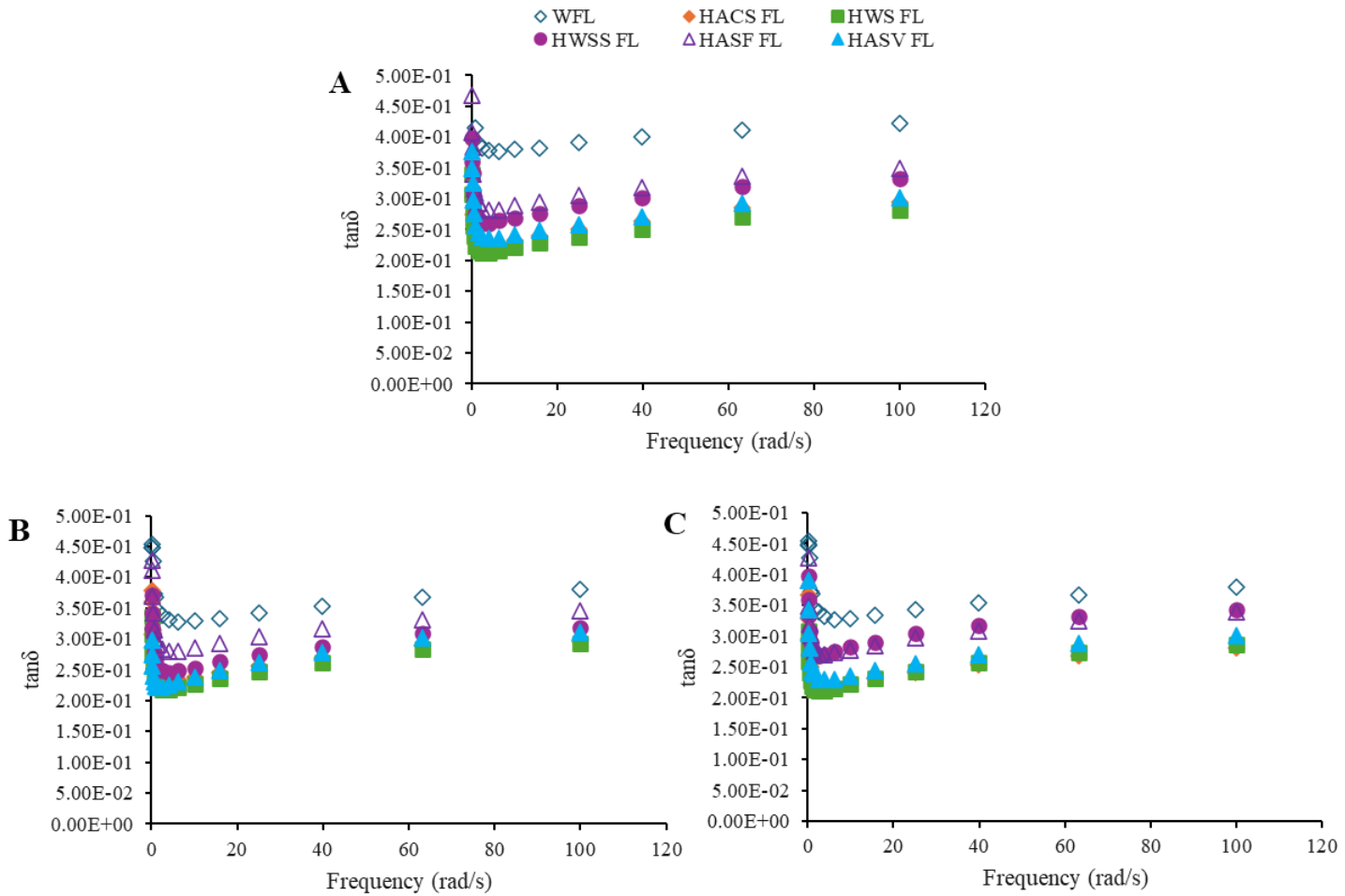

Figure 6. 5 *Loss tangent of dough from functional flour with HACS and its modified counterparts with wheat solubles at different water levels (A) 62 ml/100 g, (B) 65 ml/100 g, and (C) 70 ml/100 g. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-soluble wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-soluble wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.*

6.3.3.2 Effect of modified starches with hydrocolloids

The G', G", and tanδ behaviour of doughs from functional flours with hydrocolloid-modified starches have been shown in Figures 6.6, 6.7, and 6.8. At all the water levels, the G' of all the functional flour doughs was found to be significantly higher than wheat flour dough, similar to the previous section. At a water level of 62 ml/100 g, the functional flour doughs followed each other closely, but when the water level was increased, they showed a larger gap among their G' values. The difference between the G' values of wheat flour dough and functional flour dough was the least for HGG FL dough at a water level of 62 ml/100 g. Higher values of G' have been reported to be associated with a stronger and less extensible dough in the literature (Lazaridou et al., 2007; Song & Zheng, 2007).

Similar to the G', the difference among G" of the functional flour doughs increased with the increase in water level. Compared to HACS FL, the G" curve of all the doughs with hydrocolloid-modified starches was found to be closer to wheat flour dough, HCMC FL dough being the closest to FL at water levels of 65 ml/100 g. Moreover, when the water level was increased to 70 ml/100 g, the G" values for HACS FL were found to be lower than wheat flour dough. Among the functional flours with modified starches, HCMC FL dough was found to be the closest to WFL dough. J. Li, Y. Zhu, et al. (2019) have also reported that the addition of an anionic hydrocolloid- xanthan gum increased the G' and G" of dough. Anionic hydrocolloids could induce electrostatic interaction with gluten and improve the gluten network (Bárcenas et al., 2009; Ribotta et al., 2005). Nonionic hydrocolloids guar gum and locust bean gum have also been reported to increase the G' and G" of the dough attributed to self-association between polysaccharide chains and gluten protein (J. Li, Y. Zhu, et al., 2019).

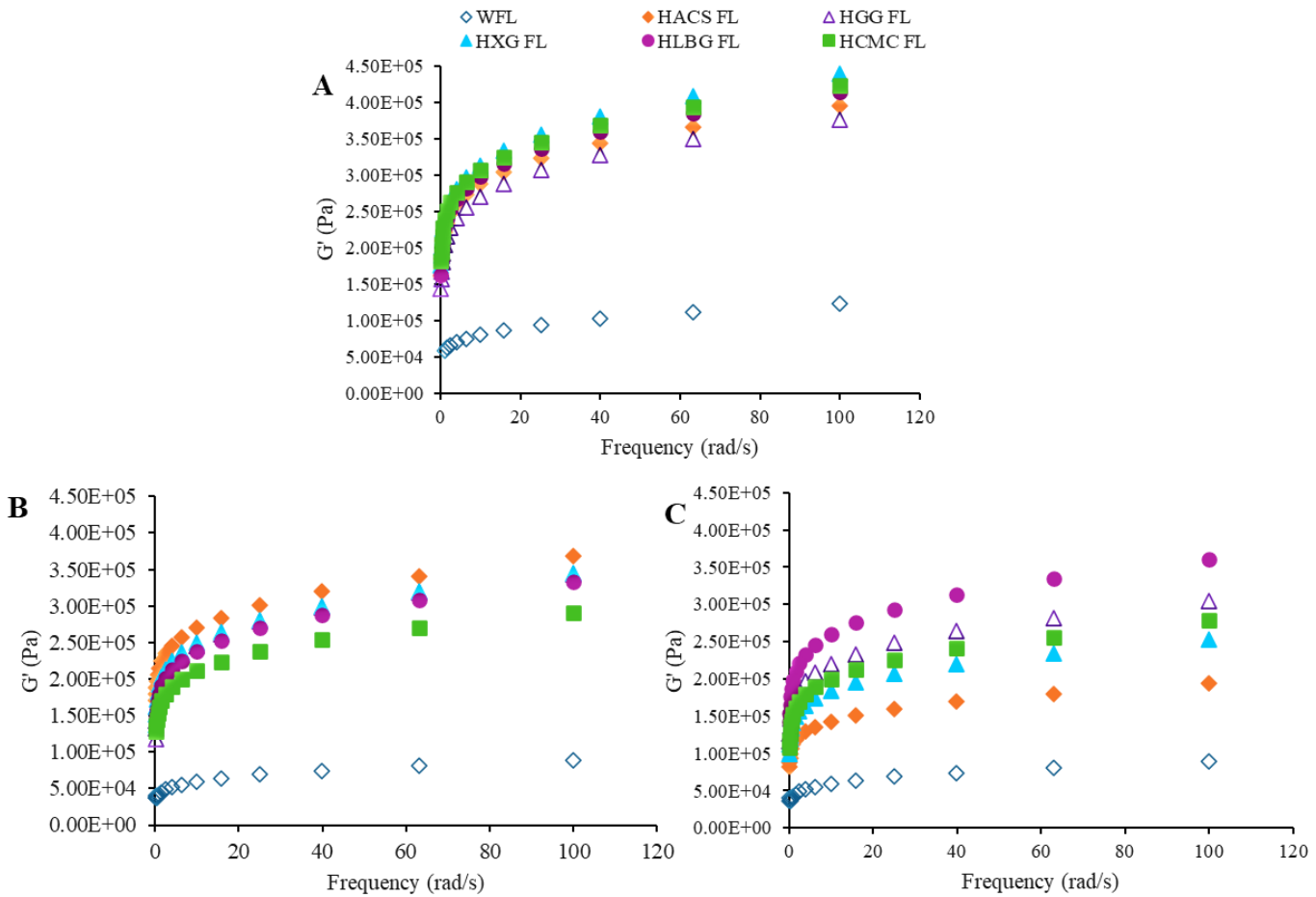

Figure 6. 6 *Storage modulus of dough from functional flour with HACS and its modified counterparts with hydrocolloids at different water levels (A) 62 ml/100 g, (B) 65 ml/100 g and (C) 70 ml/ 100 g. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.*

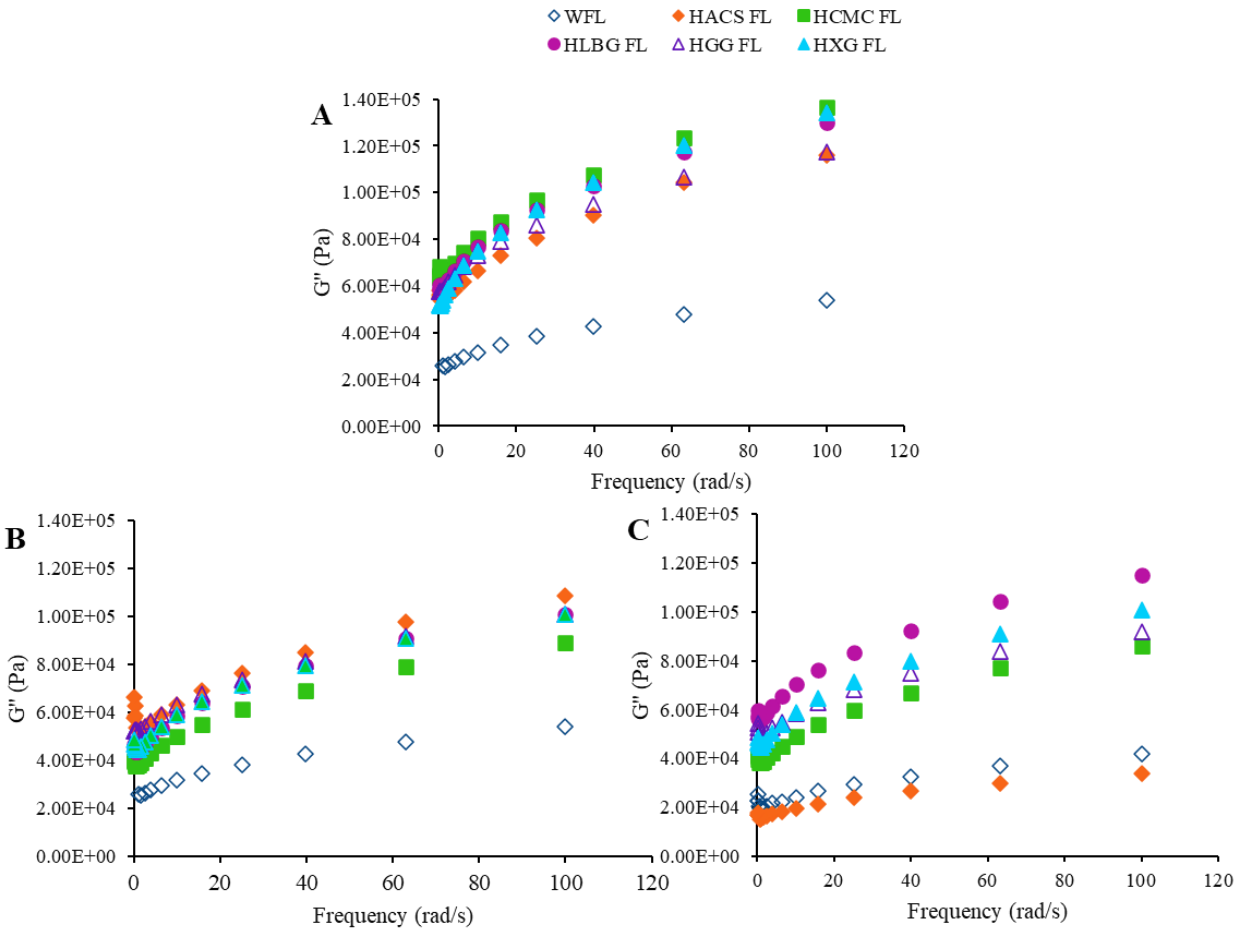

Figure 6. *7 Loss modulus of dough from functional flour with HACS and its modified counterparts with hydrocolloids at different water levels (A) 62 ml/100 g, (B) 65 ml/100 g and, (C) 70 ml/100 g. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.*

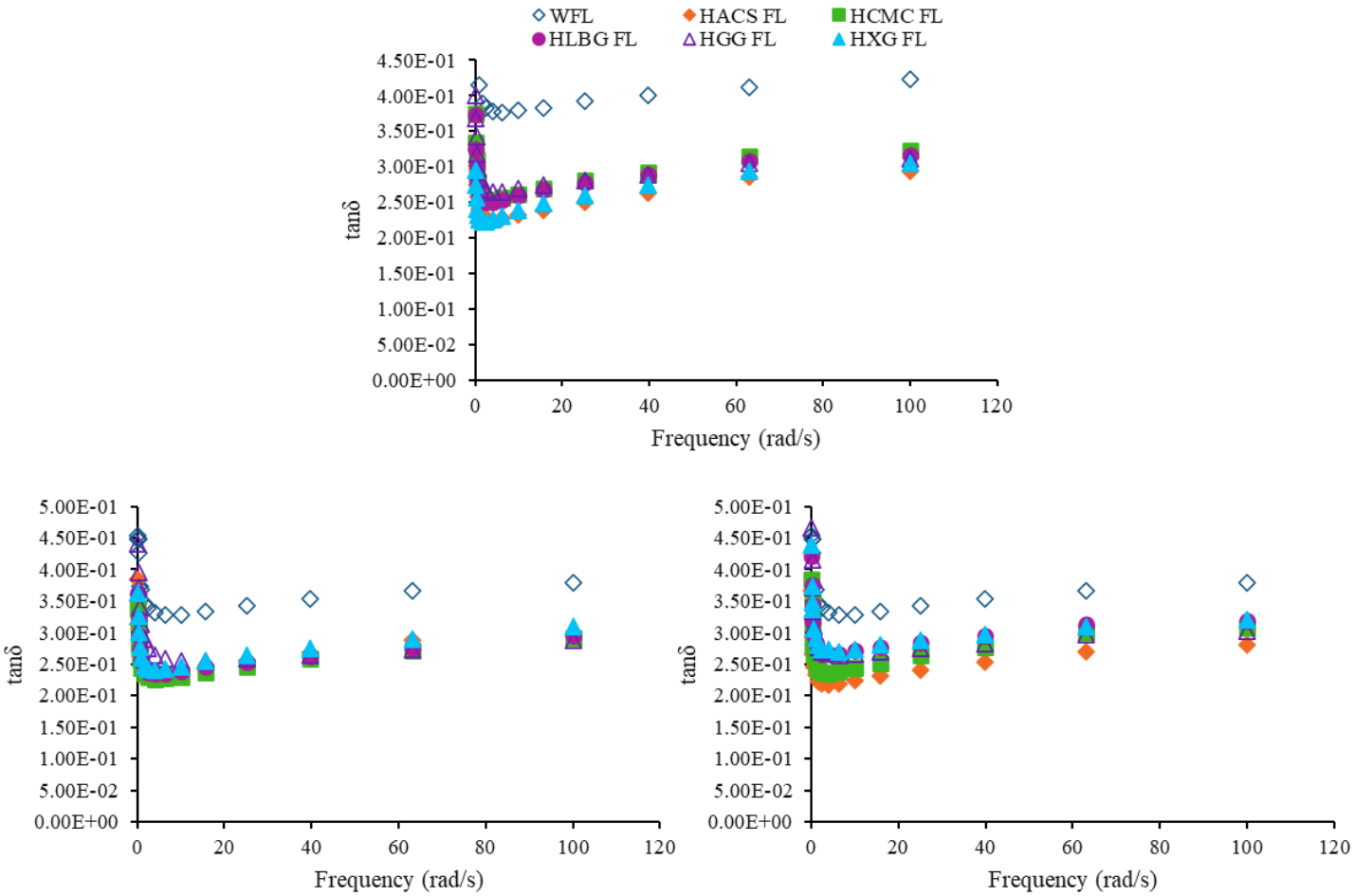

Figure 6. 8 *Loss tangent of dough from functional flour with HACS and its modified counterparts with hydrocolloids at different water levels (A) 62 ml/100 g, (B) 65 ml/100 g and (C) 70 ml /100g. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.*

The G"/ G' of all the functional flour doughs was less than 1 throughout the tested frequency range at all the water levels. It is evident from the rheological parameters of doughs from functional flours with hydrocolloids that even though the storage modulus and loss modulus varied, they behaved in a viscoelastic manner, with a loss tangent value of less than 1. Moreover, when the water content was increased, the rheology of doughs with modified starches with hydrocolloids improved. This could be attributed to the high water absorption capacity of these modified starches. Overall, starches modified with hydrocolloids behaved closer to the wheat flour dough when the water content was improved, suggesting that the optimum water content would make a better dough with these modified starches.

6.3.4 Effect of modified starches on the microstructure of functional flour dough

6.3.4.1 Effect of modified starches with wheat solubles

The micrographs of dough prepared with wheat flour and the functional flour formulations with modified starches with wheat solubles have been presented in Figure 6.9. Starch granules (in green colour) and gluten network (in yellow-orange colour) is evident in all the micrographs. The starch granules were embedded within the gluten network. A close-knit continuous network of gluten is evident in wheat flour dough (6.9-a). However, the dough from HACS FL revealed a significantly different microstructure. The gluten network presented by HACS FL dough was observed to be weaker and non-continuous. These results indicate the poor network formation in the dough as a result of the substitution of wheat flour with HACS. Even though the HACS was complemented with vital gluten, the resulting dough from their functional flour revealed weak gluten network formation. However, in a previous study by Arp et al. (2017), the microstructure of dough supplemented with a charged resistant starch (up to 30%) did not have a large variation from the control due to the ionic interaction of starch with gluten. However, in the present study, HACS did not improve the gluten network.

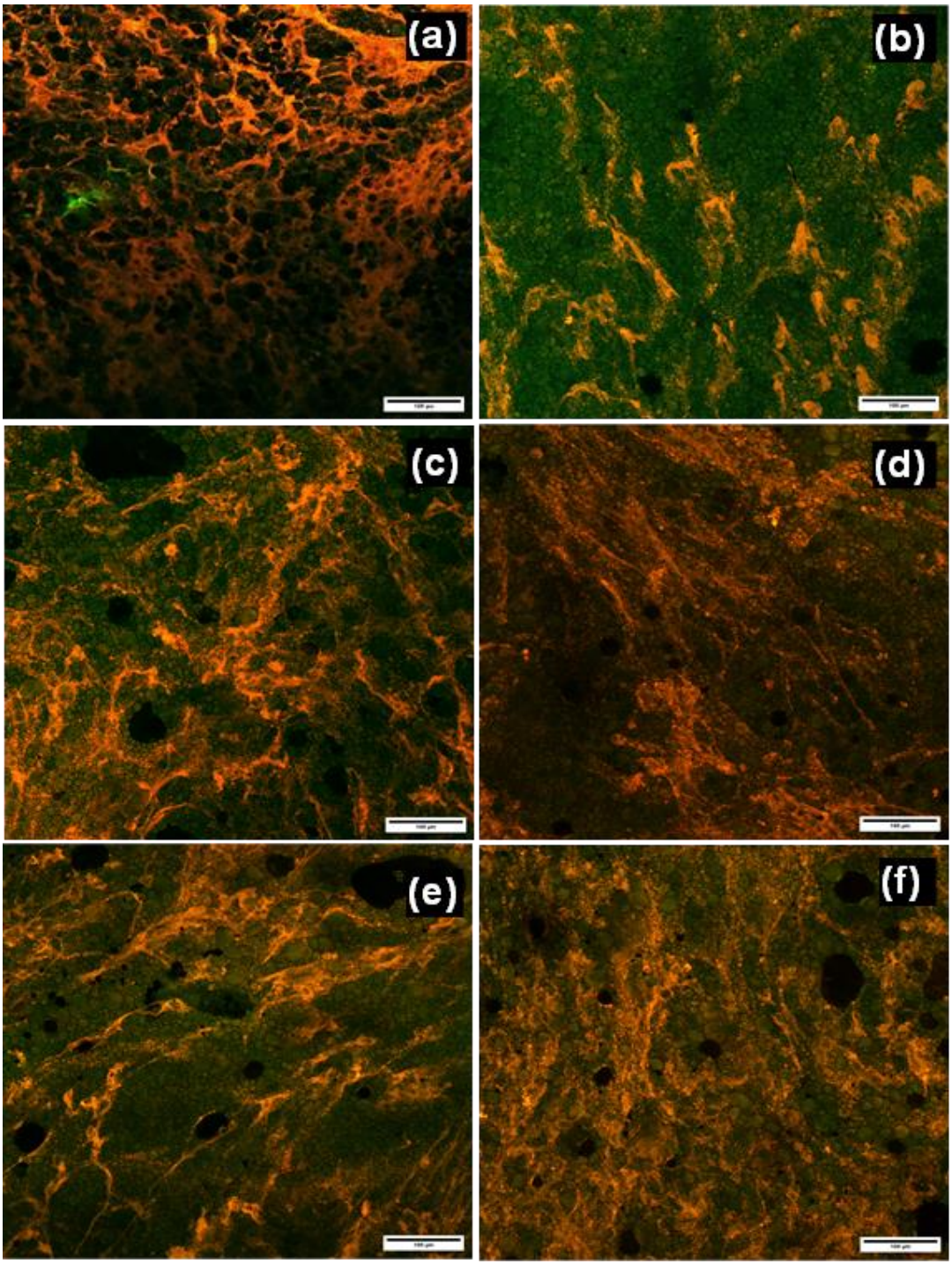

Figure 6. 9 *Confocal laser scanning micrographs of dough with WFL (a), HACS FL (b), HWS FL(c), HWSS FL(d), HASF FL (e) and HASV FL (f) (scale: 100µm). Red and green colours represent gluten and starch, respectively. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-soluble wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-soluble wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.*

Moreover, in the case of HWS FL, the network was found to be less dense compared to FL, but more continuous compared to HACS FL. These results show that the interaction of the water-soluble components from wheat flour when loaded on HACS with gluten resulted in better network formation.

Additionally, HWSS FL revealed a dense and continuous gluten network, similar to the network shown in a study by (Arp et al., 2017). These results indicate that salt-assisted water-soluble extracts containing soluble polysaccharides along with gliadin-like protein interact with wheat flour proteins in a better way and result in better network formation.

Dough from HASF FL also revealed a continuous network of gluten, however, the strands of gluten were more separated compared to other functional flour formulations. Moreover, HASV FL revealed a dense network compared to HASF FL. Even though the protein content was high in HASF FL, these results indicate better interaction of the loaded protein (in the absence of soluble polysaccharides from wheat flour) on modified starch and with the wheat flour during dough formation.

6.3.4.2 Effect of modified starches with hydrocolloids

The micrographs of dough prepared with functional flours containing hydrocolloid-modified starches have been shown in Figure 6.10. The gluten network has been observed to be improved for all the functional flour doughs with hydrocolloid-modified starches compared to HACS FL. In the case of dough with HGG FL, the guar gum present in HGG interacted with the wheat flour protein to create a continuous network with parallel strands of gluten proteins and a dense starch-protein matrix. Moreover, HXG FL dough also revealed a continuous gluten network and a compact matrix of starch and protein. A similar observation was made for dough with HLBG FL. In addition, dough with HCMC FL revealed a starch-protein matrix similar to HACS FL dough, however, it was more concentrated than HACS FL dough.

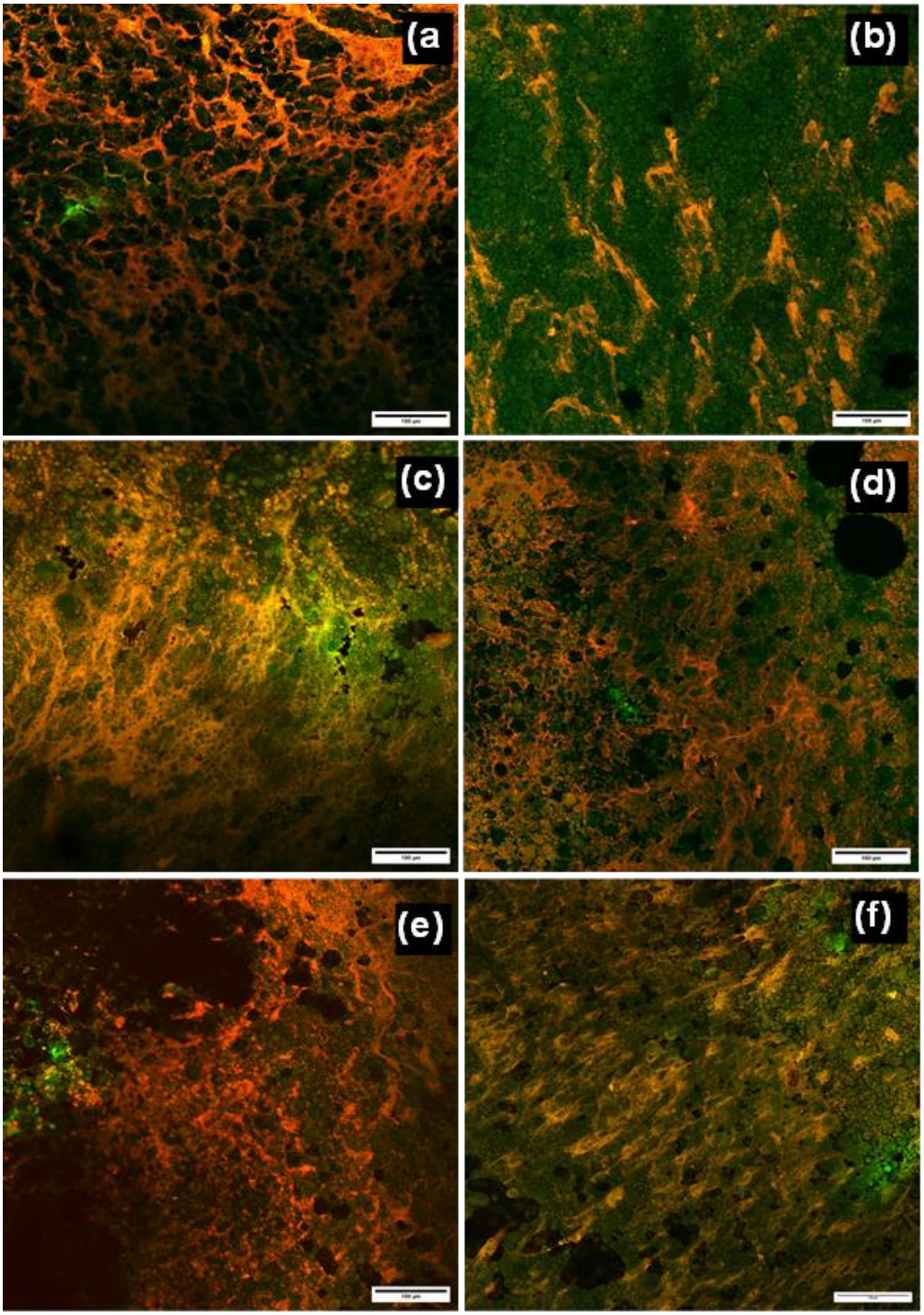

Figure 6. 10 *Confocal laser scanning micrographs of dough with WFL (a), HACS FL (b), HGG FL(c), HXG FL (d), HLBG FL (e) and HCMC FL (f) (scale: 100µm). Red and green colours represent gluten and starch, respectively. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.*

These results indicate that the hydrocolloid-modified starches improved the starch-protein matrix and gluten network. J. Li, Y. Zhu, et al. (2019) have reported that the gluten network was enhanced better in the presence of hydrocolloids with linear structure and high viscosity. This enhanced network has been attributed to the interaction between gluten proteins and polysaccharides as well as the self-association of polysaccharides (J. Li, Y. Zhu, et al., 2019).

6.3.5 Effect of modified starches on chapatti bake loss

Table 6. 3 *Bake loss in chapatti from functional flour*

Sample	Bake loss (%) (Mean±SD)
WFL	14.74±0.74 [a]
HACS FL	12.59±0.4 [b,c]
HWS FL	13.59±0.49 [a,b,c]
HWSS FL	12.08±0.5 [c]
HASF FL	12.95±0.09 [a,b,c]
HASV FL	14.39±1.17 [a,b]
HGG FL	12.84±1.15 [a,b,c]
HXG FL	13.66±0.45 [a,b,c]
HLBG FL	13.17±0.11 [a,b,c]
HCMC FL	14.46±0.52 [a,b]

Different superscripts in the same column indicate a significant difference (n=3, $p < 0.05$). WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-solubles wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-solubles wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.

The water lost during baking or cooking of chappati has been evaluated through % bake loss (Table 6.3). The bake loss for the chapattis in this study ranged from 12.08±0.5% to 14.74±0.74%. The lower bake loss in the case of HWSS FL could be attributed to the presence of soluble polysaccharides and soluble gliadin-like proteins present from salt-assisted water-soluble wheat flour extract. Moreover, a higher protein content along with soluble polysaccharides present in HASF FL resulted in lower bake loss and retained more amount of moisture during the baking of chapatti. Moreover, the hydrocolloid gums are known for higher water absorption properties, which led to more moisture retention in the chapattis in this study. However, a study by Moza and Gujral (2018) revealed higher bake loss associated with the presence of arabinoxylans from barley flours. Moreover, higher moisture retention has been associated with slower staling rates in baked products (Moza & Gujral, 2018).

6.3.6 Effect of modified starches on *in vitro* oral-gastro-small intestinal starch hydrolysis of chapatti

Chapattis were prepared using the functional flour formulations involving processing such as dough mixing, rolling into thin discs, and then baking on a high-temperature hot plate for a short time (200 °C for approximately 90 seconds) (Figure A 6.2 in Appendix). The *in vitro* starch hydrolysis of chapattis was done immediately after cooling to room temperature to avoid the effects of retrogradation of starch post-cooking.

6.3.6.1 Effect of modified starches with wheat solubles

The chapattis were subjected to *in vitro* oral-gastro-small intestinal starch digestion to study their starch hydrolysis. The oral digestion of chapattis from all the functional flour formulations resulted in starch hydrolysis in the range of 0.8 to 2%. Following oral digestion, the gastric digestion phase did not result in much starch hydrolysis as evident from the starch hydrolysis curves in Figure 6.11. Similar results have been obtained in previous studies (Abhilasha et al., 2022), and could be attributed to the absence of starch digestive enzymes in the gastric

digestion phase. However, the starch hydrolysis increased as soon as the small intestinal digestion phase started.

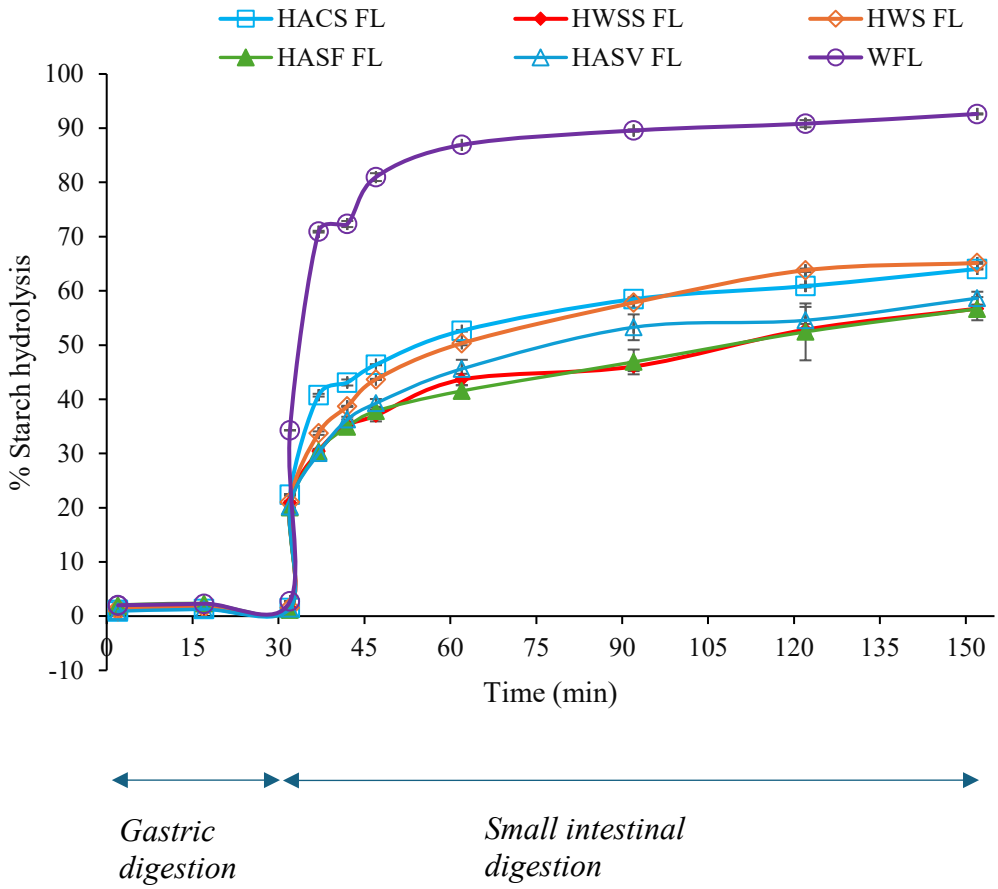

Figure 6. 11 *In vitro starch hydrolysis of chapattis prepared from functional flours with HACS and its modified counterparts with wheat solubles. Error bars represent standard deviation (n=3). WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-soluble wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-soluble wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.*

The starch hydrolysis of wheat flour chapatti was found to be the highest among all the flours. The total extent of starch hydrolysis at the end of 120 min of simulated small intestinal digestion was significantly lower for all the chapattis from functional flour formulations

compared to wheat flour chapatti. Chapattis from HWSS FL, HASF FL, and HASV FL were observed to have a lower extent of starch hydrolysis compared to HACS FL. Additionally, the starch hydrolysis of chapattis with HWS FL was lower compared to HACS until 60 min of small intestinal digestion. These results show that when the modified starches with wheat flour solubles were incorporated in a wheat flour-based system like chapatti, it led to slower digestion of the flour system. The decreased overall starch hydrolysis in all the functional flour formulations could be attributed to the presence of HACS, which is known as RS2, with high amylose content and possesses crystalline structure type B. The structure of B-type starch belongs to the outside-in digestion type, is relatively compact, surface has no pores; therefore, enzymes can not enter into the starch (Shrestha et al., 2012). Moreover, the lower starch hydrolysis of chapattis from functional flour with wheat solubles could be attributed to the soluble polysaccharides and soluble proteins from wheat flour which might act as an inhibitor to the starch digestive enzymes (Poerio et al., 1989). Another recent study has also revealed the role of soluble wheat protein in inhibiting the starch digestive enzymes and lowering starch hydrolysis (Wu & Warren, 2023). The addition of gluten has also been reported to enhance the slowly digestible starch fraction of chapatti (Giri et al., 2017). The interaction of these soluble wheat components also revealed a better dough network as shown in the dough micrographs, which embedded the starch granules in the protein network, slowing down the digestion of the starch in the chapattis.

Moreover, the hydrolysis index and eGI have been shown in Table 6.4. The eGI of chapattis with wheat flour was found to be the highest, and all the chapattis from functional flour formulations possessed significantly lower eGI. The decrease in eGI of chapattis from functional flour formulations containing wheat solubles could be attributed to both the presence of HACS-a RS2, as well as the wheat soluble components, possibly containing amylase

inhibitory activity as well as enhanced starch-protein interaction of the overall system, making starch less available to digestive enzymes.

Table 6. 4 *Total Starch hydrolysis, Hydrolysis index (HI), and estimated glycaemic index (eGI) of Chapattis prepared from functional flour with HACS and its modified counterparts with wheat solubles*

Sample	Total starch hydrolysis (%) (Mean±SD)	HI (Mean±SD)	eGI (Mean±SD)
WFL	92.61±0.09 [a]	96.07±0.18 [a]	92.45±0.1 [a]
HACS FL	64.01±0.08 [b]	64.13±0.19 [b]	74.92±0.11 [b]
HWS FL	65.1±0.13 [b]	61.16±0.38 [b]	73.29±0.21 [b]
HWSS FL	56.21±1.75 [c]	51.15±0.99 [d]	67.79±0.54 [d]
HASF FL	56.51±0.56 [c]	50.56±2.08 [d]	67.47±1.14 [d]
HASV FL	58.37±0.97 [c]	54.36±1.64 [c]	69.56±0.9 [c]

Different superscripts in the same column indicate a significant difference (n=3, $p < 0.05$). WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-soluble wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-soluble wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.

6.3.6.2 Effect of modified starches with hydrocolloids

The starch hydrolysis rate and overall extent of starch hydrolysis were decreased in all the chapattis from functional flour formulations containing hydrocolloid-modified resistant starch compared to wheat flour chapatti (Figure 6.12). The overall starch hydrolysis of chapattis from functional flour formulations was almost 0.7 times of the wheat flour chapatti.

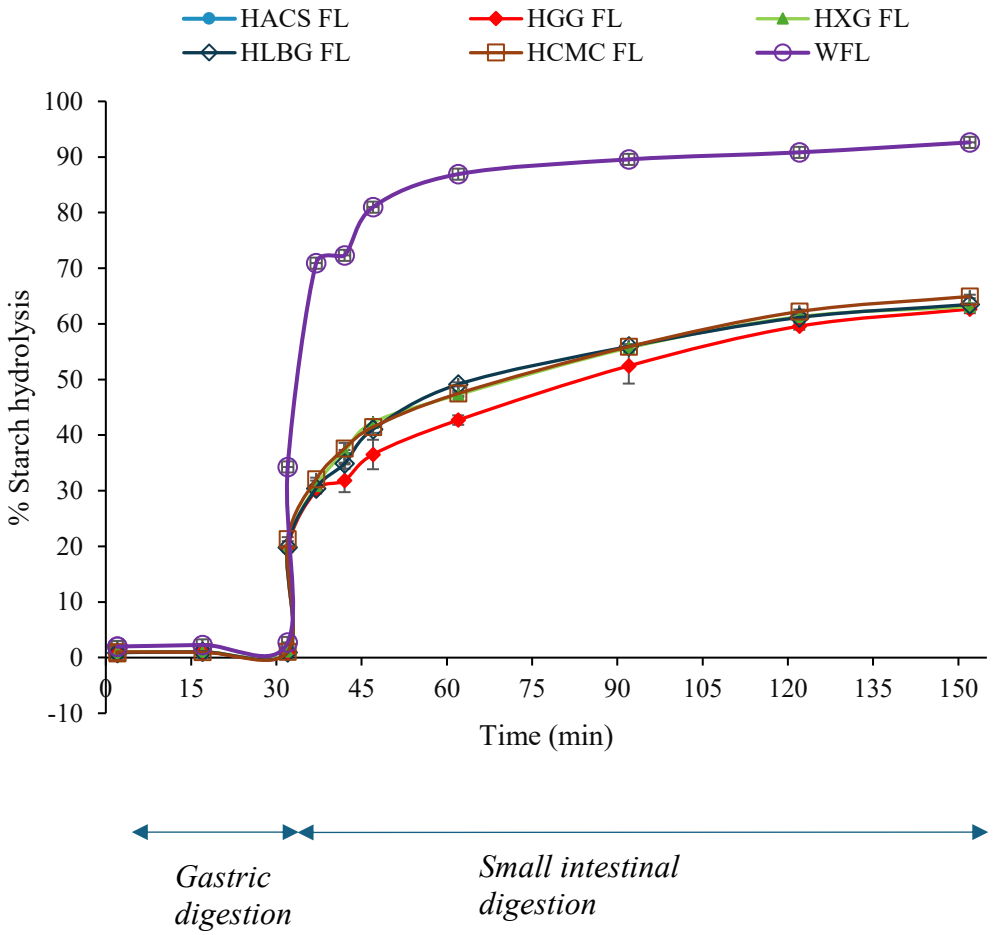

Figure 6. 12 *In vitro starch hydrolysis of chapattis prepared from functional flours with HACS and its modified counterparts with hydrocolloids. Error bars represent standard deviation (n=3). WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.*

The starch hydrolysis of chapattis from the functional flour formulations was found to be significantly lower for the wheat flour chapattis. The overall lower starch hydrolysis of the chapattis from these functional flours is mainly attributed to the presence of the resistant starch source, i.e. HACS. Moreover, the overall extent of starch hydrolysis of chapattis was lowest for HGG FL, followed by HXG FL and HLBG FL, while HCMC followed a pattern similar to

HACS. The initial rate of starch hydrolysis of the chapattis from functional flours with hydrocolloid-modified starches was lower due to the high water absorption and viscosity attributes of the hydrocolloids (Shahzad et al., 2019). The interaction of hydrocolloid-modified starches with wheat flour resulted in a dense network of starch-protein in the dough, which could have further become dense after baking the chapattis, as revealed by the RVA pasting profile of these functional flour formulations and making the starch less available to the digestive enzymes.

Table 6. 5 *Total Starch hydrolysis, Hydrolysis index (HI), and estimated glycaemic index (eGI) of Chapattis prepared from functional flour with HACS and its modified counterparts with hydrocolloids*

Sample	**SH (%) (Mean±SD)**	**HI (Mean±SD)**	**eGI (Mean±SD)**
WFL	92.61±0.09 [a]	96.07±0.18 [a]	92.45±0.1 [a]
HACS FL	64.01±0.08 [b]	64.13±0.19 [b]	74.92±0.11 [b]
HGG FL	62.48±0.61 [e]	55.08±1.4 [d]	69.95±0.77 [d]
HXG FL	62.98±0.39 [d,e]	58.63±0.2 [c]	71.9±0.11 [c]
HLBG FL	63.44±0.17 [c,d]	58.93±0.01 [c]	72.07±0.01 [c]
HCMC FL	64.86±0.26 [b]	59.33±0.05 [c]	72.28±0.03 [c]

Different superscripts in the same column indicate a significant difference (n=3, $p < 0.05$). WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.

The eGI of chapattis prepared with functional flours containing modified starches with hydrocolloids was found to be decreased compared to wheat flour chapattis as well as HACS FL chapattis (Table 6.5). The eGI was found to be the lowest for HGG, 69.95±0.77, while the

eGI of wheat flour chapatti was 92.45±0.1. These results indicate the potential of guar gum modification for reducing the GI of wheat-based products like chapattis. The modification with xanthan gum, locust bean gum, and carboxymethyl cellulose also reduced the eGI of the chapattis; however, there was no significant difference among the eGI of these chapattis. The reduced eGI of the chapattis containing hydrocolloid-modified starches could be due to the high viscosity profile, making dense products. However, these factors were not found to be effective in the later stage of the digestion, and the overall starch hydrolysis was increased to a similar extent as chapatti from HACS FL, bringing their eGI similar to HACS FL chapattis.

6.4 Conclusion

Modified resistant starches were attempted to develop in the previous chapter using resistant starch- high amylose corn starch and soluble extracts from wheat flour and gluten, and commonly used hydrocolloids. This chapter evaluated their functional and digestible properties in a wheat flour system by creating a functional flour formulation with wheat flour, vital gluten, and modified starches. Overall, the water-holding capacity of the functional flours increased, while the pasting properties were not improved much. Moreover, when doughs were prepared with these functional flours, the rheological parameters for the functional flour formulations approached wheat flour dough, improving their functionality. The dough microstructure was also observed to be improved in terms of the network of gluten and starch in the dough. Additionally, the dough was utilised to prepare chapattis, and the bake loss was reduced in the functional flour formulation, indicating better keeping quality. The overall starch hydrolysis of chapattis from all the functional flour formulations was found to be significantly lower than the wheat flour chapatti. Overall, the functionality of the functional flours was improved and starch hydrolysis was reduced by the modification of resistant starch with wheat flour solubles and hydrocolloids.

Chapter 7

Chapter 7 Overall Conclusion and Recommendations

7.1 Summary and conclusion

Low-glycaemic foods are in high demand due to their health benefits; however, due to the unavailability of raw materials with functional as well as low-digestion properties, the production of high-quality food products is limited. Therefore, it is important to indulge in research to bring a practical solution to this limitation. The research work in this thesis aimed to better understand the role of components of wheat and other ingredients in starch digestibility and functionality, and develop slowly digestible raw materials with improved functional properties to overcome the limitations mentioned above.

An extensive review of the literature was conducted in Chapter 2 to understand the available information on the role of wheat components in starch digestion and functionality. A recap of this Ph.D. research work is discussed below.

7.1.1 Impact of microstructural integrity of wheat grains on the *in vitro* starch hydrolysis

To improve the fundamental understanding of wheat grain structure and the microstructure's impact on the starch hydrolysis properties of wheat, Chapter 3 investigated the relationship of differently processed wheat grains' microstructure with the starch hydrolysis of the resulting product (Figure 7.1).

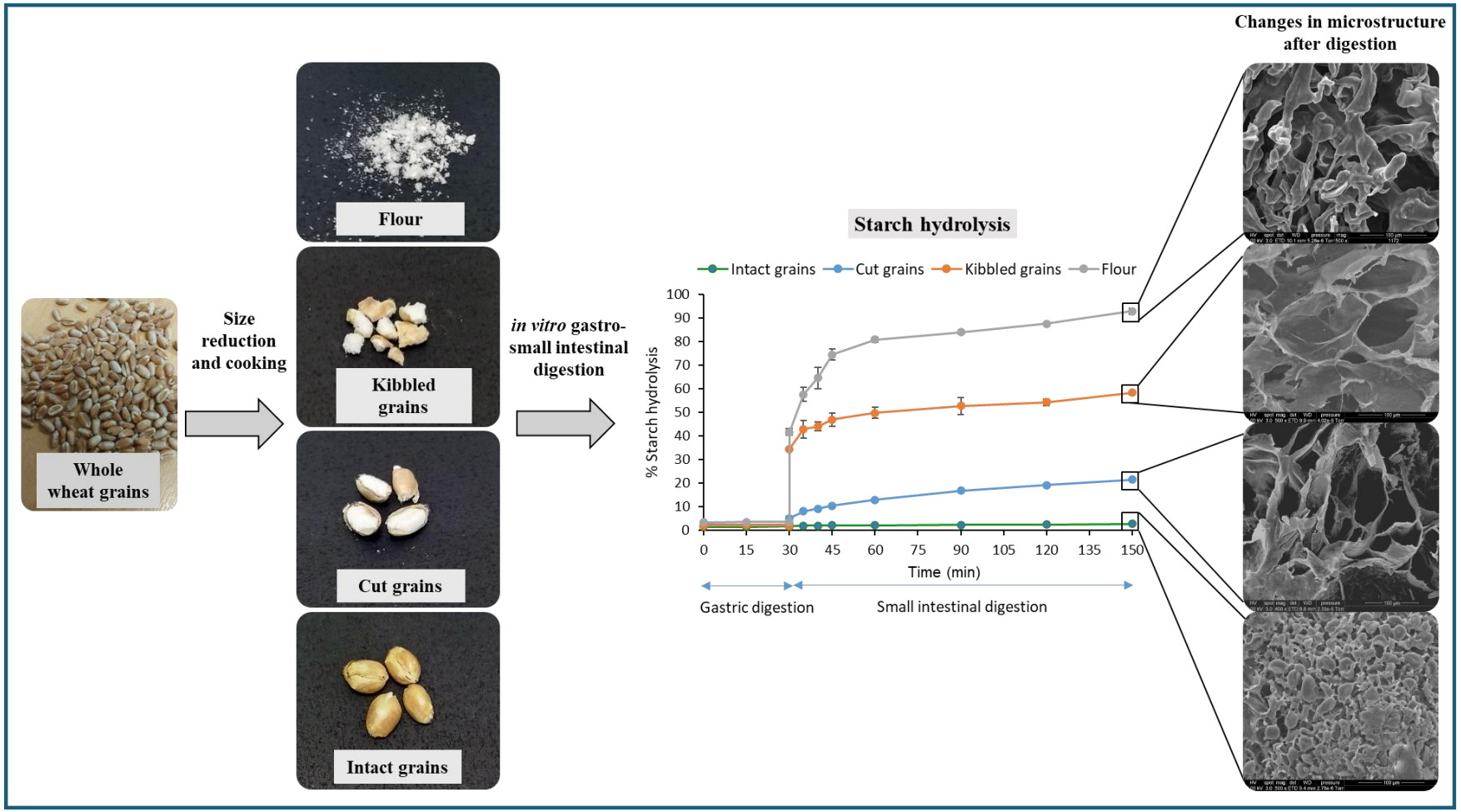

Figure 7. 1 *Summary of impact of microstructural integrity of wheat grains on the in vitro starch hydrolysis (Chapter 3)*

The microstructural changes occurring in wheat grains due to their size reduction (kibble, cut and mill), cooking and simulated gastro-small intestinal digestion, and their impact on *in vitro* starch digestion were studied. This study made evident the role of the natural microstructure of wheat grain and the state of starch in the starch hydrolysis of wheat grains. From nearly no digestion (2.19±0.06%) (in the case of whole wheat grains), size reduction and cooking led to almost complete digestion (92.85±1.21%) (in the case of cooked flour). The intact bran layer, endosperm cell wall, and protein matrix of whole intact grains helped prevent the interaction of digestive enzymes with starch. However, processing (milling and cooking) destroyed the natural intact microstructure of the grains, enhanced the area exposed to digestive enzymes and increased the starch hydrolysis of processed grain formats. SEM micrographs revealed the absence of starch granules from the grains' exposed surface areas during sequential digestion phases. This study did not include chewing (oral digestion phase) to preserve the difference in the microstructures and demonstrate the impact of microstructure during gastro-small intestinal digestion.

Overall, the findings indicated that the intact microstructure of wheat grain involving bran encapsulation is the most effective method to reduce starch hydrolysis; however, once the intactness is disturbed, the area exposed to the digestive enzymes is responsible for the increased starch hydrolysis. The outcomes of this study expound on the concept of lowering the extent of starch hydrolysis by using whole grains.

7.1.2 The impact of microstructural modification of whole wheat by hydrothermal treatment and cold storage on the starch hydrolysis and flour properties

Based on Chapter 3's findings on the role of intact whole wheat grain microstructure in starch hydrolysis, Chapter 4 explored hydrothermal treatment and low-temperature storage of intact whole wheat grains with the possibility of reducing the starch hydrolysis in different grain formats (whole, flakes, and flour) (Figure 7.2).

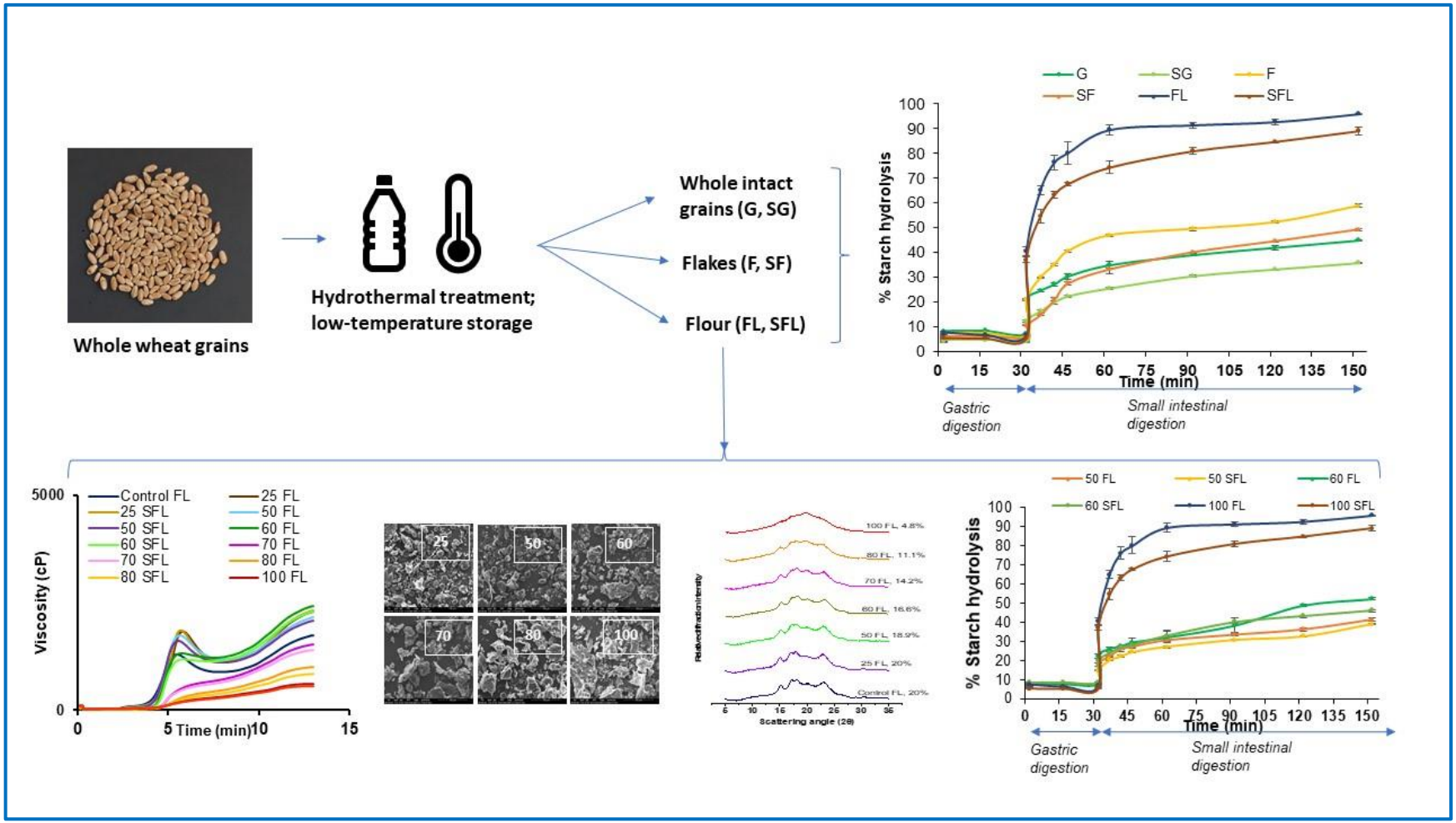

Figure 7. 2 *Summary of impact of microstructural modification of whole wheat by hydrothermal treatment and cold storage on the starch hydrolysis and flour properties (Chapter 4)*

Similar to the findings in Chapter 3, processing the hydrothermally treated whole grains into different formats, such as flakes and flour, significantly enhanced their extent of starch hydrolysis. However, low-temperature storage (4 °C /7 days) reduced their starch hydrolysis by up to 9% in different formats, such as dried whole grain, flakes, and flour, and reduced their eGI. The effect of retrogradation was visible through the SEM micrographs of whole grain in its intact grains and flake format, demonstrating a more porous structure.

The impact of hydrothermal treatment and low-temperature storage of whole wheat grains on the functional and digestible properties of resulting wheat flour was also evaluated in Chapter 4 (Figure 7.2). Hydrothermal treatment of whole wheat grains at temperatures below the starch gelatinisation temperature and up to 100 °C resulted in flours with modified pasting and digestion properties. The treatment temperatures above 60 °C led to distorted starch granules and leached starch-denatured protein matrix fragments in the flour, enhancing water absorption capacities. Moreover, the increase in the treatment temperature of the grains reduced the relative crystallinity and pasting viscosities of the resulting flours and raised their starch hydrolysis and eGI. Nevertheless, low-temperature storage of the hydrothermally treated grains resulted in reduced water absorption, pasting viscosities, the extent of starch hydrolysis, and enhanced relative crystallinity of flour, which could be attributed to the retrogradation of the starch. Overall, this study provided a fundamental understanding of the hydrothermal treatment and low-temperature storage of whole wheat grains in microstructural changes and their impact on starch hydrolysis and eGI. The treatments involved in this chapter were effective in reducing the starch hydrolysis properties of grain formats like whole grain, flakes, and flour. However, these treatments also had a detrimental impact on the functional properties of the resulting flours. Therefore, alternative strategies were investigated in the next chapter to improve the functional properties of flour while maintaining slower starch digestibility.

7.1.3 Modification and characterisation of resistant starch with soluble components from wheat, and their impact on functionality and starch digestibility of a wheat flour system

Chapter 5 utilised a resistant starch source and modified it using wheat grain components to improve functionality and reduce starch hydrolysis. Soluble extracts from wheat, i.e., water-soluble wheat flour extract, salt-assisted water-soluble wheat flour extract, acid-soluble wheat flour extract, and acid-soluble vital gluten extracts, were used to modify high amylose corn starch (HACS), a resistant starch. The resulting modified starches revealed modified physicochemical, microstructural, pasting, and *in vitro* starch hydrolysis characteristics (Figure 7.3).

The modification of HACS with solubles from wheat resulted in increased protein content up to 4.03±0.03%; moreover, their water-holding capacity increased up to 1.54 times. The SEM micrographs revealed the formation of wheat solubles-starch clusters, mimicking the wheat flour microstructure having starch-protein clusters. However, the heat treatment during spray drying reduced the relative crystallinity of the resulting starch. The RVA pasting viscosities were lower for modified starches than HACS, except with acid-soluble extract from vital gluten. Nevertheless, compared to HACS, the modification with wheat solubles helped reduce the starch hydrolysis of the cooked (100 °C for 20 min) starches. Modifications of HACS with acid-soluble extracts from wheat flour and vital gluten were found to be more effective in reducing starch hydrolysis throughout oral-gastro-small intestinal digestion. Moreover, salt-assisted water-soluble extracts reduced the rate of starch hydrolysis until 90 min of simulated digestion; however, the water-soluble wheat flour extract was ineffective in reducing the total starch hydrolysis of modified starch.

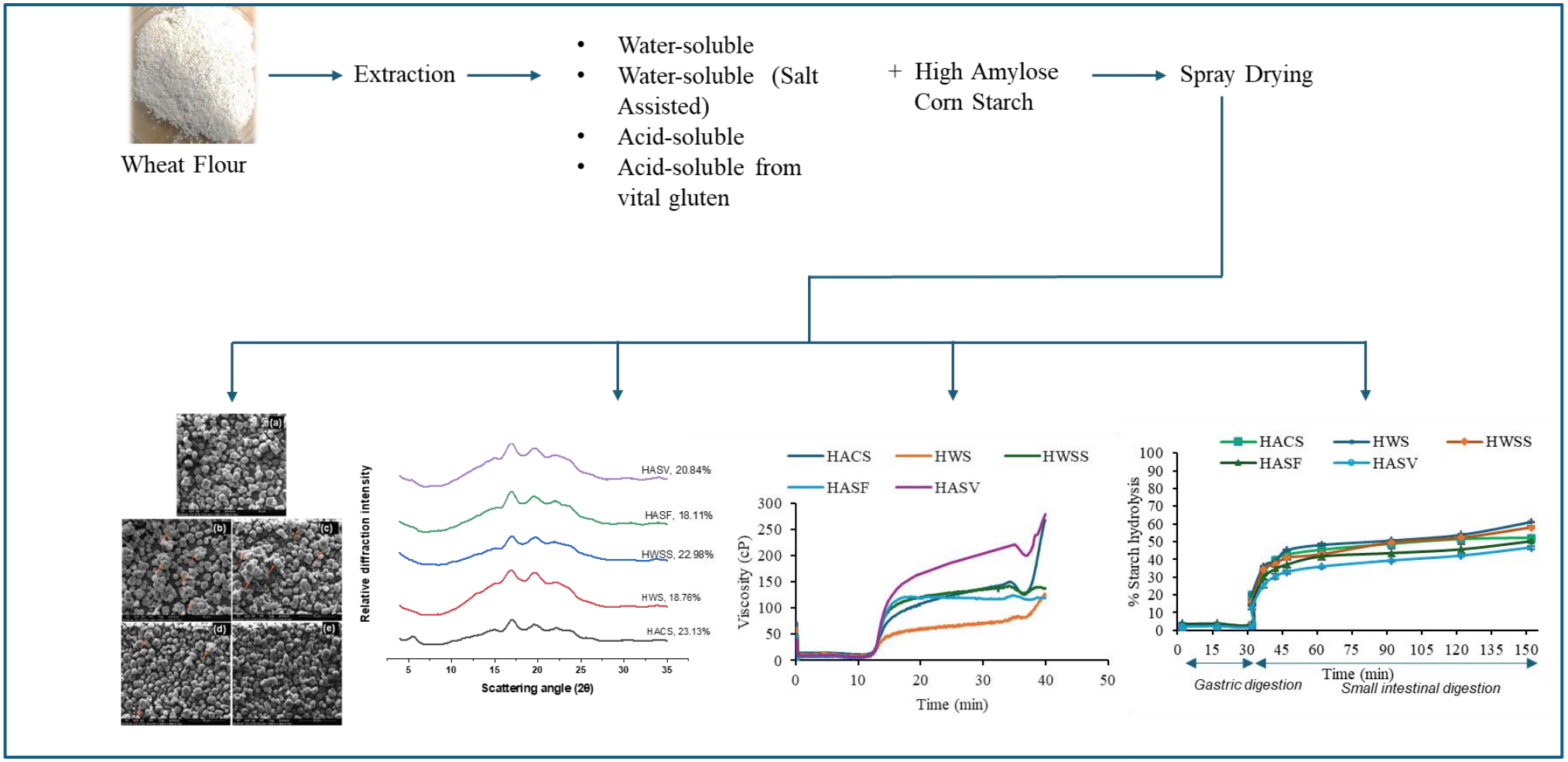

Figure 7. 3 *Summary of modification and characterisation of resistant starch with soluble components from wheat (Chapter 5)*

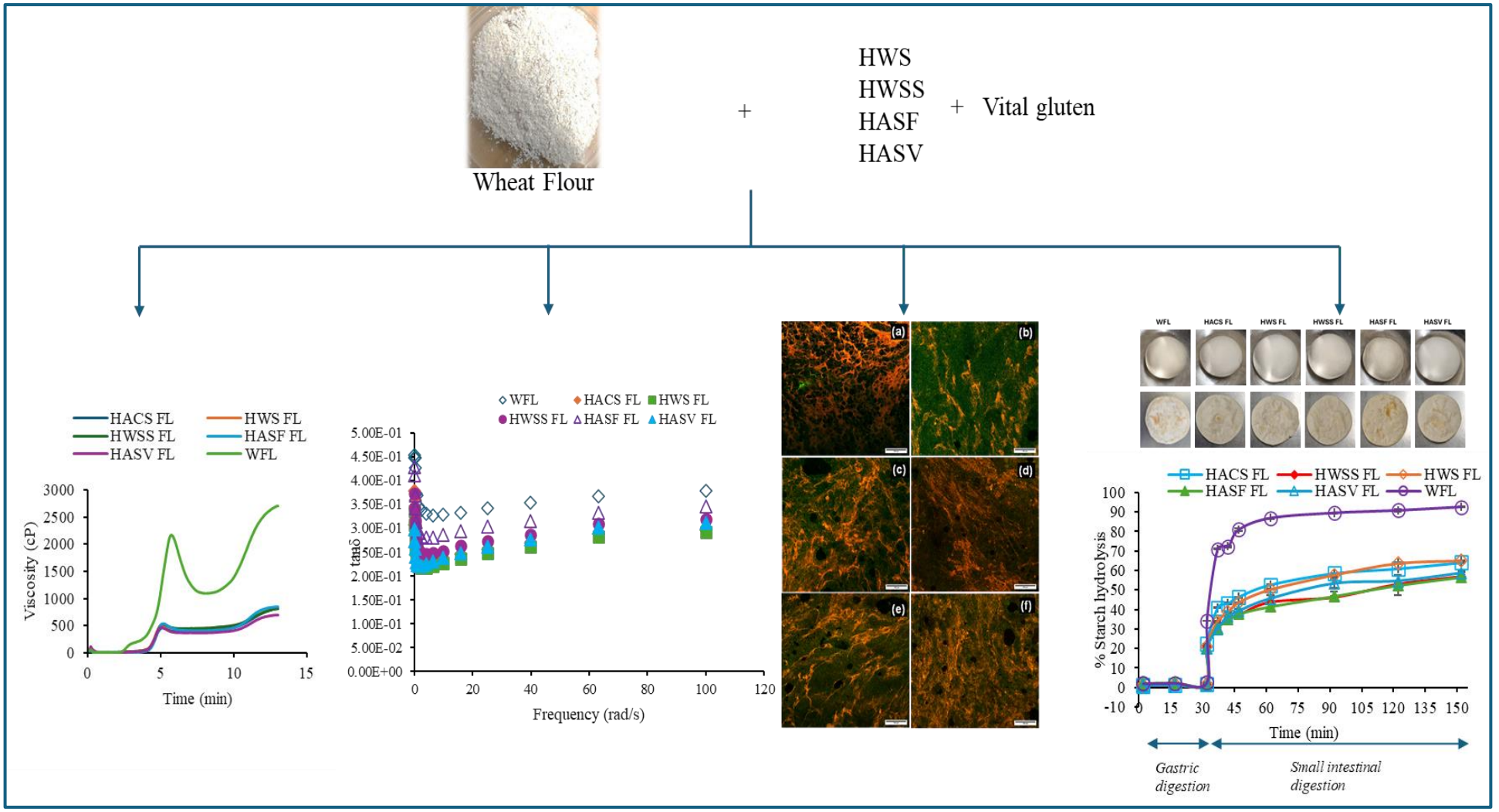

Figure 7. 4 *Summary of the functionality of resistant starch modified with hydrocolloids in a wheat flour system with partial replacement (Chapter 6)*

These modified starches were then used in a wheat flour system (Chapter 6), and their functional and digestible properties were evaluated by creating functional flour formulations with wheat flour (50%), vital gluten, and modified starches (Figures 7.4). The water-holding capacity of the functional flours containing modified starches increased approximately twofold that of wheat flour. At the same time, the pasting properties were negatively impacted due to the partial replacement of wheat flour with modified starches. However, their functionality in the dough was improved regarding the rheological parameters such as storage modulus, loss modulus and loss tangent. The storage modulus increased for doughs from functional flours with modified starches loaded with wheat solubles; acid-soluble wheat flour extract and salt-assisted water-soluble wheat flour extract showed a closer profile to wheat flour dough when the water level in the doughs was increased. Also, the improved interaction of wheat flour and acid-soluble components loaded on resistant starch resulted in a loss modulus profile similar to wheat flour. The confocal laser scanning micrographs revealed an improved dough microstructure with a continuous network of gluten and starch in the dough containing wheat-solubles modified starches compared to a weaker and non-continuous network in dough containing unmodified starch. Furthermore, the dough was utilised to prepare chapattis, and the bake loss was reduced in the functional flour formulation, indicating better keeping quality. Moreover, the starch hydrolysis of chapattis from all the functional flour formulations was significantly lower than the wheat flour chapatti. Starch hydrolysis was reduced by up to 39.3%, and chapattis' eGI reduced from 92.45±0.1 to 67.47±1.14 by partially replacing wheat flour with wheat solubles modified resistant starch and vital gluten. Overall, modified resistant starches loaded with acid-soluble wheat flour extract and salt-assisted wheat flour extract were more effective in reducing the starch hydrolysis of wheat flour chapattis while improving dough functionality.

7.1.4 Impact of hydrocolloids on resistant starch's structural, physicochemical properties, and *in vitro* digestion behaviour

Another strategy to improve the functionality while maintaining low starch digestion explored HACS modification using commonly used food hydrocolloids such as guar gum, xanthan gum, locust bean gum and carboxymethyl cellulose in Chapter 5 (Figure 7.5). The spray drying of HACS-hydrocolloid mixtures resulted in starch-hydrocolloid cluster formation. The water-holding capacity of the resulting starches increased, resulting in increased RVA pasting viscosities for most of the modified starches; however, the treatment negatively impacted the relative crystallinity. Furthermore, all the hydrocolloid-modified starches, except HCMC, had a lower starch hydrolysis profile until 90 minutes of simulated digestion compared to HACS in cooked form (100 °C for 20 min). Among all the hydrocolloids used in this study, the interaction of locust bean gum with HACS was found to be most effective in modifying microstructure and reducing starch hydrolysis throughout the simulated digestion.

The results obtained in Chapter 5 allowed these modified starches to be evaluated for their digestibility and functional properties in a wheat-based system (Figure 7.6). These modified starches and vital gluten were used in functional flour formulations with wheat flour (50%) (Chapter 6). Similar to the modified starches with wheat solubles, the pasting profile was negatively impacted due to the partial replacement of wheat flour with hydrocolloid-modified starches, however, their functionality in the dough was improved. The loss tangent, i.e. the ratio of loss modulus to storage modulus decreased for doughs from functional flours with modified starches loaded with hydrocolloids; locust bean gum followed by guar gum showed a closer profile to wheat flour dough at increased water levels. Also, compared to a weaker and non-continuous network in dough containing unmodified starch, an improved dough microstructure with a continuous network of gluten and starch in the dough containing hydrocolloid-modified starches as shown by the confocal laser scanning micrographs.

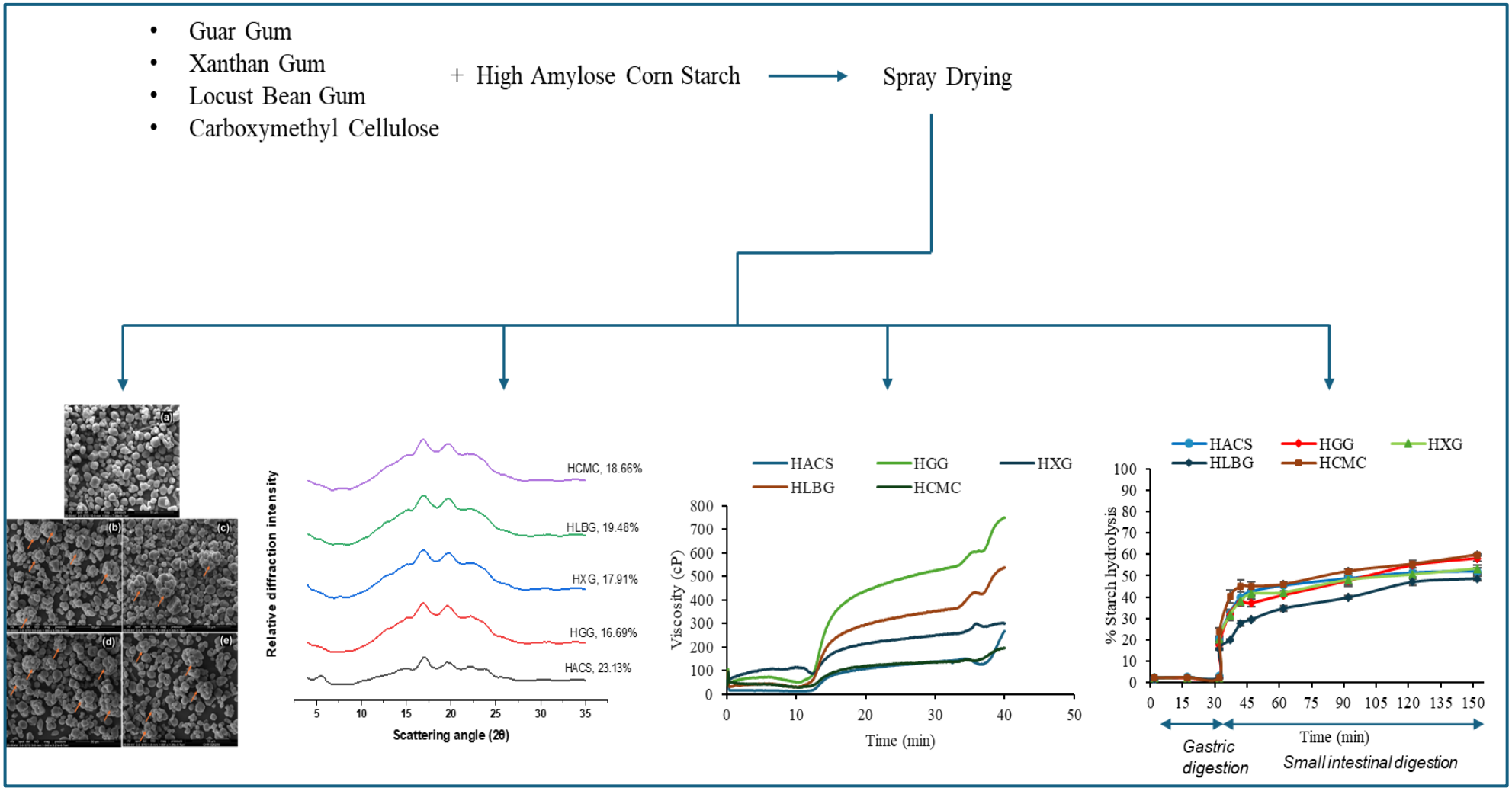

Figure 7. 5 *Summary of modification and characterisation of resistant starch with hydrocolloids (Chapter 5)*

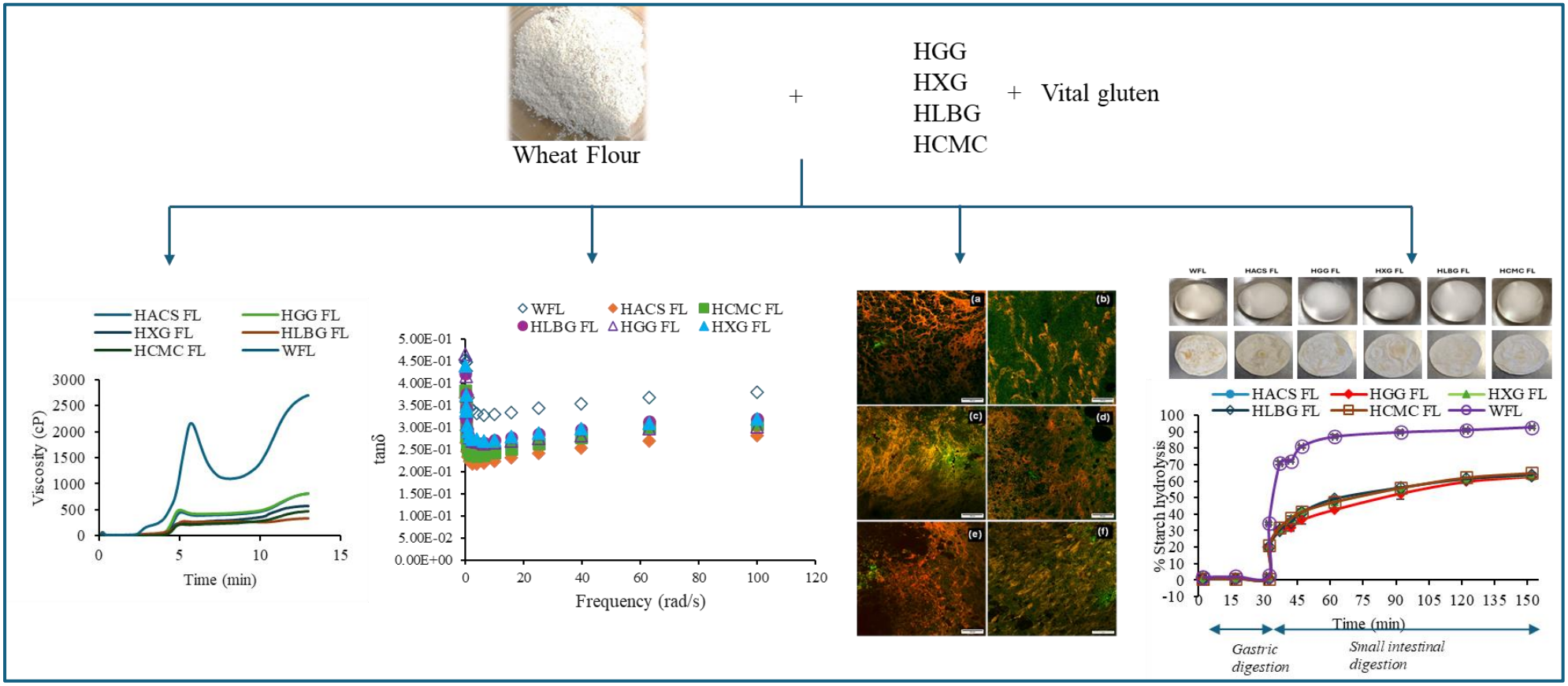

Figure 7. 6 *Summary of the functionality of resistant starch modified with hydrocolloids in a Wheat Flour System with partial replacement (Chapter 6)*

Moreover, when chapattis were prepared using these functional flours, the bake loss was reduced signifying their better-keeping quality. Likewise, all the functional flour formulations with hydrocolloid-modified starches significantly reduced the starch hydrolysis and eGI of chapattis. The starch hydrolysis was reduced from 92.61±0.09 to 62.48±0.61% while the eGI of chapattis reduced from 92.45±0.1 to 69.95±0.77 by partially replacing wheat flour with guar gum modified resistant starch and vital gluten. Similar to modified resistant starches loaded with wheat solubles, hydrocolloid-modified resistant starches were also effective in reducing the starch hydrolysis of wheat flour chapattis while improving dough functionality.

7.2 Implications for food industry and further recommendations

This study provided a fundamental understanding of the impact of structural modification of the wheat grain and different strategies employing resistant starch to reduce starch digestibility and improve the functionality of a wheat flour system. This research provides a groundwork for further investigation on developing low glycaemic wheat flour with better functionality. The findings from this thesis support the structural engineering approach to reduce the starch digestion and provide opportunities for functional and slowly digestible food formulations by utilising intact grains, resistant starches, soluble components of wheat and hydrocolloids. Also, this research work supports the multi-component strategy (using modified starches with wheat solubles or hydrocolloids along with wheat flour), ingredient-based method to achieve nutritional goals and processing stability without requiring radical changes in consumer habits or machinery.

a) Wheat grains' structural modification impacts their starch hydrolysis, and maintaining the intact microstructure was the most effective strategy for reducing this hydrolysis. The presence of an intact bran layer, endosperm cell wall, and protein matrix may protect the interaction of digestive enzymes with starch; however, processing may destroy the natural intact microstructure of the grains. Size reduction and cooking used

in combination could lead to almost complete digestion (92.85±1.21%) from nearly no digestion (2.19±0.06%). These findings inspire the development of intact or encapsulated structures of starches and proteins to be used as low-glycaemic raw materials in wheat-based products.

b) Processing the hydrothermally treated whole grains into different formats, such as flakes or flour, significantly enhanced their extent of starch hydrolysis. Nevertheless, the low-temperature storage reduced their starch hydrolysis by up to 9% in different formats such as dried whole grain, flakes, and flour, thereby reducing their eGI. The hydrothermal treatment and low-temperature storage for prolonged periods of whole wheat grains results in starch retrogradation. However, the presence of soluble fibres, proteins, and amylose-lipid complex formed during the hydrothermal treatment of the whole grains could interfere with the retrogradation of the starch, and additional processing might further interfere with their effect in reducing the overall starch hydrolysis. Therefore, it could be an effective strategy for reducing the overall starch hydrolysis of whole grain products such as whole grains and flakes; however, these treatments negatively impact the flour functionality. Further studies could involve pre-treatments like pearling or milling of whole grains to enhance the extent of retrogradation during cold-storage.

c) Resistant starch, such as high amylose corn starch, could be used in wheat flour systems to reduce their glycaemic index. Utilisation of soluble components of wheat such as water solubles, salt-assisted water solubles, and acid solubles was effective in modifying high amylose corn starch to improve their functionality and reduce digestibility when used in partial replacement in wheat flour-based products. The SEM micrographs revealed clusters of starch and soluble extracts from wheat similar to wheat flour microstructure. Utilising the modified resistant starches effectively reduced

the starch digestibility of wheat flour chapatti by reducing the hydrolysis levels from 92.61±0.09% to 56.21±1.75%, transforming it from high GI to medium GI. Further studies could involve characterisation, such as protein analysis and polysaccharide analysis of the soluble extracts from wheat, to evaluate their composition and role in modified starches' functional properties. The chemical interactions in dough need further exploration for a better understanding of the role of wheat components.

d) Hydrocolloids such as guar gum are effective in modifying high amylose corn starch to reduce digestibility, and guar gum and xanthan gum to improve the functionality of wheat flour-based products. Utilising the hydrocolloid-modified resistant starches effectively reduced the starch digestibility of wheat flour chapatti by reducing the hydrolysis levels from 92.61±0.09% to 62.48±0.61%, transforming it from high GI to medium GI. The chemical interaction between dough components needs further exploration to better understand the role of hydrocolloids in dough improvement. Hydrocolloids from different origins and varied concentrations could be explored in future studies to create better functional and slower digestion properties in food products.

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Appendix

Appendix

I. Data supporting Chapter 3

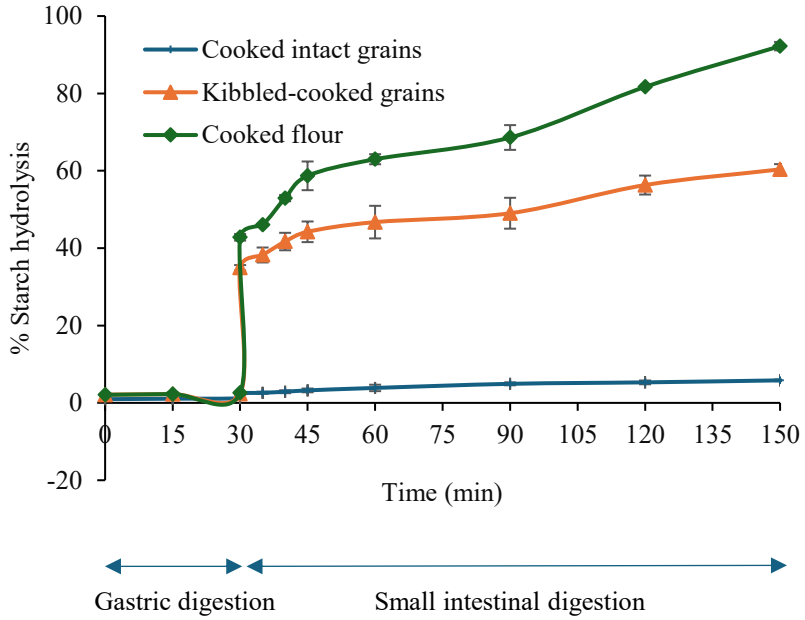

Figure A 3. 1 *Effect of processing of the whole wheat grain on starch hydrolysis during in vitro gastro-small intestinal digestion in gelatinised state (Grain variety-biscuit wheat, Ignite). Error bars represent standard deviation.*

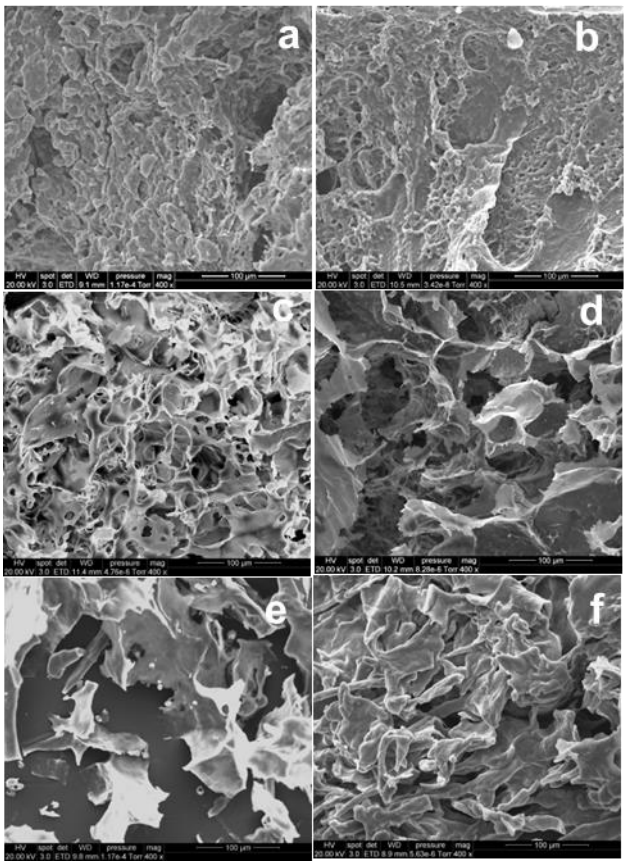

Figure A 3. 2 *SEM micrographs of the digests of cooked grain samples (Grain variety-biscuit wheat, Ignite) collected after G0 and I120 digestion phases: intact grain sectioned after freeze-drying (a,b), kibbled grain (c,d), and flour (e,f), respectively. G0-before gastric digestion, and I120-after 120 min of small intestinal digestion phase. (scale:100 µm).*

II. Data supporting Chapter 4

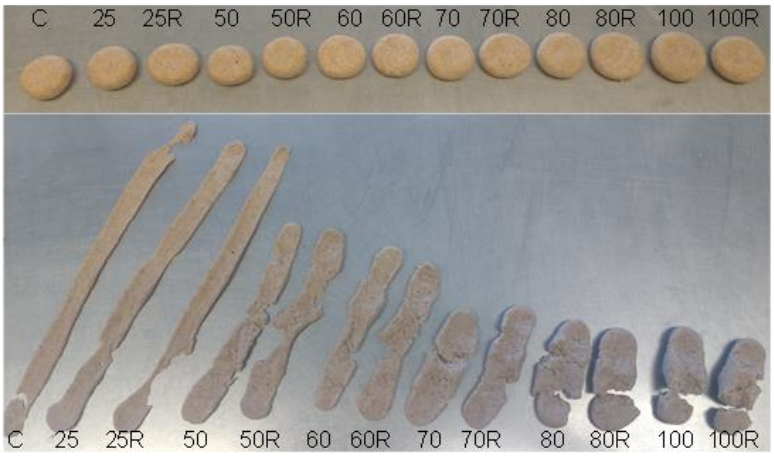

Figure A 4. 1 *Preliminary trials for flour functionality from whole wheat grains after hydrothermal treatment (25, 50, 60,70, 80 and 100) and their low-temperature stored counterparts (25R, 50R, 60R, 70R, 80R and 100R) from Chapter 4.*

III. Data supporting Chapter 5

Figure A 5. 1 *Preliminary trials for the functionality of different starch sources and gluten.*

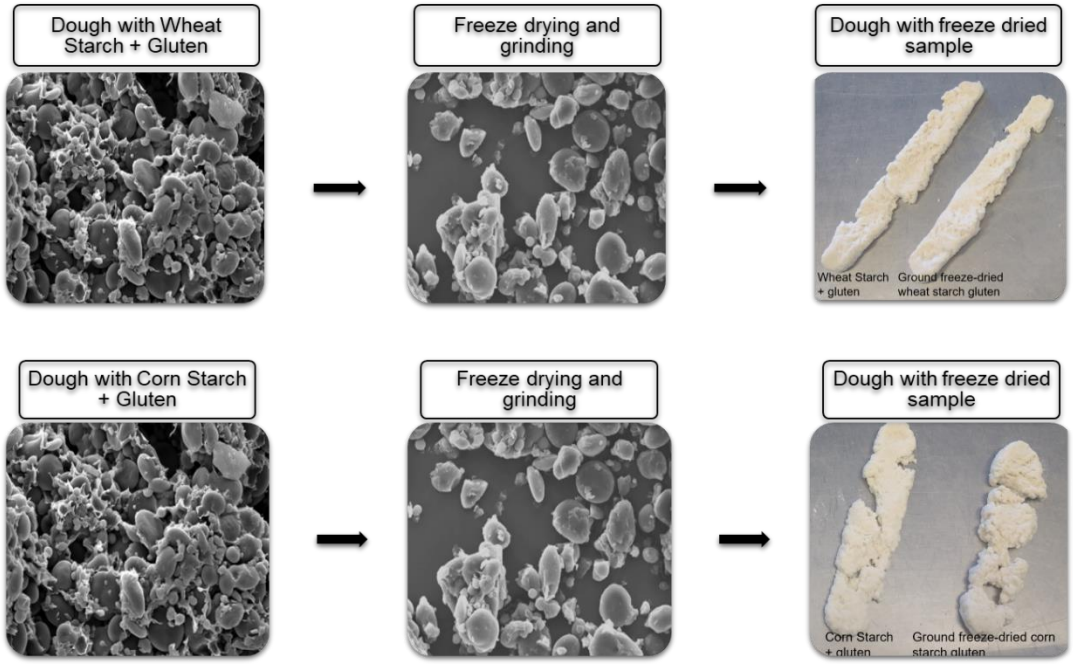

Figure A 5. 2 *Preliminary trials for the surface modification of starch to improve the functionality of different starch sources with gluten.*

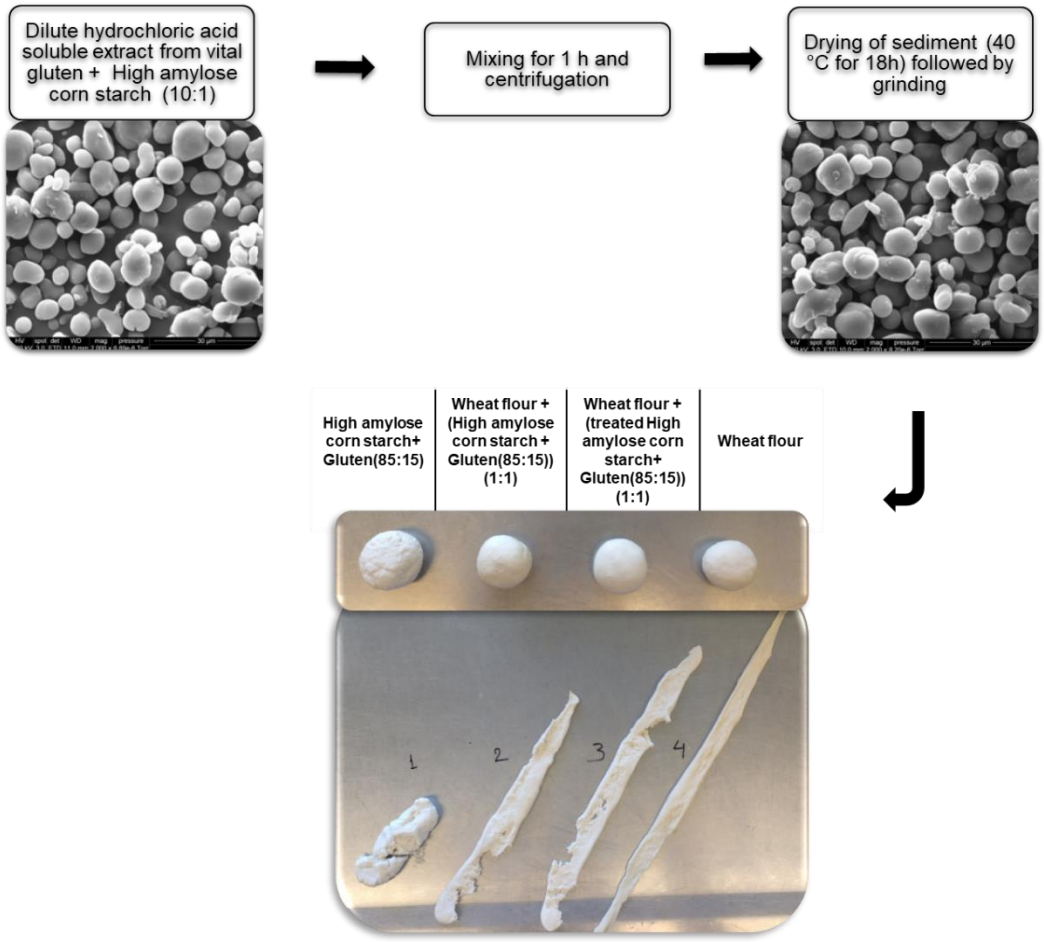

Figure A 5. 3 *Preliminary trials for the surface modification of resistant starch with acid-soluble extract from vital gluten for improvement in functionality.*

IV. Data supporting Chapter 6

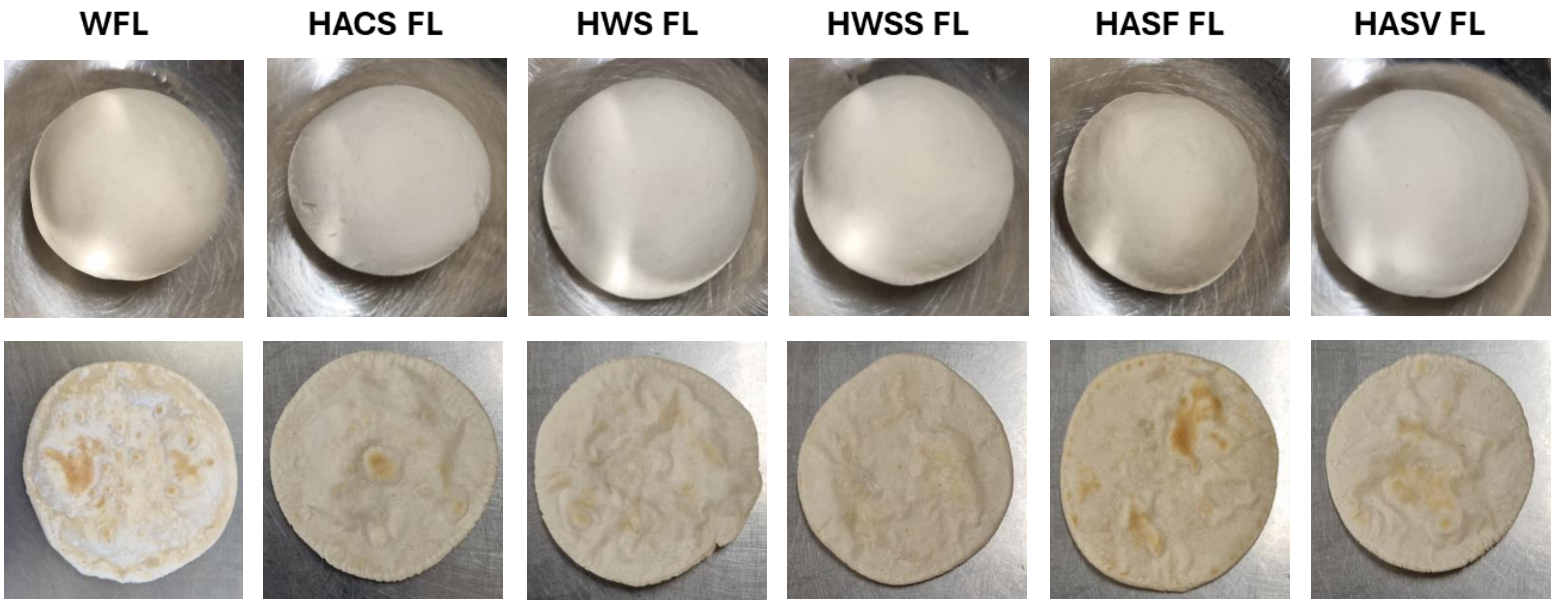

Figure A 6. 1 *Dough and chapattis prepared from wheat flour and functional flour with modified starches with wheat solubles. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HWS FL-functional flour with HACS modified with water-soluble wheat flour extract, HWSS FL-functional flour with HACS modified with salt-assisted water-soluble wheat flour extract, HASF FL- functional flour with HACS modified with acid-soluble wheat flour extract, and HASV FL- functional flour with HACS modified with acid-soluble vital gluten extract.*

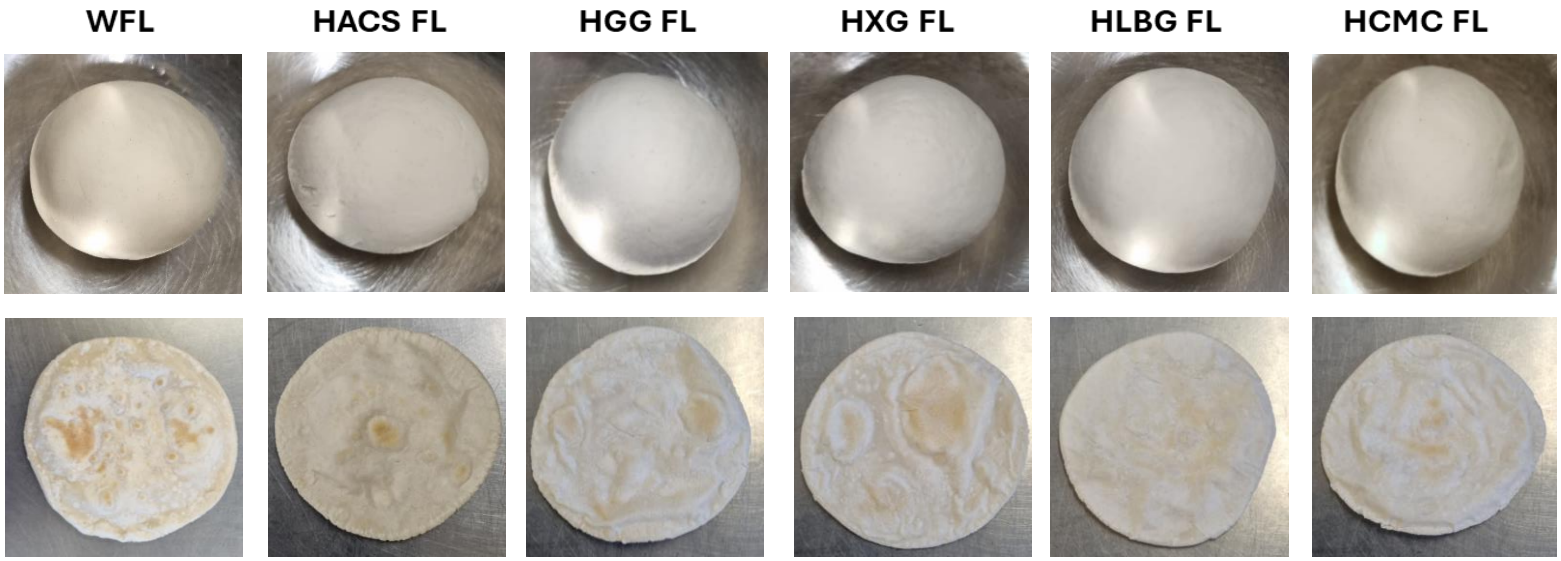

Figure A 6. 2 *Dough and chapattis prepared from wheat flour and functional flour with modified starches with hydrocolloids. WFL-wheat flour, HACS FL-functional flour with high amylose corn starch, HGG FL-functional flour with HACS modified with guar gum, HXG FL-functional flour with HACS modified with xanthan gum, HLBG FL-functional flour with HACS modified with locust bean gum, HCMC FL-functional flour with HACS modified with carboxymethyl cellulose.*

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Exploration of the literature review, preparation of original draft and revisions of the draft was done by the student. The main supervisor edited and reviewed the manuscript, guided for the idea and the modifications. Co-supervisor Assoc. Prof. Lovedeep Kaur reviewed, and edited the manuscript.

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We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Abhilasha
Name and title of main supervisor:	Professor Jaspreet Singh

In which chapter is the manuscript/published work?	Chapter 5 and 6

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Conceptualization, data curation, formal analysis, methodology, writing -original draft and -review and editing has been done by the candidate. The main supervisor helped in conceptualization, planning, methodology, analysis, results, original draft and revised drafts. Co-supervisor Assoc. Prof. Lovedeep Kaur helped in conceptualization, methodology, analysis, reviewing and editing the manuscript. Co-supervisor Dr. John Monro helped in reviewing and editing the manuscript.

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