Assessment of Physicochemical Properties, Nutritional Values, and Sensory Acceptability of Pigeon Pea Flour Substitution in Chapati Flat Bread

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Food and Nutrition
Department of Nutrition and Dietetics
FACULTY OF ALLIED HEALTH SCIENCES
Chulalongkorn University
Academic Year 2021

การประเมินคุณสมบัติทางเคมีฟิสิกส์ คุณค่าทางโภชนาการและการยอมรับทางประสาทสัมผัสของการทดแทนแป้งถั่วมะแฮะในขนมปังจาปาตี

น.ส.ศิรินทร์ สัจจนฤหล้า

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอาหารและโภชนาการ ภาควิชาโภชนาการและการกำหนดอาหาร
คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2564

Thesis Title	Assessment of Physicochemical Properties, Nutritional Values, and Sensory Acceptability of Pigeon Pea Flour Substitution in Chapati Flat Bread
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Field of Study	Food and Nutrition
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ศิรินทร์ สัจจนฤหล้า : การประเมินคุณสมบัติทางเคมีฟิสิกส์ คุณค่าทางโภชนาการและการยอมรับทางประสาทสัมผัสของการทดแทนแป้งถั่วมะแฮะในขนมปังจาปาตี . (Assessment of Physicochemical Properties, Nutritional Values, and Sensory Acceptability of Pigeon Pea Flour Substitution in Chapati Flat Bread) อ.ที่ปรึกษาหลัก : ดร.แพรว จันทรศิลปิน, อ.ที่ปรึกษาร่วม : ศ. ดร.สิริชัย อดิศักดิ์วัฒนา

ในช่วงปีที่ผ่านมา ความสนใจในโปรตีนจากพืชเพิ่มขึ้นอย่างมาก ถั่วมะแฮะ (*Cajanus cajan*) เป็นแหล่งโปรตีนจากพืชชั้นดี การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาแป้งผสมจากถั่วมะแฮะ (PPF) และแป้งโฮลวีต (WWF) ซึ่งถูกนำมาใช้ทำขนมปังจาปาตี ระดับการทดแทน PPF ใน WWF อยู่ระหว่าง 10%–40% w/w จากนั้นทำการศึกษาคุณสมบัติทางกายภาพและคุณค่าทางโภชนาการของแป้งผสมและจาปาตี นอกจากนี้ ได้ทำการประเมินการยอมรับทางประสาทสัมผัสของจาปาตีที่ทำจากแป้งผสมอีกด้วย การวิเคราะห์ proximate analysis เผยให้เห็นปริมาณโปรตีนใน PPF (26.10%) สูงกว่า WWF สองเท่า (13.52%) PPF มีความเหลือง (ค่า *b**) สูงกว่าอย่างมีนัยสำคัญ ในขณะที่มีค่าสีแดง (ค่า *a**) และความสว่าง (ค่า *L**) ที่ต่ำกว่าเมื่อเทียบกับ WWF (p<0.05) การแทนที่ WWF ด้วย PPF ที่ 20%–40% ทำให้ค่าสีแดงลดลงอย่างมีนัยสำคัญ (p<0.05) PPF มีปริมาณแป้งทั้งหมด (total starch; TS) และการย่อยได้ของแป้งที่ต่ำกว่า ในขณะที่มีการปลดปล่อยของสารประกอบที่มีหมู่อะมิโนสูงกว่าเมื่อเปรียบเทียบกับ WWF (p<0.05) หลังจากนั้น การแทนที่ WWF ด้วย PPF ในจาปาตีส่งผลให้ความเหลืองและความแข็งของจาปาตีที่สูงขึ้นเมื่อเปรียบเทียบกับจาปาตีควบคุม จาปาตีที่มีการแทนที่ PPF ที่ 20% และ 40% มีการปลดปล่อยกลูโคสที่ลดลงภายใต้การย่อยแบบจำลอง ซึ่งสอดคล้องกับดัชนีระดับน้ำตาลในเลือดที่คาดการณ์ไว้ (predicted glycemic index; pGI) ที่ลดลงเมื่อเปรียบเทียบกับกลุ่มจาปาตีควบคุม (p<0.05) ซึ่งอาจเนื่องมาจากปริมาณแป้งรวม (TS) ที่ต่ำกว่าอย่างมีนัยสำคัญ และปริมาณแป้งต้านทาน (resistant starch, RS) ที่สูงขึ้นด้วยการแทนที่ PPF ที่เพิ่มขึ้น (p<0.05) ในการสอบวิเคราะห์นินไฮดริน สารประกอบที่มีหมู่อะมิโนเพิ่มขึ้นอย่างเห็นได้ชัดในจาปาตีที่มีการแทนที่ PPF อยู่ที่ 40% เมื่อเปรียบเทียบกับจาปาตีควบคุม (p<0.05) รสชาติ เนื้อสัมผัส รสที่ค้างอยู่ในคอ และการยอมรับโดยรวมของจาปาตีที่มี PPF 40% ลดลงอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับจาปาตีควบคุม (p<0.05) เมื่อเปรียบเทียบระหว่างประเภทของผู้บริโภค ผู้ที่เคยบริโภคจาปาตีมาก่อนให้คะแนนที่สูงกว่าอย่างมีนัยสำคัญในทุกพารามิเตอร์สำหรับจาปาตีที่มี 20% PPF เมื่อเทียบกับผู้บริโภคใหม่ (p<0.05) อย่างไรก็ตาม การยอมรับโดยรวมของ จาปาตีที่มี 20% PPF พบว่าไม่มีความแตกต่างกันระหว่างกลุ่มผู้บริโภค การศึกษานี้ชี้ให้เห็นว่า PPF สามารถเป็นส่วนผสมที่ใช้ในการปรับปรุงคุณค่าสารอาหารของจาปาตีและผู้บริโภคยอมรับได้ดี

สาขาวิชา	อาหารและโภชนาการ	ลายมือชื่อนิสิต ....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

6176852737 : MAJOR FOOD AND NUTRITION

KEYWORD: Pigeon pea, Starch digestion, Chapati, Protein digestibility

Sirin Sachanarula : Assessment of Physicochemical Properties, Nutritional Values, and Sensory Acceptability of Pigeon Pea Flour Substitution in Chapati Flat Bread. Advisor: Praew Chantarasinlapin, Ph.D. Co-advisor: Prof. SIRICHAI ADISAKWATTANA, Ph.D.

In recent years, an interest in plant–based protein increased dramatically. Pigeon pea (*Cajanus cajan*) is recognized as a good source of plant protein. The current study was aimed to develop pigeon pea flour (PPF) and whole wheat flour (WWF) blends, which were then used to make chapatis. The substitution levels of PPF for WWF ranged from 10%–40% w/w. The physical properties and nutritional values of the flour blends and the chapatis were investigated. The chapatis were also evaluated for sensory acceptability. Proximate analysis of the flours revealed protein content in PPF (26.10%) two times higher than that in WWF (13.52%). PPF had significantly higher yellowness (*b** value), whereas had lower redness (*a** value) and lightness (*L** value) as compared to WWF ($p<0.05$). Substitution of WWF with PPF at 20%–40% caused a significant decrease in redness values of the flour blends ($p<0.05$). PPF showed lower total starch content and starch digestibility; had a higher release of amino–group–containing compounds as compared to WWF flour samples ($p<0.05$). Thereafter, the substitution of PPF for WWF into chapati presented higher yellowness and hardness of chapati as compared to the control ($p<0.05$). Chapati with PPF substitution at 20% and 40% attenuated glucose release under simulated digestion, corresponding to decreased predicted glycemic index (pGI) when compared to the control chapati ($p<0.05$). This may be due to the significantly reduction of total starch contents, and increase in resistant starch contents with the increased substitutions of PPF in the chapati ($p<0.05$). In ninhydrin assay, amino–group residues markedly elevated in chapati with 40% PPF substitution as compared to the control ($p<0.05$). Sensory evaluation revealed that taste, texture, aftertaste, and overall acceptability of chapati with 40% PPF were significantly decreased when compared to the control ($p<0.05$). For sensory evaluation, when compared between the types of consumers, regular consumers gave significantly higher scores in all parameters for 20% PPF chapati as compared to the new consumers ($p<0.05$). However, the overall acceptance of 20% PPF chapati showed no significant difference between consumer groups. These findings suggest that PPF can serve as a promising ingredient to improve nutrient values of plant–based chapatis with adequate consumer acceptability.

Field of Study:	Food and Nutrition	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....
		Co-advisor's Signature ....

ACKNOWLEDGEMENTS

First and foremost, I would like to take this opportunity to thank His Holiness Sri Satguru Uday Singh Ji, for His blessings, strength, and guidance in helping me surpass all obstacles encountered throughout to complete this study successfully.

Next, I would like to sincerely thank my advisor, Dr. Praew Chantarasinlapin, and my co-advisor, Professor Dr. Sirichai Adisakwattana for their continued support, invaluable time, and for nurturing me till the end of this research work. I will remain grateful to you, for your effective supervision and understanding. It is a great privilege and honor to work and study under their guidance.

I am also grateful to my thesis committees: Associate Professor Dr. Suwimol Sapwarobol and Dr. Thavaree Thilavech for offering their priceless time and sharing their expertise by giving constructive recommendations and suggestions in uplifting the technicality of this study.

Additionally, I thank my colleagues and all my labmates in the Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University, for their friendship, support, help, suggestions and encouragement throughout the journey.

Lastly, these acknowledgments would not be complete without mentioning my family and my loved ones. I would like to thank them for their love, care, prayers, and support. They give me the strength, opportunity, and inspiration to achieve all my goals in life. I love you.

Sirin Sachanarula

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CHAPTER I

INTRODUCTION

Background of study

A new trend of food is to reduce consumption of animal–based meat, which has led to a dramatic increase in the needs for plant–based sources of proteins (1). Plant–based diet consists of less or no animal consumption, also known as a vegetarian or vegan diet (2). It has shown various benefits such as weight loss and lower risks of obesity, high blood pressure, diabetes, and heart disease (3). Plant protein sources account for up to 65% of the world's supply of edible protein, with cereal grains accounting for 47% and pulses, nuts and oilseeds accounting for 8% (4).

Wheat is a grass widely cultivated for its seeds, and a staple food consumed worldwide. It is the third most important crop after rice and maize in terms of global production (5). The consumption of wheat significantly increased in Nigeria, China, and India (5). Whole wheat is most commonly found in a form of flour, which is extensively used for the production of staple foods, including flat breads (6). In India, over 85% of wheat consumption is in the form of unleavened flat bread, namely chapati (6). Wheat serves as a primary source of carbohydrate and energy. It also provides proteins, dietary fiber, vitamins, and phytochemicals. Even though wheat has a respectively high amount of protein (10%–15%), its protein quality is low. This is due to the fact that it has lysine and threonine as its limiting amino acids (5).

On the other hand, pigeon pea (PP, *Cajanus cajan*) is a legume crop grown widely in Africa, Central America, and India (7). It was reported that pigeon pea has high protein content of up to 24%, and it is a rich source of amino acid lysine (8). Moreover, it is relatively high in fiber, vitamins, and minerals. Pigeon pea is classified as a low glycemic food, of which consumption has been shown to reduce the risk of non-communicable diseases (9).

Proteins can be characterized by their nutritional values, deduced from the essential amino acids presented. Animal proteins are usually nominated with almost 100%, while most vegetal proteins are classified with values between 50% to 90% (10). Generally, legume proteins are high in lysine and lack sulfur–containing amino acids, whereas cereal proteins are deficient in lysine but have an adequate amount of sulfur–containing amino acids. By combining different protein sources with different essential amino acids it becomes possible to reach 100% or more (10).

Given that, combining grains with legume protein would provide a better overall balance of essential amino acids. However, to date studies conducted to incorporate pigeon pea into staple flat bread “chapati” as a novel ingredient is still rare. Therefore, the current research aimed to develop composite flour and flat bread by partially substituting different proportions of pigeon pea flour into whole wheat flour. Then, physicochemical properties, nutritional values, and sensory acceptability of the flour blends and flat bread made from whole wheat–pigeon pea flour blends were investigated. This contributes to a better understanding of the utilization of pigeon pea flour in chapatis. Additionally, the effect of partial pigeon pea flour substitution on nutritional values, digestibility, and overall sensory acceptance by the consumers of the chapatis was also explained.

Research Objective

1. **Product development**
    - 1.1 To develop a staple food product made from different levels of pigeon pea flour substitution.
2. **Physicochemical properties**
    - 2.1 To investigate the effects of different levels of pigeon pea flour substitution on physicochemical properties of the whole wheat–pigeon pea composite flour, dough, and flat bread.
3. **Nutritional analysis**
    - 3.1 To investigate the effects of different levels of pigeon pea flour substitution on digestibility of whole wheat–pigeon pea composite flour and flat bread.
4. **Sensory evaluation**
    - 4.1 To investigate the effects of different levels of pigeon pea flour substitution on the acceptability of flat bread.

Research Question

1. **Product development**
    - 1.1 Can a staple food product be developed from different levels of pigeon pea flour substitution?
2. **Physicochemical properties**
    - 2.1 How does the different proportions of pigeon pea flour substitution affect the physicochemical properties of the whole wheat–pigeon pea composite flour?

2.2 How does the different proportions of pigeon pea flour substitution affect the physicochemical properties of the whole wheat–pigeon pea composite dough?

2.3 How does the different proportions of pigeon pea flour substitution affect the physicochemical properties of the whole wheat–pigeon pea composite flat bread?

3. Nutritional analysis

3.1 How does the different proportions of pigeon pea flour substitution affect the digestibility of the whole wheat–pigeon pea composite flour?

3.2 How does the different proportions of pigeon pea flour substitution affect the digestibility of the whole wheat–pigeon pea composite flat bread?

4. Sensory evaluation

4.1 How does the different proportions of pigeon pea flour substitution affect acceptability of flat bread?

Research Hypothesis

1. Product development

1.1 A staple food product flat bread will be developed from different levels of pigeon pea flour substitution.

2. Physicochemical properties

2.1 The whole wheat–pigeon pea composite flour will have color similar to that of the control.

2.2 The whole wheat–pigeon pea composite dough will have color similar to that of the control.

2.3 The whole wheat–pigeon pea composite flat bread will have color similar to that of the control.

2.4 The whole wheat–pigeon pea composite flour will have moisture content similar to that of the control.

2.5 The whole wheat–pigeon pea composite dough will have moisture content similar to that of the control.

2.6 The whole wheat–pigeon pea composite flat bread will have moisture content similar to that of the control.

2.7 The whole wheat–pigeon pea composite flat bread will have texture profile similar to that of the control.

3. Nutritional value

3.1 Increasing pigeon pea flour substitution will simultaneously increase protein content of composite flour as compared to the control.

3.2 Increasing pigeon pea flour substitution will simultaneously increase protein content of flat bread as compared to the control.

3.3 The whole wheat–pigeon pea composite flour will have slower starch digestibility as compared to the control.

3.4 The whole wheat–pigeon pea composite flat bread will have slower starch digestibility as compared to the control.

3.5 The whole wheat–pigeon pea composite flour will have lower predicted glycemic index as compared to the control.

3.6 The whole wheat–pigeon pea composite flat bread will have lower predicted glycemic index as compared to the control.

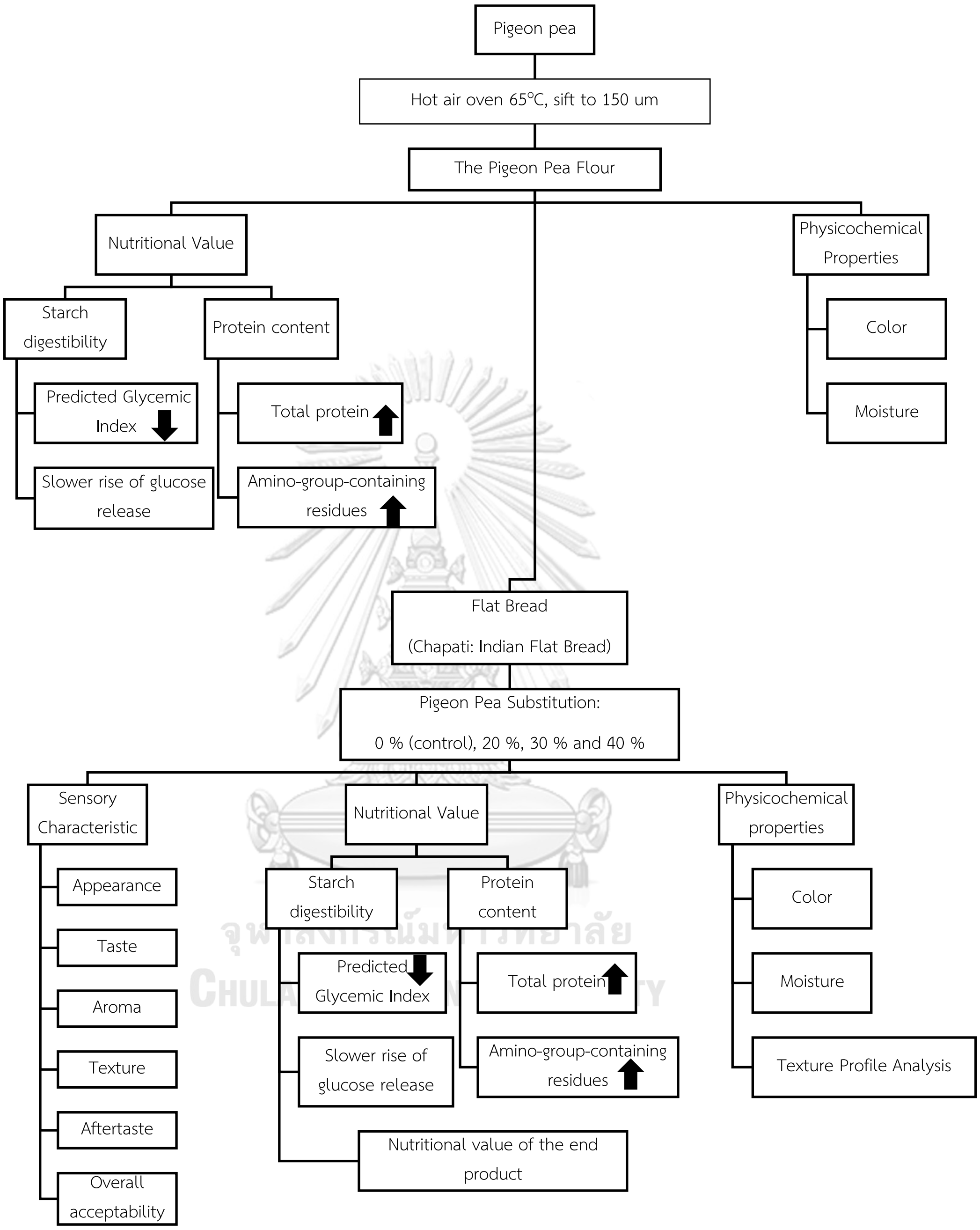
3.7 Increasing pigeon pea flour substitution will simultaneously increase the amino–group–containing compound of composite flour as compared to the control.

3.8 Increasing pigeon pea flour substitution will simultaneously increase the amino–group–containing compound of composite flat bread as compared to the control.

4. Sensory analysis

4.1 The whole wheat–pigeon pea composite flat bread will have levels of appearance, texture, aroma, taste, aftertaste, and overall acceptability similar to the control.

Conceptual framework



CHAPTER II

LITERATURE REVIEW

Protein

Protein participates in various body functions, including maintenance, growth, regulation of body processes; repairs and structures; and energy provision. Proteins are large chemical structures made up of smaller building blocks called amino acids. There are 20 amino acids that make up most of the body’s proteins (Table 2.1). Healthy humans can endogenously produce several amino acids, such as alanine, cysteine, and glutamine. These are known as non-essential amino acids. On the other hand, the human body cannot synthesize some amino acids, such as lysine, isoleucine, and leucine. These are called essential amino acids, which must be supplied from the diet. Body proteins, as well as other nitrogen-containing substances including peptide hormones, creatine, and certain neurotransmitters, require amino acid for production. Therefore, proper intake of total protein and essential amino acids is vital for maintaining good health.

Table 1 Amino Acids in Human

Essential Amino Acids	Non-Essential Amino Acid
Lysine	Tyrosine
Leucine	Serine
Isoleucine	Proline
Histidine	Glycine
Tryptophan	Glutamine
Threonine	Aspartate
Valine	Cysteine
Phenylalanine	Glutamate
Methionine	Arginine
	Asparagine
	Alanine

The quality of a protein is determined by the ratio of essential amino acids. There are two main sources of protein, namely animal protein, and plant protein. Animal protein is considered complete or excellent quality because it contains all the essential amino acids which humans need. On the other hand, plant proteins are regarded as incomplete or poor-quality proteins due to the lack of certain essential amino acids. Considering protein quality, meat and dairy products are excellent sources of essential amino acids. However, it may not be a suitable dietary component due to its costs and cultural restrictions. Therefore, maintenance and adequate intake of essential amino acids require attention, especially in a population with high dependence on plant protein (11).

The ninhydrin reaction is a widely used method for analysis and characterization of amino–group–containing residues such as amino acids, peptides, and proteins. The ninhydrin assay is actively applied for research in environmental, food and clinical chemistry, toxicology, microbiology, and pharmacology. The major strengths of the protein ninhydrin assay are its ability to analyze insoluble tissue and soluble protein, uniformity of the color reaction of the protein hydrolysate, the relatively high sensitivity and specificity of the ninhydrin reaction, and the applicability without protein hydrolysis (12).

Previous studies demonstrated that the ninhydrin reaction can be used to analyze chemically and nutritionally available lysine in food proteins. Protein hydrolysis followed by amino acid analysis can theoretically be used to determine total protein content. It has also been used to figure out how much amino–groups are in vegetables and fruits during ripening, browning, dehydration, and storage. The amino acid composition of colored proteins separated by SDS gel electrophoresis was determined, as well as the quantification of total protein based on the amino acid content of the protein hydrolysates (13).

Plant–based protein

A plant-based diet includes all food made from whole grains, legumes, fruits, vegetables, herbs and spices, nuts and seeds, and excludes all animal products (14). Interest in plant-based consumption was raised in recent years. In 2015, it was reported that approximately 0.4% to 3.4% American adults, 1% to 2% of British adults, and 5% to 10% of German adults ate predominantly plant-based diets (15). Similarly, the frequency of publication with the term ‘plant–based’ increased over 42 times, from 10 publications in 2007 to 425 publications in 2017 (15).

Plant protein provides up to 65% of the world's edible protein supply (16), with other major sources being cereal grains (47%), and legumes, nuts and oil seeds (8%) (4). Plant-based diets have shown various beneficial effects on human health, such as maintaining desirable body mass index (BMI) and improving plasma cholesterol concentrations (17). It may also reduce risks of non–communicable diseases such as type 2 diabetes (18), obesity, hypertension, cardiovascular mortality, hyperlipidemia, and cancer (3). Plant sources of protein may differ from animal sources in regard to digestibility and amino acid composition (Table 2.2). They also differ in the presence of anti-nutritional factors, which negatively affect digestibility and safety; and phytoprotective factors, which may be beneficial in mediating defense against disease (19).

Table 2 Amino acid content of dietary protein source and human skeletal muscle (g/100 g)

Amino Acid	Wheat	Soy	Pea	Milk	Egg	Human muscle
Threonine	1.8	2.3	2.5	3.5	2.0	2.9
Methionine	0.7	0.3	0.3	2.1	1.4	1.7
Phenylalanine	3.7	3.2	3.7	3.5	2.3	3.8
Histidine	1.4	1.5	1.6	1.9	0.9	2.8
Lysine	1.1	3.4	4.7	5.9	2.7	6.6
Valine	2.3	2.2	2.7	3.6	2.0	4.3
Isoleucine	2.0	1.9	2.3	2.9	1.6	3.4
Leucine	5.0	5.0	5.7	7.0	3.6	6.3
Total EAA*	18.0	19.9	23.6	30.3	16.5	31.8
Serine	3.5	4.3	3.6	4.0	3.3	2.3
Glycine	2.4	2.7	2.8	1.5	1.4	3.1
Glutamic acid	26.9	12.4	12.9	16.7	13.1	5.1
Proline	8.8	3.3	3.1	7.3	1.8	0.0
Cysteine	0.7	0.2	0.2	0.2	0.4	0.0
Alanine	1.8	2.8	3.2	2.6	2.6	4.1
Tyrosine	2.4	2.2	2.6	3.8	1.8	2.0
Arginine	2.4	4.8	5.9	2.6	2.6	4.4
Total NEAA**	48.9	31.9	34.4	38.6	19.0	29.0

*Total Essential Amino Acid. **Total Non-Essential Amino Acid. (20)

Whole Wheat

Wheat (43%), rice (39%), and maize (12%) are the three cereals that contribute the most to the world's edible protein supply. Wheat is a grass that is widely grown for its seeds, which functions as a global staple food. Many types of wheat together make up the genus *Triticum*. Common wheat (*T. aestivum*) is the most widely grown. Wheat's contribution to total calories increased significantly in Nigeria (less than 1% to 6.64%), India (11.85% to 20.41%), and China (12.20% to 17.83%) (5). It is extensively used in the form of flour as refined wheat flour and whole wheat flour. Commonly, refined wheat flour is used for the production of bakery products such as bread, cakes, biscuits, cookies, crackers, breakfast cereals, and noodles, while whole wheat flour is used for the preparation of traditional flat breads such as puri, roti, tandoori and chapati (6). In India, up to 85% of wheat consumption is in the form of chapati, which is an unleavened flat bread (21).

Wheat serves as a major source of carbohydrates and energy. It also provides other ingredients that are important and beneficial to our health such as fiber, large amounts of protein, vitamins, and phytochemicals. Even though wheat has respectively high amount of protein (10%–15%), the protein quality is considered to be low as lysine and threonine are its limiting amino acid (5).

Legumes

Figure 1 Pigeon pea seeds

Legumes are another major source of plant-based protein. Pigeon pea (*Cajanus cajan* (L.) Millsp.) is a legume plant grown in subtropical and tropical regions. It is also known as congo pea, red gram, no eye pea, and gungo pea (22). Pigeon pea is highly tolerant to drought and low/high temperatures.

Regarding nutritional values, pigeon peas are rich in protein, carbohydrates, and several minerals such as iron, magnesium, calcium, phosphorus, potassium, and sulfur, but is low in sodium (23). India serves as one of the major producers of pigeon pea (24). Its demand in India is high as it can provide relatively high quality protein in the diet, especially for vegetarians (22). For whole grain samples, the protein content of widely cultivated pigeon peas varies from 17.9 to 24.3 g/100 g (25). Its protein content is a rich source of lysine, but contains relatively few sulfur–containing amino acids, especially cysteine and methionine (Table 2.3) (25). However, it does contain proteins with a relatively similar amino acid profile to soybeans (26). In a recent study, pigeon peas can replace soybeans without affecting rabbit performance (27).

Pigeon peas are increasingly being used as a novel ingredient in food products such as biscuits (28), noodles (29), pasta (30), sausages (31), and doughnuts (32). This may be due to its high protein and fiber content, gluten free, antioxidant, low glycemic index, and functional properties such as water-binding capacity and fat absorption (33, 34). Sahu and colleagues (2014) reported that pigeon peas contained flavonoids, alkaloids, anthraquinone, reducing sugars, tannins, phenols, saponins, and triterpenoids. Biological activities and medicinal properties such as anti-inflammatory, antinociceptive, immunomodulatory, and antioxidant activities of pigeon pea were also studied (35-37). Given that pigeon pea is a novel promising source of protein, many studies investigated the flour properties of pigeon pea. Ohizua et al. studied the quality properties of flour blends of sweet potato, pigeon pea and unripe cooking banana. The study revealed that crude fiber, protein, ash, least gelation, and foaming capacity of the flour blends increased as level of pigeon peas increased (38).

Table 3 Crude protein and amino acid content of wheat, whole wheat, soy and pigeon pea

Crude protein and Amino Acids	Wheat flour (whole grain) (g/100 g)	Wheat flour (all-purpose, unenriched) (g/100 g)	Soybean (mature raw seed) (g/100 g)	Pigeon pea (mature raw seed) (g/100 g)
Crude protein	13.21	10.33	36.49	21.7
Tryptophan	0.174	0.127	0.591	0.212
Threonine	0.367	0.281	1.766	0.767
Isoleucine	0.443	0.357	1.971	0.785
Leucine	0.898	0.71	3.309	1.549
Lysine	0.359	0.228	2.706	1.521
Methionine	0.228	0.183	0.547	0.243
Cysteine	0.275	0.219	0.655	0.25
Phenylalanine	0.682	0.52	2.122	1.858
Tyrosine	0.275	0.312	1.539	0.538
Valine	0.564	0.415	2.029	0.937
Arginine	0.648	0.417	3.153	1.299
Histidine	0.357	0.23	1.097	0.774
Alanine	0.489	0.332	1.915	0.972
Aspartic acid	0.722	0.435	5.112	2.146
Glutamic acid	4.328	3.479	7.874	5.031
Glycine	0.569	0.371	1.88	0.802
Proline	2.075	1.198	2.379	0.955
Serine	0.62	0.516	2.357	1.028

(39-42)

The application of pigeon pea in foods was investigated in various studies. Torres et al. (2007) examined the effect of fermented pigeon pea flour as an ingredient for making pasta in the proportions of 5%, 10% and 12%. It was found that the enhanced pasta with pigeon pea flour required a longer cooking time, higher water absorption, higher protein loss, and higher cooking loss than control pasta made from 100% semolina (43). Another study by Martinez-Villaluenga et al. (2010) incorporated fermented and germinated pigeon pea flour into semolina. The results showed that pigeon pea seeds fermentation and germination improved some essential amino acids like valine, leucine, lysine, glycine, and alanine (30). Furthermore, Yadav, Yadav and Kumar (2011) investigated the potential of pigeon pea substitution for rice starch in noodles. The results revealed that noodles with 70% pigeon pea scored the highest for overall acceptability (44). Many researchers also examined the effect of pigeon pea substitution in biscuits (9, 28, 37). The results showed that substitution of pigeon pea flour up to 35% had higher scores for flavors, textures, and acceptability as compared to millet flour alone (28) or wheat flour alone (9). These studies suggest that processing reduces non-nutritive factors and, in comparison, causes the emergences of health-promoting compounds such as bioactive peptides and non-protein amino acids (i.e., γ-aminobutyric acid (GABA)) when compared to raw legumes (45). The findings also suggested that pigeon pea flour can be incorporated into food products up to 70% and still be acceptable.

Carbohydrates

Carbohydrates are one of the most important sources of energy for our bodies. Glucose provides energy to the body. Glucose is found in the blood as blood glucose and is stored as glycogen in the muscles and liver. Carbohydrates are the primary energy-metabolizing substrate, influencing satiety, insulin, blood glucose, and lipid metabolism. Carbohydrates also have a big influence on colonic function because of fermentation. These properties have impacts for general health, contributing to body weight management, diabetes and aging, large bowel cancer, bone mineral density, cardiovascular disease, resistance to gut infection, and constipation (46).

Carbohydrates are mainly found in plants. Starch is a form of glucose storage in plants. A total of 70%–80% of the carbohydrate in food is starch. Starch is divided into 3 categories for nutritional purposes based on the rate of digestion: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (47). Rapidly digested starch is a starch that causes the blood sugar level to rise rapidly after ingestion. Slowly digestible starch is a starch that is slowly but completely digested in the human small intestine. Resistant starch is the part of starch that “resists” digestion and absorption in the small intestine and passes through the large intestine, where it is fermented by good bacteria into short-chain fatty acids. There is strong evidence that resistant starch may be important in reducing the risk of colon cancer, lowering cholesterol, hypoglycemic effect, inhibiting fat accumulation, and increasing mineral absorption (48).

The quality and digestibility of carbohydrates can affect the postprandial plasma glucose levels and the inflammatory response, which are now known to underlie the development of metabolic syndrome, insulin resistance, and type 2 diabetes (49). The glycemic index (GI) of food is classified as low (<55), medium (56–69), or high (>70) depending on its effect on postprandial glucose release (50). The GI is calculated by dividing the area under the curve (AUC) of blood glucose after eating a test food by the AUC of a control food (i.e., glucose) (51). Additionally, glycemic load (GL) refers to the quality and quantity of carbohydrates in food. It is calculated by multiplying the carbohydrate content (in grams) with the GI of the food and dividing by 100 (52). It has been reported that foods with high GI and GL have been linked to a higher risk of diseases (49, 53, 54). Therefore, reducing GI and GL in the diet can improve metabolic control (55-60).

The predicted glycemic index (pGI) is a widespread way to determine the rate of hydrolysis of carbohydrate in food (54). Moreover, *in-vitro* methods for classifying foods based on their digestive properties were found to be similar to the *in-vivo* situation (61).

Sensory evaluation

The development of food products and the introduction of new products require some assessment of whether the products appeal to the target consumers. Many rating scales developed to measure the degree of affect, of which the labeled hedonic scale is used for recent developments. The most widely used sensory evaluation’s scientific method scale is the 9–point hedonic scale (62). It has been used in many bakery products such as cookies, breads, and flat breads (33, 63). The verbal categories are usually assigned numerical values for quantitative and statistical analysis, ranging from ‘like extremely’ as ‘9’ to ‘dislike extremely’ as ‘1’(Figure 2.4) (62).

(a)

DISLIKE EXTREMELY	DISLIKE VERY MUCH	DISLIKE MODERATELY	DISLIKE SLIGHTLY	NEITHER LIKE NOR DISLIKE	LIKE SLIGHTLY	LIKE MODERATELY	LIKE VERY MUCH	LIKE EXTREMELY
1	2	3	4	5	6	7	8	9

(b)

1	2	3	4	5	6	7	8	9
LIKE LEAST or DISLIKE MOST				NEITHER LIKE NOR DISLIKE				LIKE THE MOST

Figure 2 Versions of the 9–point hedonic scale.

Part (a) shows the traditional "words only" version, with the numbers assigned to the words for statistical analysis. Part (b) shows the numerical "numbers only" scale that is sometimes presented to consumers and is labeled at the ends and sometimes in the middle (62).

Food products are commonly evaluated for the following attributes:

Appearance – by eyes perceive color, size, shape, texture, consistency and capacity.

Aroma – odor-active, volatile compounds that trigger a sensory response by stimulating the olfactory epithelium at the tip of the nasal cavity.

Taste – identified by taste buds on the tongue; the main characteristics of this category are bitter, sour, and sweet.

Aftertaste – determination of a sensation (as of flavor or a feeling) after the stimulating agent or experience has gone.

Texture – the impression of texture through oral sensation and skin.

Overall acceptability – overall scoring of like and dislike considering all the above attributes.

The desirable characteristics for flat breads are a smooth, soft, pliable texture with slight chewiness, wheaty aroma, and light creamish brown in color. Chapati is evaluated for its taste, color and appearance, flavor, and overall acceptability (63). The chapati should look attractive with no surface cracks. Light brown spots should be evenly distributed across the surface. The texture should be soft, smooth, and supple, with these properties lasting at least 2–3 hr. The chapati should also have a sweet wheat flavor and a baked wheat aroma to it. When chewed, it should not be conceived as leathery and hard (64).

Unleavened flat breads, namely chapatis, are made from whole wheat flour and serves as a staple diet to the population of India. Due to the limited amount of some essential amino acids, the combination of wheat with other plant-based proteins would provide better overall essential amino acids. Therefore, composite flour may be used as a better substitute for wheat flour alone without affecting its physicochemical, sensory, and textural properties. Previous studies suggest that substitution of pigeon pea caused an increase in the nutritional quality such as the level of proteins and digestible carbohydrates with acceptable sensory ratings in the end products (37, 65). This leads to an opportunity to study the incorporation of pigeon pea into staple bakery products such as flat bread. However, such a study remains scarce. Therefore, the purpose of this study was to investigate the effects of partial substitution of pigeon pea flour (10%-40% w/w) for whole wheat flour in the development of composite flour and chapati. Firstly, the physicochemical properties, including color, moisture content, and cutting force of the chapati were evaluated. The nutritional values of the flour blends and flat breads such as their protein and starch digestibility were determined. Then, the sensory analysis of composite flat breads was performed to determine the acceptable level of pigeon pea substitution. This would provide a better understanding on application of pigeon pea and propose a new plant-based product with improved overall nutritional values and good acceptability.

CHAPTER III

METHOD AND MATERIALS

Materials and equipment

Material	**Company**
Pigeon pea (*Cajanus cajan*) seeds	Local farm (Tak, Thailand)
Whole wheat flour (Hukamchand)	Local grocery store (Bangkok, Thailand)

Chemicals	**Company**
Sodium Bicarbonate ($NaHCo_3$)	Ajax Finechem (Taren Point, Australia)
Sodium carbonate anhydrous (Na_2Co_3)	Ajax Finechem (Taren Point, Australia)
Sodium acetate 3-hydrate	Elago Enterprise Pty. Ltd. (Cherrybrook, Australia)
Glacial acetic acid (CH_3COOH)	Merck (Darmstadt, Germany)
Hydrochloric acid (HCl)	Merck (Darmstadt, Germany)
Sodium hydroxide (NaOH)	Ajax Finechem (Taren Point, Australia)
Tin (II) chloride dehydrate ($SnCl_2$)	Ajax Finechem (Taren Point, Australia)
Ethylene glycol ($C_2H_6O_2$)	Elago Enterprise Pty. Ltd. (Cherrybrook, Australia)
Ninhydrin ($C_9H_6O_4$)	Ajax Finechem (Taren Point, Australia)
L-Lysine ($C_6H_{14}N_2O_2$)	Ajax Finechem (Taren Point, Australia)
D-Glucose ($C_6H_{12}O_6$)	Ajax Finechem (Taren Point, Australia)
Potassium hydroxide (KOH)	Ajax Finechem (Taren Point, Australia)
Glucose liquicolor, GOPOD	HUMAN GmbH (Wiesbaden, Germany)

Enzymes	**Company**
Porcine α–amylase (Sigma A-3176, Type VI – B)	Sigma-Aldrich CO. (St. Louis, Missouri, USA)
Amyloglucosidase (*Aspergillus niger*)	Megazyme International (Illinois, USA)
Pancreatin (Sigma P-1750, porcine pancreas)	Sigma-Aldrich CO. (St. Louis, Missouri, USA)
Pepsin (porcine stomach mucosa)	Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India)

Equipment	Company
Herb Grinder (DXM–500)	DXFill Machine (Bangkok, Thailand)
Electric mixer (Model 5K45SS)	Heavy-Duty, KitchenAid (Michigan, USA)
Colorimeter (Color–flex EZ)	Hunter Lab (Virginia, USA)
Infrared Moisture Analyzer (FD610)	Kett Electric Laboratory (Tokyo, Japan)
Texture analyzer (TA.XT. Plus)	Stable Micro System (London, UK)
Shaking water bath (NB-304)	N-Biotek Co., Ltd. (Gyeonggi, Korea)
Dry bath incubator (AccuBlock D1200)	Labnet International, Inc. (New Jersey, USA)
pH meter (Orion 2-star)	Thermo Fisher Scientific, Inc. (Massachusetts, USA)
Vortex mixer	Gemmy Industrial (Taipei, Taiwan)
Sonicate	GT SONIC (Guangdong, China)
Centrifuge, (ROTINA-380R)	Hettich (Tuttlingen, Germany)
Hot plate	IKA-works (Staufen, Germany)
Electronic weighing balance	Sartorius Co. Ltd. (Gottingen, Germany)
Microplate Spectrophotometer (PowerWave XS2)	BioTek Instruments, Inc. (Vermont, USA)
Electric Stove (HW-116A2)	House Worth (Bangkok, Thailand)

Protocol for making pigeon pea flour

Pigeon peas were made into flour by the method outlined by Gayle et al., (66) with slight adjustments. Briefly, the dry seeds were cleaned, handpicked, and boiled for 1 min, then soaked in that water for 1 hr and manually dehulled. The dehulled seeds were then blended in an herb grinder (DXM–500, DXFill Machine, Thailand) into a slurry paste, spread on a tray lined with aluminum foil, and dried in air dry oven at 65°C for 14 hr. After drying, the flour was blended, sieved through 150 μm screen mesh, and stored in an aluminum zip lock bag at room temperature until used (66).

Proximate analysis

The pigeon pea flour (PPF) and purchased whole wheat flour (WWF) were sent to the Food Research and Testing Laboratory (FTRL) at the Faculty of Science of Chulalongkorn University, Thailand for proximate analysis. Total calorie, total carbohydrate (67), protein (N x 6.25) (68), total fat, total dietary fiber, and ash content (69) were measured using a standard method approved by AOAC.

Product development

Preparation of composite flour blends

Substitution levels of PPF for WWF were selected at 10%, 20%, 30%, and 40% based on the previous studies where substitution of legume flour up to 40% improved the overall nutrient contents and had acceptable satisfaction when used in food products (70, 71). The substitution was made in weight-by-weight basis per 100 g of flour as shown in Table 3.1. All composite flour blends were mixed very well before used. Whole wheat flour was used as a control.

Table 4 Formulation of whole wheat-pigeon pea composite flour blends per 100 g of flour

Formulations	WWF (g)	PPF (g)
WWF (Control)	100	0
10% PPF	90	10
20% PPF	80	20
30% PPF	70	30
40% PPF	60	40

WWF: Whole wheat flour; PPF: Pigeon pea flour

Protocol for making chapati

A total of 5 sample formulations, including the control flat bread and 4 whole wheat–pigeon pea composite flat bread was prepared by using flour blends according to the substitution levels in Table 3.1.

Chapati was prepared using 60 ml of water for each 100 g of flour (21). It was mixed in an electric mixer (Model 5K45SS Heavy Duty, KitchenAid, USA) for approximately 5 min until a dough was formed. The final dough was hand-kneaded for 2 min and rested covered with a wet cloth for 30 min at room temperature before use. The dough was then divided into 40 g pieces and rolled into a sheet of 15 cm in diameter with a thickness of 2 mm. The non-stick pan was preheated on an electric hot plate for 10 min. The dough was then heated using a nonstick pan which was preheated (10 min) on an electric stove set at max level (～200°C) (HW-116A2, House Worth, Thailand) for 30 sec on each side. Finally, slight pressure was applied to sheets until they puffed (20 sec) and then allowed to cool at room temperature (72).

Physicochemical properties

Color measurement

The color of flour, dough, and chapati was measured using a colorimeter by Hunter Lab Color Measuring System (Color–flex EZ, Hunter Lab, Virginia, USA). The instrument was calibrated using the standard tiles. Then, samples were placed in the sample holder and the reflectivity was recorded in triplicates. The results were reported as an average and expressed according to the CIE L^* a^* b^* system, where:

L^* is known as lightness [L^*=0 (black), L^*=100 (white)]

a^* (–a=greenness, +a=redness)

b^* (–b^* values=blueness, +b^* value=yellowness)

Moisture measurement

Moisture contents of flour, dough, and chapati were measured using an infrared moisture balance (FD610, Kett, Tokyo, Japan). Approximately 3 g of samples were placed into the machine with temperature set at 170°C. The dough and chapati samples were placed into the machine approximately 5 min after preparation.

Cutting force

The cutting force of chapati samples was evaluated using a texture analyzer TA.XT. Plus (UK) and method outlined by Hemalatha et al. (73) with slight modification. The chapatis were cut into strips measuring 4 cm x 2 cm and packed in a polypropylene pouch until used. One strip of chapati after another was placed in the middle of the sample holder and the Warner-Bratzler blade (HDP/BSW) was allowed to cut the strip. The maximum force (i.e., hardness) needed to cut the chapati strip in half was recorded. Speed was kept constant at 1.70 mm/s. A total of 10 strips per chapati sample were tested and average values were reported (74).

Nutritional value

Simulated gastrointestinal digestion

Samples were passed through simulated gastrointestinal digestion, which includes a total of 4 flour samples (WWF, PPF, 20% PPF, and 40% PPF) and 3 chapati samples (WWF, 20% PPF, and 40% PPF).

Digestion was performed according to the method outlined in a previous study with slight adjustments (75). Briefly, 500 mg of flour (mixed with 5 ml water and boiled at 100°C for 20 min) or chapati samples were measured. Then, 1 ml of artificial saliva containing porcine α-amylase (250 U/ml in 0.2 M pH 7 carbonate buffer) was added for 15–20 sec followed by 5 ml of pepsin (4500 U/ml) (1 ml/ml in 0.02 M pH 2 HCl), incubated at 37°C in a shaking water bath (100 rpm) for 1 hr (gastric phase). The mixture was then neutralized by adding 5 ml of 0.02 M aq. NaOH before adjusting the pH to 6 (25 ml of 0.2 M sodium acetate buffer). Next, 5 ml of pancreatin (2 mg/ml in 0.2 M pH 6 acetate buffer) and amyloglucosidase (28 U/ml in 0.2 M pH 6 acetate buffer) mixture was added, and incubation was continued for 180 min (intestinal phase). Digesta were collected at the end of the gastric phase and at different time points in the intestinal phase (0–180 min). To stop enzymatic reactions, the collected digesta was immediately heated at 90°C for 10 min and centrifuged at 4°C, 10000 rpm for 15 min. The supernatant of the digesta was collected and kept at −20°C until required for further analysis.

In-vitro starch digestibility and predicted glycemic index (pGI)

Glucose content in the digesta was measured by using an enzymatic colorimetric GOPOD method (Glucose liquicolor test, HUMAN, GmbH, Germany). In brief, the working reagent (500 μl) was mixed with the digesta (5 μl) of the samples and incubated at room temperature for 10 min. The absorbance was measured at

500 nm. Glucose (100 mg/dl) was used as a standard. The amount of glucose was calculated using the following equation:

$$\mathrm{C} = \frac{\Delta \mathrm{Abs\ sample}}{\Delta \mathrm{Abs\ STD}}\ \text{(mg/dl)}$$

C: Glucose concentration

ΔAbs sample: Absorbance of sample subtracted by absorbance of the reagent blank

ΔAbs STD: Absorbance of standard subtracted by absorbance of the reagent blank

The rate of starch digestibility was expressed as the glucose concentration at different time intervals (0, 10, 20, 30, 60, 90, 120, and 180 min).

The glucose values (0–180 min) were plotted as a line graph and areas under hydrolysis curves (AUC) were calculated using the trapezoidal rule. The hydrolysis index (HI) was calculated by dividing the sample's area under the hydrolysis curve by the area under the glucose standard curve:

$$\mathrm{HI} = (\mathrm{AUC}_{\mathrm{sample}}/\mathrm{AUC}_{\mathrm{glucose}}) \times 100$$

The predicted glycemic indices (pGI) of the samples were estimated using the following equation:

$$\mathrm{pGI} = 39.71 + 0.549\ \mathrm{HI}$$ (50, 61).

Total starch and starch fraction

Total starch was determined based on the method previously reported by Goni et al. (76) with slight modification. Accurately measured 50 mg of flour (mixed with 5 ml water and boiled at 100°C for 20 min) or chapati was added with 6 ml of 2 M KOH and shaken energetically for 30 min. Then, 3 ml of 0.4 M of sodium acetate buffer pH 4.75 was added and the pH was adjusted to 4.5 using 6 M HCL. Amyloglucosidase (3260 U/ml, 60 μl) was added to the mixture and incubated in a shaking water bath at 60°C 100 rpm for 45 min. Finally, 1 ml of the solution was collected and heated at 90°C for 10 min to stop the enzyme reaction, then centrifuged at 4°C 10000 rpm for 15 min. Starch was measured as glucose with the enzymatic colorimetric GOPOD method, which the absorbance was read at 500 nm. The concentration of glucose was multiplied by 0.9 to convert to the amount of starch in the samples (76). Total starch (TS) content was reported in mg per 50 mg sample.

The starch fraction was calculated according to the *in-vitro* digestibility of the starch in the samples (61). The percentage starch fraction was calculated based on the study of Englyst et al. (77), where the amount of glucose present in the sample during the first 20 min was known as rapidly digestible starch (RDS); the difference between glucose measured at 120 min and 20 min was known as slowly digestible starch (SDS); and the amount of glucose that was not digested in 120 min was known as resistant starch (RS).

$$\%RDS = [(G_{20}-G_0)/TS] \times 0.9 \times 100$$

$$\%SDS = [(G_{120}-G_{20})/TS] \times 0.9 \times 100$$

$$\%RS = [(TS-RDS-SDS)/TS] \times 100$$

G0: Glucose released at time 0 min

G20: Glucose released at time 20 min

G120: Glucose released at time 120 min

0.9: Factor conversion from glucose to starch

TS: Total Starch

Amino-group-containing residue

The digesta collected at the end of the gastric phase and in the intestinal phase (at 0, 10, 15, 30, 40, 45, 60, 90, 120, 150, and 180 min) for every sample was used for ninhydrin assay with slight modification (78, 79).

Briefly, 20 μl of each sample was mixed with 380 μl of distilled water followed by 200 μl of ninhydrin reagent. A blank sample with 400 μl of distilled water and 200 μl of ninhydrin reagent was prepared. The mixtures were incubated in a heat block at 100°C for 10 min and then allowed to cool for 10 min. The absorbance of the mixtures was read at 568 nm using a microplate reader. Lysine diluted over the range from 1.5625 to 200 μg/ml was used as a standard (79).

Sensory evaluation

A total of 80 untrained panelists were recruited by convenience sampling from staffs and students in Chulalongkorn University, Bangkok, Thailand. The sample size was sufficient to detect a difference of 0.5-unit between the acceptance of flatbread on a 9-point categorical hedonic scale used for sensory evaluation (62).

Inclusion criteria:

- Healthy
- Male or female
- Age 18–50 years
- Voluntarily participate in the study

Exclusion criteria:

- Colorblind
- Have common cold symptoms such as runny nose, sore throat, or cough
- Having dietary allergies to gluten, nuts, or any other food source
- Being pregnant or breastfeeding

- Smokers
- Refuse to participate or withdraw from the study

Eligible participants were invited to the sensory lab at the Faculty of Allied Health Sciences, Chulalongkorn University. The participants were asked “Have you ever eaten chapati?” or “Are you familiar with chapati?”. Sensory evaluation was carried out while the subject sat in an individual cabin at room temperature and in daylight equivalent brightness. To obtain the most accurate evaluation possible, panelists were asked not to eat or drink (other than water) for 1 hr prior to evaluation to cleanse their palate. The chapati samples (WWF, 20% PPF, 30% PPF, and 40% PPF) cut into even slices, labeled with a random 3–digit coding were given to the panelists in random order.

The participants were instructed to cleanse their palate before tasting each sample with water and then evaluate the samples for acceptability of appearance, color, flavor, texture, and overall acceptance using the 9–point hedonic scale (dislike extremely=1; dislike very much=2; dislike moderately=3; dislike slightly=4; neither like nor dislike=5; like slightly=6; like moderately=7; like very much=8; like extremely=9). Panelists were given time to ask questions for more information if any and also allowed to withdraw from the study anytime. Approximately 10–15 min were required to complete the test.

During the test, if participants had any possible adverse effects, such as headache and nausea, they were allowed to quit the study immediately and safety precautions to health were taken accordingly.

Statistical analysis

All experiments were performed in triplicates or as stated. The data were analyzed using SPSS program version 23. Data were analyzed using one–way analysis of variance (ANOVA) followed by Duncan’s multiple range test and reported as Mean±Standard Error of Mean (SEM).

For sensory analysis, the test of normality was performed. Kruskal–Wallis test was used to compare data among different formulations, whereas Mann-Whitney U test was used for comparison between two types of consumers, regular and new. Data were expressed as median with interquartile range.

The graphs were generated using Sigma–Plot software version 12.0. Results were statistically significant if the p-value is <0.05.

CHAPTER IV

RESULTS

Proximate analysis

The proximate analysis including total calories, total carbohydrate, ash, moisture, protein, total dietary fiber, and total fat of whole wheat flour (WWF) and pigeon pea flour (PPF) are presented in Table 4.1. The total calorie ranged between 363 kcal/g and 374 kcal/g for WWF and PPF, respectively. The total carbohydrate content was lower for PPF (60.53%) as compared to WWF (71.82%). The protein content was found to be two times higher for PPF (26.10%) than WWF (13.52%). Moreover, PPF had a total fat content of 25.41%, ash content of 21.8%, and dietary fiber 3.3% higher than that of WWF.

Table 5 Proximate analysis of pigeon pea flour and whole wheat flour

Parameters	PPF	WWF
Total calories (kcal)	374.06	363.32
Total carbohydrate (g)	60.53	71.82
Moisture (g)	8.80	10.98
Ash (g)	1.51	1.24
Total fat (g)	3.06	2.44
Protein (N x 6.25) (g)	26.10	13.52
Total dietary fiber (g)	10.41	10.08

Results are shown per 100g of flour. PPF: Pigeon pea four; WWF: Whole wheat flour

Physicochemical properties

Color measurement

Table 4.2 and Figure 4.1 illustrate the color attributes of the flour samples. The lightness (*L**) and redness (*a**) were significantly lower for PPF, whereas the yellowness (*b**) was significantly higher for PPF when compared to WWF ($p<0.05$). Significant reduction in redness values was also observed with increased substitution of PPF at 20%–40% ($p<0.05$). Even though not significant, increasing PPF substitution increased yellowness and decreased lightness of the flour blends ($p>0.05$).

Table 4.3 and Figure 4.2 illustrates the color attributes of the dough samples. All samples with PPF substitution showed significantly lower redness when compared to WWF ($p<0.05$). A slight reduction in lightness and increase in yellowness was also observed for dough with PPF substitution from 10% to 40%, however, the difference was not considered to be statistically significant as compared to WWF dough ($p>0.05$).

The color attributes of the chapati samples are presented in Table 4.4 and Figure 4.3. The lightness of 30% PPF chapati (23.31±0.32) and 40% PPF chapati (23.49±0.32) were significantly higher than WWF control (21.79±0.65, $p<0.05$). Moreover, 40% substitution of PPF (9.17±0.15) caused a significant increase in yellowness of the chapati when compared to WWF (8.63±0.06, $p<0.05$). The redness of the chapati reduced with increasing substitution of PPF. It was found that PPF substitution at 30% (2.66±0.02) and 40% (2.47±0.14) was significantly reduced the redness of the chapati when compared to WWF chapati (3.10±0.09, $p<0.05$).

Moisture content

The moisture contents of the flour, dough, and chapati samples are presented in Tables 4.2, 4.3, and 4.4, respectively. For the flour samples, the moisture content was significantly lower for PPF (7.13±0.15) as compared to WWF (10.23±0.34, $p<0.05$). Even though not statistically significant, an increasing trend in moisture content was observed with increasing PPF substitution up to 40% ($p>0.05$). In the dough and chapati samples, the moisture levels were slightly elevated with increased PPF substitution, however, it was not significantly different from the control ($p>0.05$).

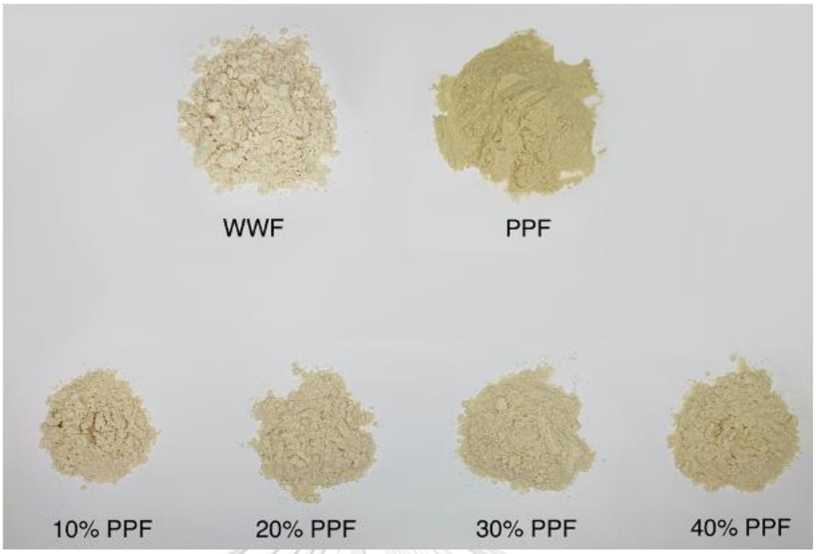


Figure 3 The appearance of flour samples.

WWF: Whole wheat flour; PPF: Pigeon pea flour.

Figure 4.2 The appearance of dough samples.

WWF: Whole wheat flour; PPF: Pigeon pea flour.

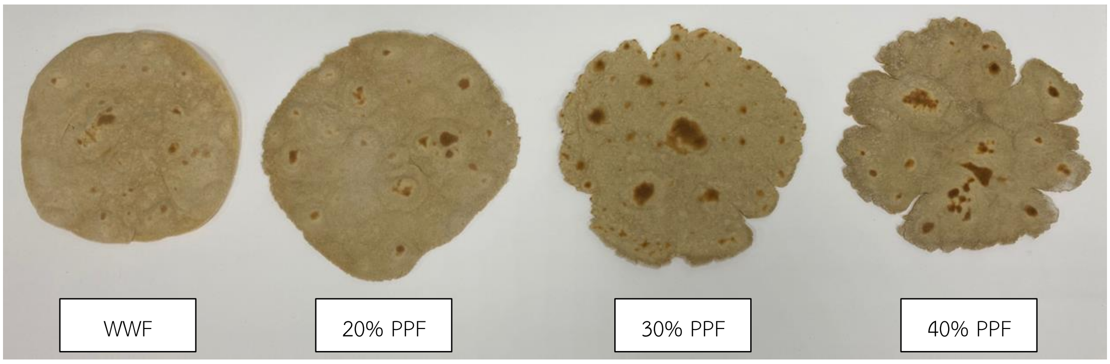


Figure 4.3 The appearance of chapati samples.

WWF: Whole wheat flour; PPF: Pigeon pea flour.

Table 6 Color attributes and moisture content of whole wheat–pigeon pea composite flour

Samples	*L**	*a**	*b**	Moisture (%)
WWF	36.96 ± 0.32^{a}	1.31 ± 0.02^{a}	6.26 ± 0.08^{a}	10.23 ± 0.34^{a}
10% PPF	36.94 ± 0.25^{a}	1.25 ± 0.03^{ab}	6.42 ± 0.18^{ab}	10.20 ± 0.44^{a}
20% PPF	36.92 ± 0.23^{a}	1.13 ± 0.03^{bc}	6.46 ± 0.07^{ab}	10.17 ± 0.19^{a}
30% PPF	36.90 ± 0.30^{a}	1.05 ± 0.07^{c}	6.62 ± 0.11^{ab}	9.97 ± 0.33^{a}
40% PPF	36.79 ± 0.15^{ab}	0.93 ± 0.05^{d}	6.69 ± 0.20^{ab}	9.60 ± 0.51^{a}
PPF	35.98 ± 0.35^{b}	0.78 ± 0.02^{e}	6.84 ± 0.12^{b}	7.13 ± 0.15^{b}

Data expressed as Mean±Standard Error of Mean (SEM)

$^{a\text{-}b}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p<0.05$ based on one-way ANOVA and Duncan’s multiple range post hoc analysis (n=3).

WWF, 100% Whole Wheat Flour; PPF, 100% Pigeon Pea Flour; 10% PPF, 10% pigeon pea substitution; 20% PPF, 20% pigeon pea substitution; 30% PPF, 30% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution

Table 7 Color attributes and moisture content of whole wheat–pigeon pea composite dough

Samples	*L**	*a**	*b**	Moisture (%)
WWF	25.89±0.34^{a}	2.92±0.11^{a}	10.01±0.30^{a}	14.53±0.18^{a}
10% PPF	25.69±0.33^{a}	2.48±0.09^{b}	10.14±0.08^{a}	15.07±0.55^{a}
20% PPF	25.68±0.41^{a}	2.42±0.07^{b}	10.22±0.15^{a}	15.63±0.72^{a}
30% PPF	25.61±0.32^{a}	2.37±0.06^{b}	10.46±0.07^{a}	16.33±1.49^{a}
40% PPF	25.45±0.39^{a}	2.36±0.04^{b}	10.56±0.23^{a}	17.67±2.11^{a}

Data expressed as Mean±Standard Error of Mean (SEM)

$^{a\text{-}c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p<0.05$ based on one-way ANOVA and Duncan's multiple range post hoc analysis (n=3).

WWF, 100% Whole Wheat Flour; 10% PPF, 10% pigeon pea substitution; 20% PPF, 20% pigeon pea substitution; 30% PPF, 30% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution

Table 8 Color attributes moisture content and texture profile analysis of whole wheat–pigeon pea composite chapati

Samples	*L**	*a**	*b**	Moisture (%)
WWF	21.79 ± 0.65^{a}	3.10 ± 0.09^{a}	8.63 ± 0.06^{a}	20.73 ± 0.95^{a}
10% PPF	22.49 ± 0.20^{ab}	2.83 ± 0.16^{ab}	8.66 ± 0.19^{ab}	21.37 ± 1.57^{a}
20% PPF	22.55 ± 0.47^{ab}	2.77 ± 0.05^{abc}	8.77 ± 0.10^{ab}	22.30 ± 3.04^{a}
30% PPF	23.31 ± 0.32^{b}	2.66 ± 0.02^{bc}	8.83 ± 0.22^{ab}	22.77 ± 0.62^{a}
40% PPF	23.49 ± 0.32^{b}	2.47 ± 0.14^{c}	9.17 ± 0.15^{b}	25.10 ± 0.86^{a}

Data expressed as Mean±Standard Error of Mean (SEM)

$^{a\text{-}c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p<0.05$ based on **o**ne-way ANOVA and Duncan’s multiple range post hoc analysis (n=3).

WWF, 100% Whole Wheat Flour; 10% PPF, 10% pigeon pea substitution; 20% PPF, 20% pigeon pea substitution; 30% PPF, 30% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution

Cutting force

The cutting force of the whole wheat–pigeon pea composite chapati is illustrated in Table 4.5. The cutting force of chapati samples ranged from 31.06±0.84 N to 42.58±0.83 N. The force required to cut the chapati strips increased corresponding to the increasing ratio of PPF replacement (p<0.05).

Table 9 Cutting force of whole wheat–pigeon pea composite chapati

Samples	Cutting Force (Newton)
WWF	31.06±0.84^{a}
10% PPF	34.11±0.40^{b}
20% PPF	36.49±0.18^{c}
30% PPF	38.24±0.06^{d}
40% PPF	42.58±0.83^{e}

Data expressed as Mean±Standard Error of Mean (SEM)

$^{a\text{-}e}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p<0.05$ based on one-way ANOVA and Duncan's multiple range post hoc analysis (n=10).

WWF, 100% Whole Wheat Flour; 10% PPF, 10% pigeon pea substitution; 20% PPF, 20% pigeon pea substitution; 30% PPF, 30% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution

Nutritional Value

In-vitro starch digestion and predicted glycemic index (pGI)

Figure 4.4 illustrates the glucose release of the flour samples. A significant reduction of glucose release was observed at 20 min for PPF (56.56±1.47) when compared to WWF (70.35±5.78, $p<0.05$). Partial substitution of PPF at 20% and 40% caused a slight reduction in glucose release, however, the results were not considered to be statistically significant when compared to WWF ($p>0.05$).

Figure 4.5 illustrates the glucose release of the chapati samples. It was observed that 40% PPF substituted chapati had significantly lower glucose release at all time points above 20 min when compared to WWF ($p<0.05$). A decreasing trend of glucose release in 20% PPF substituted chapati was also recognized when compared to WWF, however, it was not statistically significant ($p>0.05$).

To evaluate the predicted glycemic index (pGI), glucose was used as a standard reference. Table 4.6 represents the pGI, hydrolysis index (HI), and area under the curve (AUC) of the flour samples. Even though not statistically significant, a decreasing trend was noticed for these parameters with increasing substitution of PPF ($p>0.05$).

For the chapati samples, the pGI, HI, and AUC values are presented in Table 4.7. As compared to the control chapatis (WWF), a significant reduction in all parameters was found in the chapatis with PPF substitution at 20% and 40% ($p<0.05$), respectively.

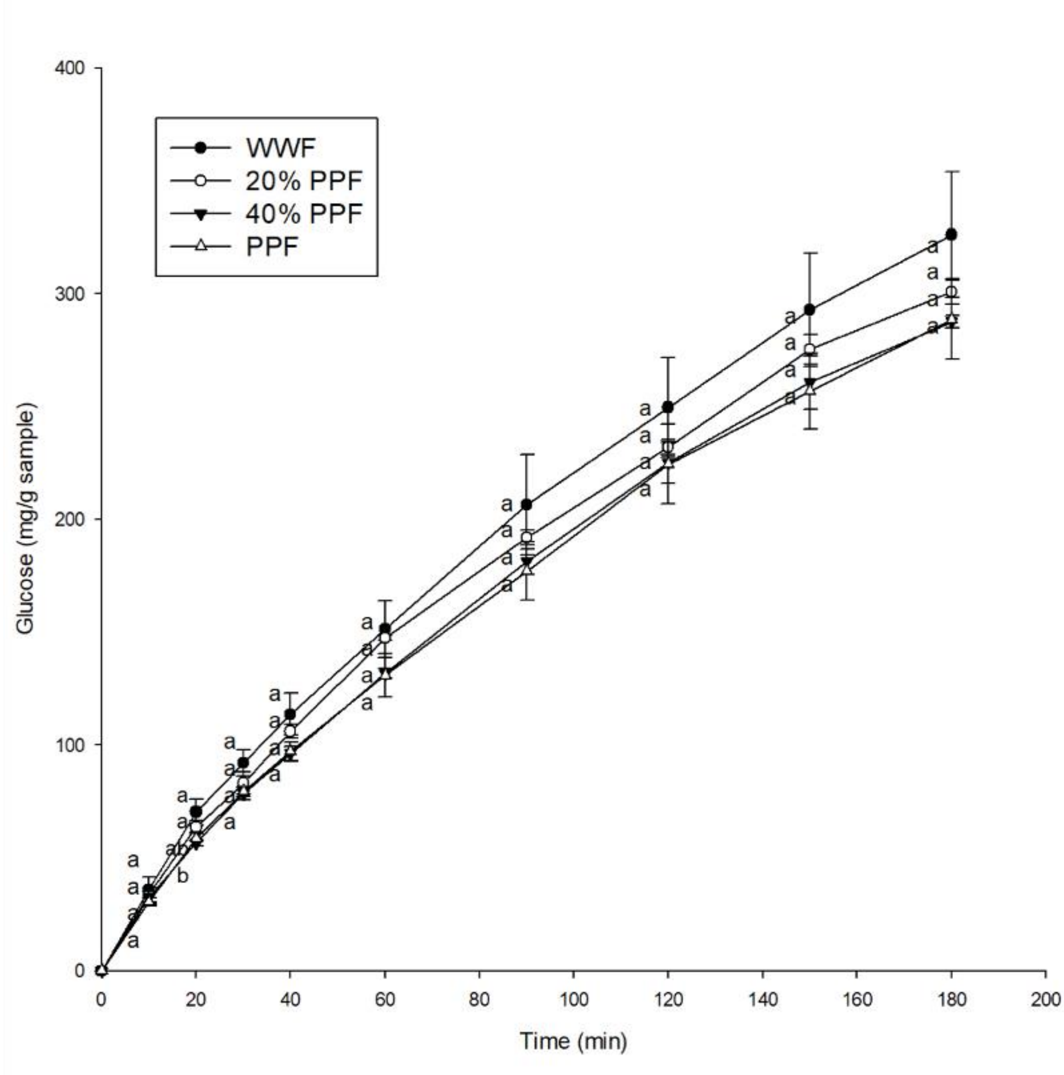


Figure 4 The effects of pigeon pea flour (PPF) and its partial substitution for whole wheat flour (WWF) on glucose release.

Different superscript alphabets on the same time interval denote statistically significant difference in the mean values among the groups at $p<0.05$ (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

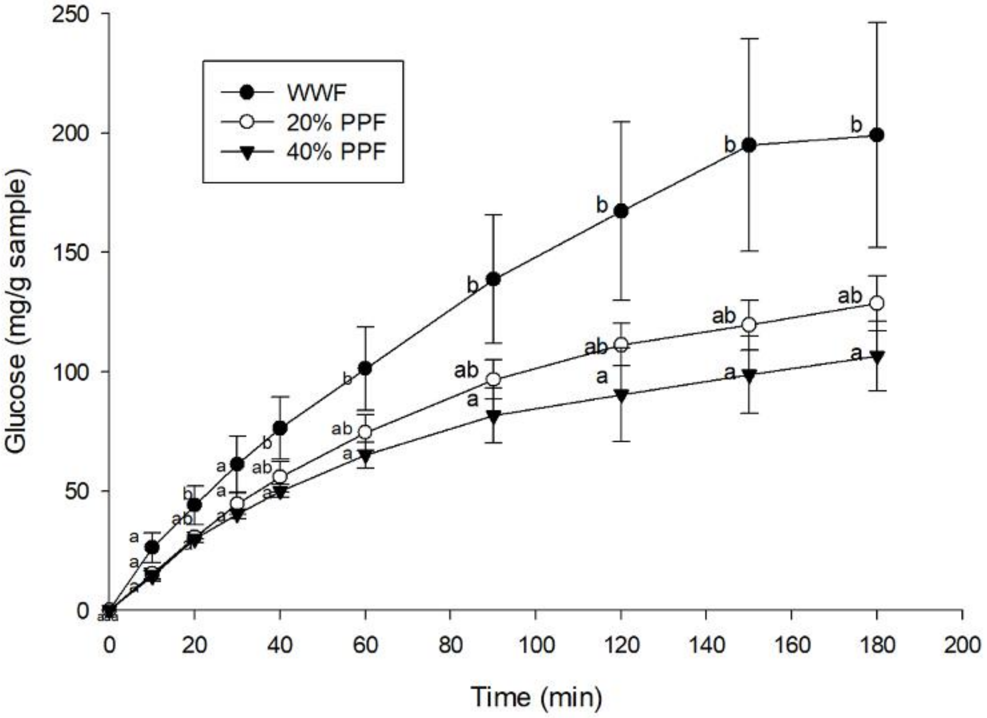


Figure 5 The effects on glucose release of chapati samples developed from pigeon pea flour (PPF) and its partial substitution for whole wheat flour (WWF). Different superscript alphabets on the same time interval denote statistically significant difference in the mean values among the groups at $p<0.05$ (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

Table 10 Predicted glycemic index (pGI), hydrolysis index (HI), and area under the curve (AUC) of flour samples

Samples	pGI	HI (%)	AUC
WWF	61.80 ± 1.53^{a}	40.24 ± 2.79^{a}	57144.92 ± 3704.40^{a}
20% PPF	60.58 ± 0.09^{a}	38.02 ± 0.17^{a}	54024.20 ± 800.51^{a}
40% PPF	59.65 ± 0.30^{a}	36.31 ± 0.55^{a}	51617.48 ± 1328.91^{a}
PPF	59.15 ± 1.11^{a}	35.40 ± 2.02^{a}	50299.16 ± 2794.61^{a}

Data expressed as Mean±Standard Error of Mean (SEM). Glucose was used as a standard.

$^{a\text{-}b}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p<0.05$ based on one-way ANOVA and Duncan's multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution.

Table 11 Predicted glycemic index (pGI), hydrolysis index (HI), and area under the curve (AUC) of chapati sample

Sample	pGI	HI (%)	AUC
WWF	51.55±0.20^{a}	21.57±0.58^{a}	30644.34±818.86^{a}
20% PPF	49.55±0.68^{b}	17.93±1.24^{b}	25475.88±1767.09^{b}
40% PPF	47.19±0.31^{c}	13.62±0.56^{c}	19356.81±795.26^{c}

Data expressed as Mean±Standard Error of Mean (SEM). Glucose was used as a standard.

$^{a-c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at *p*<0.05 based on one-way ANOVA and Duncan's multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution.

Total starch and starch fraction

Table 4.8 and Figure 4.6 represent the total starch content of the flour samples. The results showed that the total starch content was significantly lower for PPF (26.88±0.48 mg/50 mg sample) as compared to WWF (29.94±0.08 mg/50 mg sample, *p*<0.05). Substitution of PPF at 20% and 40% significantly decreased the amount of total starch as compared to WWF (*p*<0.05). The total starch content of 40% PPF (27.05±0.25 mg/50 mg sample) did not significantly differ from that of PPF (26.88±0.48 mg/50 mg sample, *p*>0.05).

Table 4.9 and Figure 4.7 illustrate the total starch content of chapati samples. The total starch content significantly reduced in the chapatis with PPF substitution at 20% PPF (20.54±0.04 mg/50 mg sample) and 40% PPF (18.55±0.09 mg/50 mg sample) as compared to the control chapati (WWF, 21.55±0.27 mg/50mg sample, *p*<0.05).

Table 4.8 and Figure 4.8 display the starch fraction, including rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) contents of flour samples. Substitution of PPF at 20% and 40% caused a slight reduction in the RDS content and increase in SDS and RS content. However, the results were not considered to be statistically significant (*p*>0.05).

Table 4.8 and Figure 4.9 demonstrate the starch fraction for the chapati samples. It was found that RDS contents in the chapatis were slightly reduced, corresponding to an increased PPF substitution (*p*>0.05). Proportions of SDS significantly decreased for 40% PPF (14.82±2.77%) as compared to the control chapatis (WWF 30.20±1.97%, *p*<0.05). On the other hand, RS content significantly increased for 40% PPF (70.84±2.10%) as compared to the control chapatis (WWF, 51.93±2.72%, *p*<0.05).

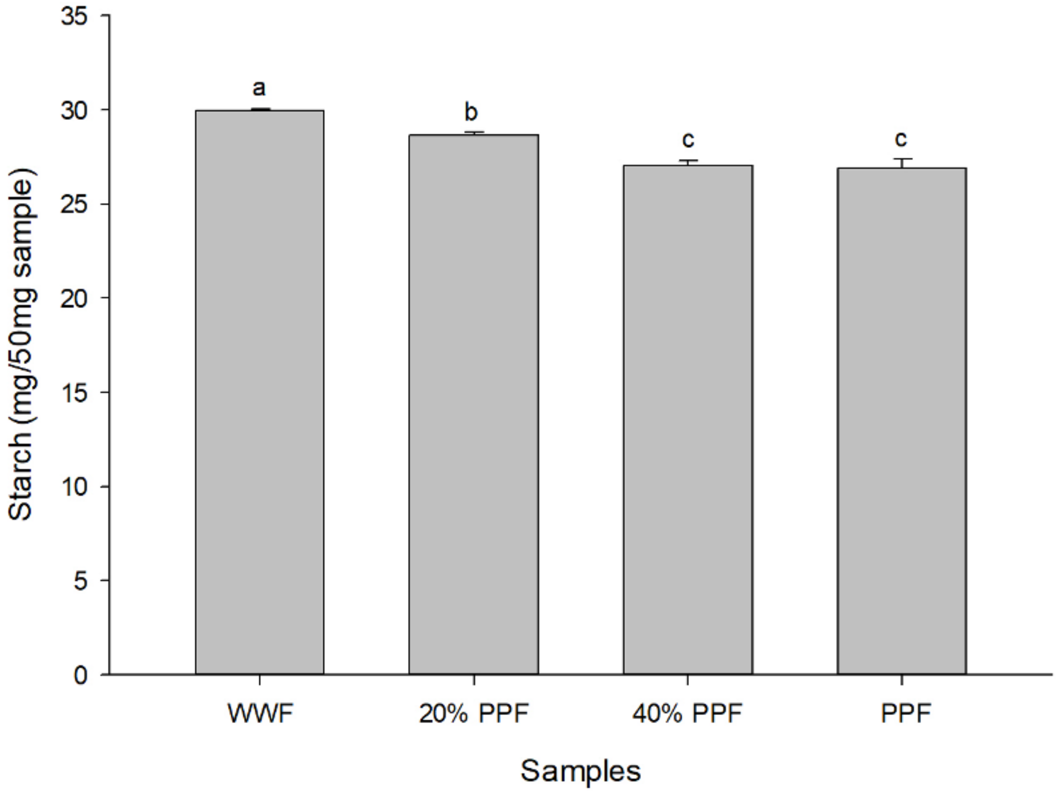


Figure 6 The effects of pigeon pea flour (PPF) and its partial substitution for whole wheat flour (WWF) on starch content.

Different superscript alphabets denote statistically significant difference in the mean values among the groups at *p*<0.05 (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

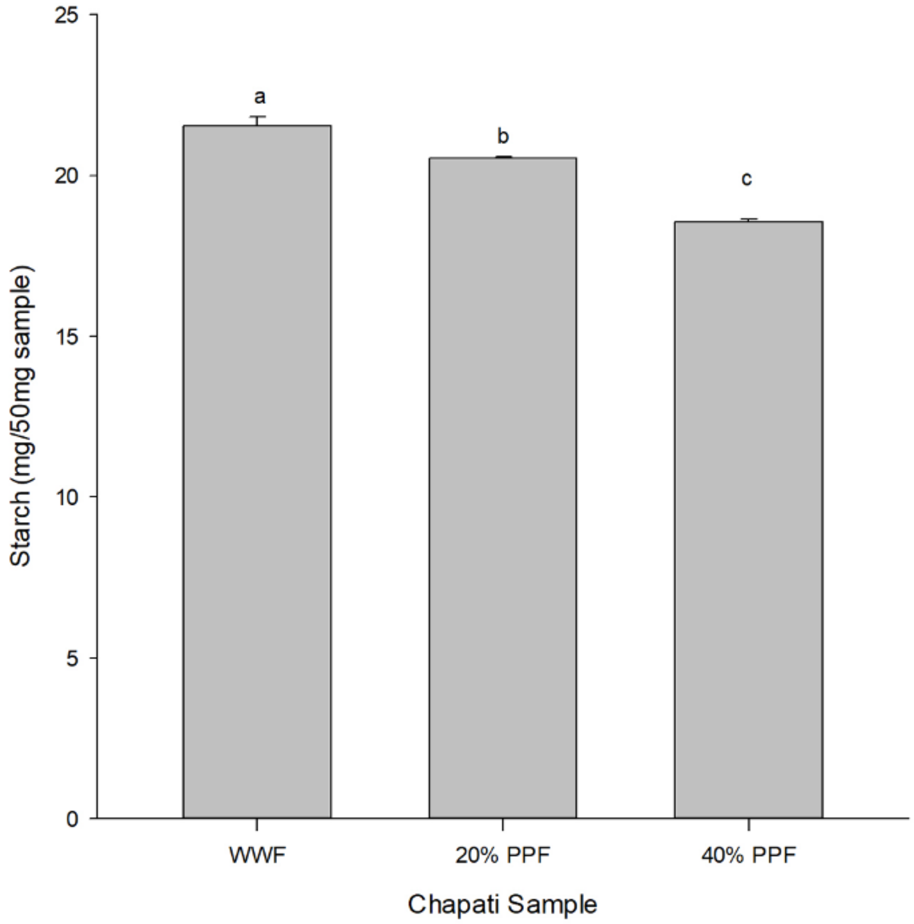


Figure 7 The effects on starch content of chapati samples developed from pigeon pea flour (PPF) and its partial substitution for whole wheat flour (WWF). Different superscript alphabets denote statistically significant difference in the mean values among the groups at $p<0.05$ (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

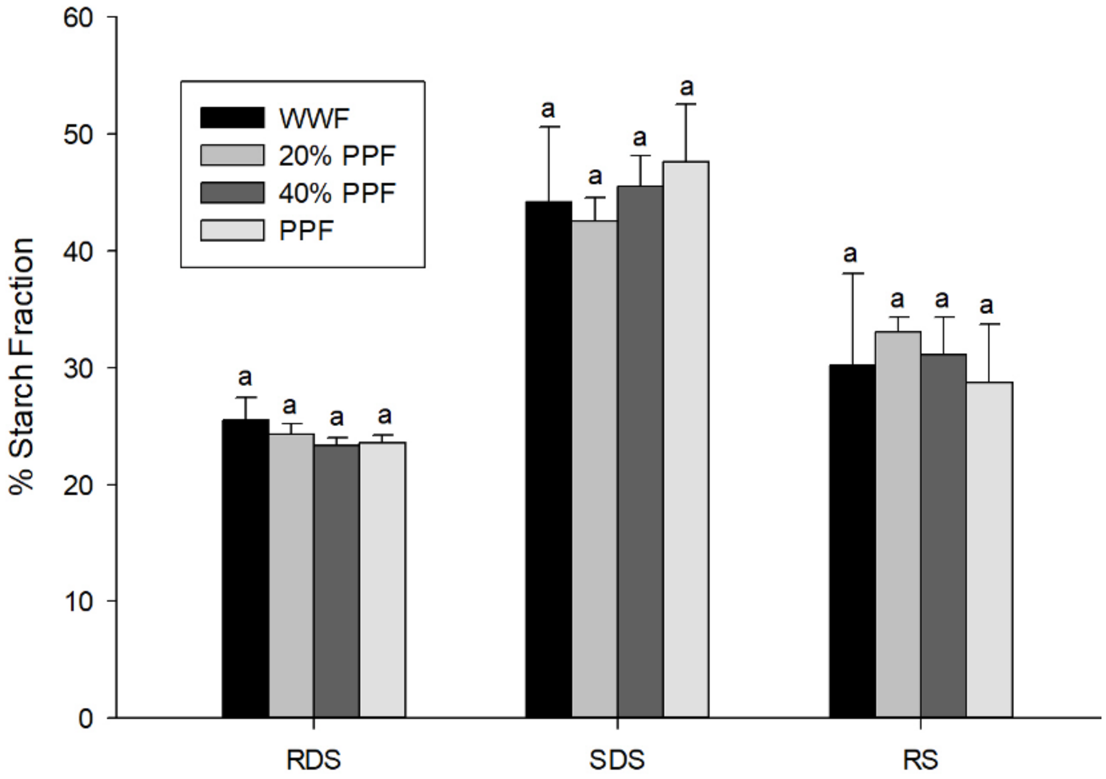


Figure 8 The effects of pigeon pea flour (PPF) and its partial substitution for whole wheat flour on starch fraction.

Different superscript alphabets denote statistically significant difference in the mean values among the groups at $p<0.05$ (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

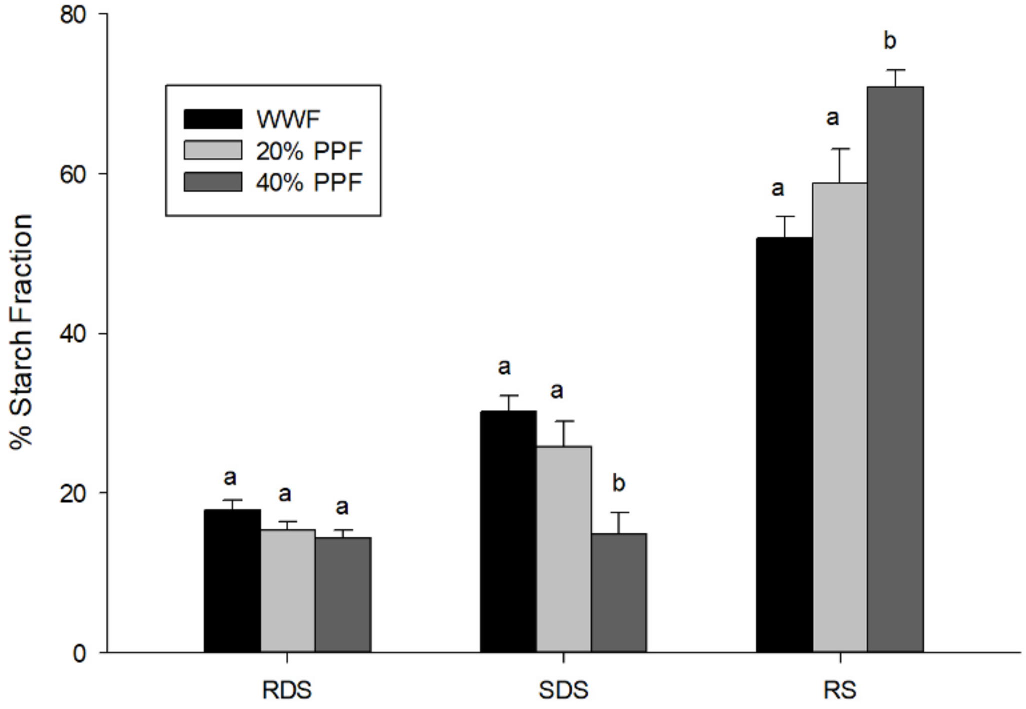


Figure 9 The effects on starch fraction of chapati samples developed from pigeon pea flour (PPF) and its partial substitution for whole wheat flour (WWF). Different superscript alphabets denote statistically significant difference in the mean values among the groups at $p<0.05$ (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

Table 12 Total starch and starch fraction of flour samples

Samples	Starch (mg/50mg sample)	RDS (%)	SDS (%)	RS (%)
WWF	29.94±0.08^{a}	25.53±1.92^{a}	44.20±6.43^{a}	30.27±7.79^{a}
20% PPF	28.67±0.15^{b}	24.35±0.88^{a}	42.57±2.01^{a}	33.08±1.26^{a}
40% PPF	27.05±0.25^{c}	23.33±0.72^{a}	45.56±2.59^{a}	31.11±3.22^{a}
PPF	26.88±0.48^{c}	23.60±0.66^{a}	47.64±4.94^{a}	28.76±5.00^{a}

Data expressed as Mean±Standard Error of Mean (SEM).

$^{a-c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at p<0.05 based on one-way ANOVA and Duncan's multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.

Table 13 Total starch and starch fraction of chapati samples

Sample	Starch (mg/50mg sample)	RDS (%)	SDS (%)	RS (%)
WWF	21.55±0.27^{a}	17.87±1.30^{a}	30.20±1.96^{a}	51.93±2.72^{a}
20% PPF	20.54±0.04^{b}	15.38±1.07^{a}	25.80±3.22^{a}	58.81±4.27^{a}
40% PPF	18.55±0.09^{c}	14.54±0.98^{a}	14.82±2.77^{b}	70.84±2.10^{b}

Data expressed as Mean±Standard Error of Mean (SEM).

$^{a-c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at p<0.05 based on one-way ANOVA and Duncan’s multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution. RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.

Amino-group-containing residues

Figure 4.10 illustrates the amount of amino-group-containing compounds equivalent to lysine in the flour samples. It was observed that PPF had a significantly higher release of amino-group residues at all time points as compared to WWF ($p<0.05$). In the gastric phase to 180 min of digestion, the amino-group residues ranged from 17.72±0.18 to 41.61±1.26 mg lysine/g sample for WWF, and from 41.61±1.26 to 100.98±21.18 mg lysine/g sample for PPF, respectively ($p<0.05$). Even though not statistically significant, an increasing trend of amino-group residue was observed for 20% PPF and 40% PPF as compared to WWF ($p>0.05$).

Figure 4.11 shows the amount of amino-group-containing compounds equivalent to lysine in the chapati samples. In the gastric phase, 20% PPF (22.15±0.66 mg lysine/g sample) and 40% PPF (25.46±1.23 mg lysine/g sample) chapatis had a significantly higher release of amino-group-containing compounds than the WWF control chapatis (18.98±0.47 mg lysine/g sample, $p<0.05$). Moreover, 40% PPF chapati showed a significantly higher release of amino-group residues in the first 30 min as compared to WWF ($p<0.05$). Even though not statistically significant, an increasing trend in the release of amino-group residues was observed in the 20% PPF chapatis at all time points ($p>0.05$).

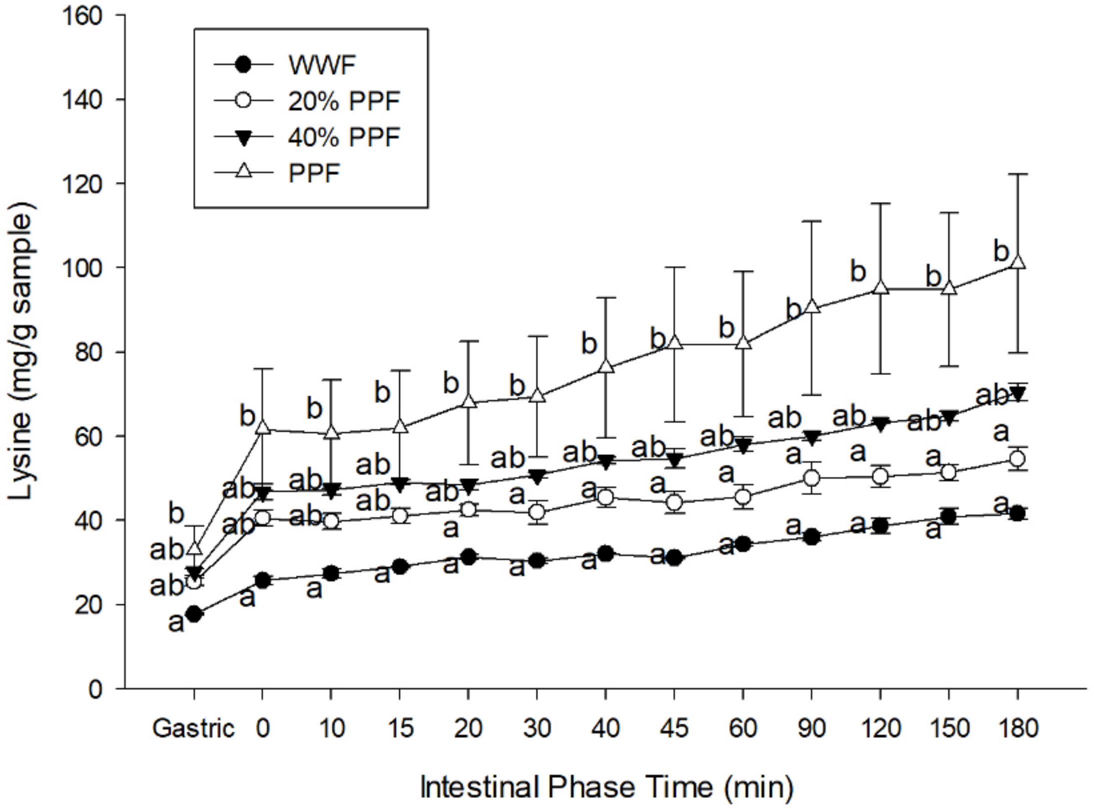


Figure 10 The effects of pigeon pea flour (PPF) and its partial substitution for whole wheat flour (WWF) on amino–group–containing residues equivalent to lysine. Different superscript alphabets on the same time interval denote statistically significant difference in the mean values among the groups at *p*<0.05 (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

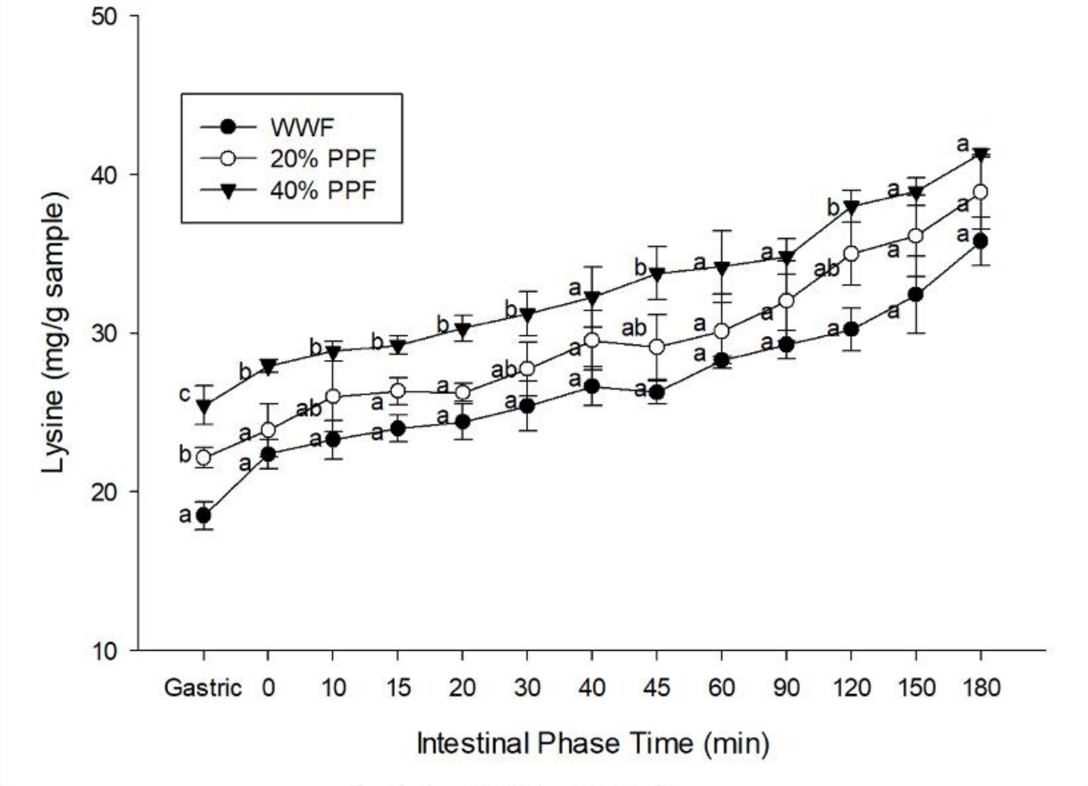


Figure 11 The effects on amino–group–containing residues equivalent to lysine of chapati developed from pigeon pea flour (PPF) and its partial substitution for whole wheat flour (WWF).

Different superscript alphabets on the same time interval denote statistically significant difference in the mean values among the groups at *p*<0.05 (n=3). (20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution).

Sensory analysis

On the day of analysis, 4 out of 80 participants failed to show up at the Faculty of Allied Health Sciences, therefore, they were excluded from the study. The sensory evaluation of the chapati samples is presented in Table 4.10. Appearance, taste, aroma, texture, aftertaste, and overall acceptability of the chapatis were evaluated and compared between types of consumers. For all consumers, the appearance and aroma were not significantly influenced by PPF substitutions as compared to the WWF control chapatis (p>0.05). On the other hand, the taste, texture, aftertaste, and overall acceptability scores were markedly reduced by the PPF levels (p<0.05). Chapati with 40% PPF substitution showed significantly lower scores in taste, texture, aftertaste, and overall acceptability as compared to the control chapatis (p<0.05).

In regular consumers, a significant reduction in scores of taste, aftertaste, and overall acceptability were shown at 40% PPF chapatis as compared to the control (p<0.05). On the other hand, no significant difference was found among chapati samples in new consumers (p>0.05). When compared between types of consumers, regular consumers gave significantly higher scoring of taste and texture for 30% PPF chapatis as compared to the new consumers (p<0.05). Moreover, regular consumers had significantly higher overall acceptability for all chapati samples when compared to new consumers (p<0.05).

Table 14 Sensory evaluation of the composite chapati comparing between the types of consumers and separated by type of consumer and formulation

		Consumer			
	Formulation	All (n=76)	Regular (n=38)	New (n=38)	*P* value$^{¥}$
Visual	WWF	**7.00 (6.00–8.00)**^{a}	8.00 (6.00–9.00)^{a}	7.00 (6.00–8.00)^{a}	0.054
	20% PPF	**7.50 (6.00–8.00)**^{a}	8.00 (7.00–8.75)^{a}	6.00 (5.25–8.00)^{a}	0.001
	30% PPF	**7.00 (5.00–8.00)**^{a}	8.00 (5.25–8.75)^{a}	7.00 (5.00–7.00)^{a}	0.035
	40% PPF	**7.00 (5.25–8.00)**^{a}	7.00 (6.00–8.00)^{a}	7.00 (5.00–7.00)^{a}	0.024
	P value†	**0.267**	0.501	0.447	
Taste	WWF	**7.00 (5.00–8.00)**^{a}	7.00 (6.00–8.75)^{a}	6.00 (4.25–7.00)^{a}	0.001
	20% PPF	**6.00 (5.00–8.00)**^{ab}	7.00 (6.00–8.00)^{a}	5.00 (4.00–6.75)^{a}	<0.001
	30% PPF	**6.00 (4.00–7.00)**^{ab}	6.50 (4.00–8.00)^{ab}	5.00 (4.00–6.00)^{a}	0.034
	40% PPF	**5.00 (4.00–7.00)**^{b}	5.50 (4.00–7.75)^{b}	5.00 (4.00–6.75)^{a}	0.139
	P value†	**0.004**	0.005	0.417	
Aroma	WWF	**7.00 (5.25–8.00)**^{a}	8.00 (6.00–9.00)^{a}	6.00 (5.00–7.00)^{a}	0.002
	20% PPF	**6.50 (5.25–8.00)**^{a}	7.50 (6.00–9.00)^{a}	6.00 (5.00–7.00)^{a}	<0.001
	30% PPF	**6.00 (5.00–8.00)**^{a}	7.00 (5.00–9.00)^{a}	6.00 (5.00–7.75)^{a}	0.124
	40% PPF	**6.50 (5.00–8.00)**^{a}	7.00 (5.00–8.00)^{a}	6.00 (5.00–7.00)^{a}	0.045
	P value†	**0.346**	0.177	0.880	
Texture	WWF	**7.00 (5.00–8.00)**^{a}	6.00 (6.00–9.00)^{a}	7.00 (5.00–7.00)^{a}	0.336
	20% PPF	**6.00 (5.00–8.00)**^{ab}	8.00 (6.00–8.50)^{a}	6.00 (4.00–7.00)^{a}	<0.001
	30% PPF	**6.00 (4.00–7.00)**^{ab}	6.00 (5.00–8.00)^{a}	5.00 (4.00–7.00)^{a}	0.046
	40% PPF	**6.00 (4.00–7.00)**^{b}	6.00 (4.00–8.00)^{a}	6.00 (4.00–7.00)^{a}	0.124
	P value†	**0.025**	0.076	0.083	
After-taste	WWF	**7.00 (5.00–8.00)**^{a}	8.00 (6.00–9.00)^{a}	6.00 (5.00–7.00)^{a}	0.002
	20% PPF	**7.00 (5.00–8.00)**^{ab}	7.00 (6.00–8.00)^{ab}	6.00 (5.00–7.00)^{a}	<0.001
	30% PPF	**6.00 (4.00–8.00)**^{ab}	7.00 (4.25–8.00)^{ab}	5.00 (4.00–7.00)^{a}	0.071
	40% PPF	**5.50 (4.00–7.00)**^{b}	7.00 (4.00–8.00)^{b}	5.00 (3.25–6.00)^{a}	0.020
	P value†	**0.002**	0.016	0.127	
Overall Accept-ability	WWF	**7.00 (5.25–8.00)**^{a}	8.00 (6.00–8.75)^{a}	6.00 (5.00–7.00)^{a}	0.001
	20% PPF	**7.00 (6.00–8.00)**^{ab}	7.00 (6.25–8.00)^{ab}	6.00 (5.00–7.00)^{a}	<0.001
	30% PPF	**6.00 (5.00–7.00)**^{ab}	7.00 (5.00–8.00)^{ab}	6.00 (5.00–7.00)^{a}	0.043
	40% PPF	**6.00 (4.00–7.00)**^{b}	7.00 (4.25–7.50)^{b}	6.00 (4.00–7.00)^{a}	0.035
	P value†	**0.007**	0.009	0.489	

Data expressed as median (Q1-Q3). WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 30% PPF, 30% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution.

$^{a\text{-}b}$Different superscript alphabets on the same row denote statistically significant differences between formulations based on †Kruskal-Wallis test at $p<0.05$ (n=76).

$^{¥}$Data comparison between new consumer (n=38) and regular consumer (n=38) based on Mann-Whitney U Test at significance levels of 0.05.

CHAPTER V

DISCUSSION

The current study was aimed to investigate whether partial substitution of pigeon pea flour (PPF) for whole wheat flour (WWF) influenced characteristics of the flour blends and the subsequent developed chapatis. A hot air oven and a high-speed universal grinder were used to make the PPF, which was then sieved through a 150 um screen mesh. The levels of PPF substitution for WWF were at 10%-40%. Physical properties and nutritional values of the composite flour blends were evaluated, which WWF was considered the control. Furthermore, the flour blends were used for the development of chapatis. Then, physical properties, nutritional values, and sensory evaluation of the chapatis were performed.

Proximate Analysis

Proximate analysis is the quantitative analysis of the macromolecules in foods, including total calorie, total carbohydrate, total fat, total dietary fiber, protein, moisture, and ash. The results indicated that protein content in PPF (26.10 g/100g) was two times higher than that in WWF (13.52 g/100g). The current study found higher protein content in PPF than previously reported, ranging between 17.9 and 24.3 g/100g (23, 25). These variations in protein contents may be due to differences in growing conditions, methods of analysis and sampling, and storage duration and conditions (80). Given high protein content, PPF can be regarded as a good novel ingredient for the development of plant-based protein products. Legume proteins have relatively high lysine content as compared to cereal proteins, however, when consumed individually it has incomplete amounts of essential amino acids. Therefore, the combination of wheat with other plant-based proteins would be more beneficial for consumers (37). The total carbohydrate of PPF in this study was 60.53 g/100g, which was similarly reported at 60.4% in pigeon pea (dhal) by Singh and colleagues (81). Total fat ranged from 2.44 g to 3.06 g in WWF and PPF. These results corresponded to the previous findings where starch (54.3–55.6%) and fat contents (2.5–2.6%) in high-protein line cultivars of pigeon pea were relatively less than that of pigeon peas with lower protein contents (80). The total dietary fiber content was slightly higher for PPF (10.41 g/100g) as compared to WWF (10.08 g/100g). The high fiber content in PPF may be advantageous to the body, as eating high fiber foods has been reported to reduce the risks of hemorrhoids (82), diabetes (83), high blood pressure (84), and obesity (85).

Physicochemical properties

Color Measurement

Color is an important characteristic as it can stimulate a person's appetite. It is one of the parameters used as a control process during roasting, since brown pigments can be formed in browning and caramelization reactions. The current results showed that PPF had lightness (*L**) and redness (*a**) lower, whereas yellowness (*b**) was higher than that of WWF. Corresponding to the previous findings (38, 86), increasing PPF substitution elevated the yellowness, while reduced the lightness and redness of the flour samples. Similarly, chapati with PPF substitution showed an increase in yellowness, lightness, and a decrease in redness as compared to control chapati. Several factors can affect the color of the product surface, such as temperature, moisture, cooking time, and the composition of reducing sugars, amino acids, or proteins on the product surface (38). A previous study reported that brown color change in color resulted after about 6 to 8 mins of roasting peanuts (87). Roasting time lesser than that showed an increase in lightness and reduction in the redness of the food samples (87). In the present study, chapati samples were developed by roasting on a non-stick pan above 200°C for 3 min. Therefore, the results of the current study agreed with a previously reported study where roasting peanuts within the first 5 min resulted in a slight increase in lightness and reduction of redness of the samples (87).

Moisture content

Moisture content of flour is an important parameter as it affects the shelf life of food. The moisture content of PPF was 19.9% lower than that of WWF. The moisture content of composite flours ranged between 9.60% for 40% PPF and 10.20% for 10% PPF, respectively. Codex Alimentarius 2016 suggest that flour blends should have moisture less than 15.5% (63, 88), which the current findings meet this specification. Previous studies also reported similar results that PPF had moisture of 7.80% (89), and lower than other flours such as unripe banana flour (10.20%), sweet potato flour (10.00%) (90), chickpea (10.70%), and cowpea (11.70%) (91).

The moisture content increased as the supplementation of PPF increases for the dough and chapati samples. This could be due to the high water absorption capacity of the PPF, which maintained a higher moisture content in the final product (92). Previous studies have shown that dough containing soy flour has a higher water absorption capacity, which is suggested to be due to the high level of soluble protein in the flour (93-95). Given that, the increasing moisture trend seen in the current study may be due to the high water absorption capacity of the PPF.

Cutting force

The texture of food is one of the most commonly measured quality attributes during consumption and processing, measured using instruments or sensory means (96). Cutting force is described as the force required to break food into pieces during the first bite by the molar teeth (97), which is generally associated with the hardness of the food. The chapati with PPF substitution showed higher hardness with increased substitution of PPF. The finding was in accordance with many previous studies where the hardness of composite chapatis increased progressively with increased substitution of defatted rice bran (98), jeering seed flour (99), cowpea flour (100), chickpea flour (101), and mung bean flour (102).

The increased hardness of chapati with PPF substitution may be related to the decrease in wheat gluten and the increase in water absorption capacity caused by the higher protein content and gluten-free property of PPF (103). Gluten plays an important role to determine the baking quality of the product. During dough fermentation, the gluten network traps CO_2 bubbles to make the dough rise. Given that PPF is gluten-free, it cannot entrap CO_2 and generate a viscoelastic network, resulting in the tight structure of chapati (104).

Nutritional Value

In-vitro starch digestion

The effects of partial PPF substitution on starch digestibility of flour and chapati were evaluated by analyzing the glucose released during simulated digestion. Flour samples (WWF, 20% PPF, 40% PPF, and PPF) and chapati samples (WWF, 20% PPF, and 40% PPF) were subjected to simulated gastrointestinal digestion. A reducing trend in starch digestibility was observed with increased substitution of PPF in both flour and chapati samples.

The flour samples showed a reduction in starch digestibility with increased substitution of PPF. It was found that PPF had lower total starch content as compared to WWF. Moreover, PPF had lower content of rapidly digestible starch

(RDS: the amount of glucose released in the first 20 min of digestion), and a higher amount of slowly digestible starch (SDS: the amount of glucose released between 20 and 120 min of digestion) when compared to the WWF. The results indicated that the hydrolysis index of PPF was lower than that of WWF. Simultaneously, PPF had the lowest pGI value when compared to the WWF.

The findings revealed that increasing PPF substitution reduced RDS and increased the SDS content of the flour blends, even though not statistically significant. Belen and colleagues reported that pigeon pea starch had the lowest amount of pGI due to the presence of lower RDS and higher SDS contents (105). SDS is the more preferable type of dietary starch because it is thoroughly yet slowly digested in the small intestine (106). Also, it has been suggested that reduced RDS content in legume starches is beneficial for people who have type 1 diabetes (106). A decreasing trend was seen for RDS in flours with PPF substitution, however, the result was not considered to be statistically significant. This may be due to the difference in the particle size of the WWF and PPF (107). In the current study, PPF had a relatively small particle size (≤150 μm), whereas an average particle size of purchased WWF was approximately 210 μm (88). The effect of particle size is often propotional to the surface area available for enzymatic action (107). The smaller particle sizes of PPF possibly increased hydrolysis by the interaction with the digestive enzymes. This may contribute to high digestibility and, in turn, cause similar levels of starch digestibility to WWF.

In the present study, chapati samples exhibited a significant reduction of starch digestibility at 40% PPF substitution as compared to the control. Total starch contents in chapati with 20% and 40% PPF substitution markedly decreased compared to the control. Chapati with 40% of PPF had the lowest proportion of RDS and SDS, while showed the highest resistant starch (RS) contents. Consequently, 40%

PPF chapati had the lowest pGI when compared to the control chapati. This could be explained by the difference in amylose:amylopectin ratio between WWF and PPF. Available data reported that one of the major factors influencing the digestibility and its physiological response of starch is related to its ratio of amylose:amylopectin (108). Amylose, a linear polymer in which the glucose residues are shared by alpha-D-(1-4) bonds; and amylopectin, a larger branched molecule with alpha-D-(1-4) and alpha-D-(1-6) bonds, are the two main structural components of starch. It has been suggested that higher amylose content reduces starch digestibility because of the positive association between amylose content and RS production. Previous finding reported that PPF has lower amylopectin and higher amylose content when compared to WWF (109). Amylose is slowly digested by digestive enzymes, while amylopectin is swiftly digested owing to its branched structure (110). Given that, the higher value of amylose in PPF can help slow down the digestion of starch into glucose to some extent.

It has also been reported that starch structures and digestibility are influenced by processing methods such as boiling, cooking, roasting, frying, baking, and drying. These methods affect the glucose release of the food products and consequently influence glycemic response (111). A previous study showed that complete gelatinization of starch during boiling reduces RS and improves digestibility (112). This may explain the high pGI of the flour samples observed in the present study. On the other hand, chapati samples were roasted prior to digestion. During food processing, retrogradation of amylose occurs causing the formation of cross-linkages and derivatization of starch resulting in recrystallization, making the food inaccessible for digestion (113). This may contribute to a lower digestibility, glycemic index, and higher RS content of chapati incorporated with PPF.

Furthermore, previous studies have reported that increasing the protein-carbohydrate ratio can reduce blood glucose (114) and inflammation caused by dietary changes (46, 115). Protein can inhibit the digestibility of starch by creating a protective layer around the starch, in turn reducing the access for enzymes. A previous study has also reported that RDS contents was higher in rice samples that had lower amount of protein (116). The current study presents lower glucose release in samples with PPF substitution. This may be due to the increased protein content caused by increased levels of PPF in the samples.

The presence of fiber in PPF may impact starch digestion, as evidenced by the glucose release results. Glucose digestion and absorption are aided by dietary fiber (117). Previous research has suggested that fiber viscosity can help with glucose management (118, 119). Increased viscosity in the food matrix caused by fiber might cause digestive enzyme interactions to change, delaying the glucose digestion and absorption.

Amino-group-containing residue

Protein is an important part of the diet that humans and animals need to survive. The quality of protein is determined by the amino acid content, digestion, absorption, and bioavailability in the food. The effects of PPF substitution on protein digestibility of flour and chapati were evaluated using ninhydrin assay, with lysine as a standard. Ninhydrin assay is a method widely used to characterize and analyze amino-group-containing compounds such as amino acids, peptides, and proteins. An increasing trend in the release of amino-group residues was observed with increased substitution of PPF in both flour and chapati samples. This is possibly due to the remarkably higher content of proteins in PPF.

Significantly higher levels of amino–group residues were seen in PPF as compared to WWF. This higher number of amino–group residues in PPF could indicate higher protein digestibility than that of WWF. Moreover, PPF in the current study had smaller particle size as compared to WWF, which possibly influenced the results. Smaller particle size is more susceptible to interaction with digestive enzymes, therefore, improving protein digestibility.

Chapati sample with 40% PPF substitution showed significant increase in the release of amino–group–containing compounds as compared to the control. Similarly, Klunklin and Savage (2018) found an increase in *in-vitro* protein digestibility of biscuits with increased substitution of green-lipped mussel powder (120). Moreover, many studies have reported that processing methods such as dehulling (121), roasting (122), cooking (123), autoclaving (121) and microwaving (124) treatments of legumes may possess a positive effect on the protein digestibility of the seeds. These processing methods reduce the anti-nutritional factors such as trypsin inhibitors and tannins present on the seed coat (125). A previous report also suggested that the trypsin inhibitor activity of pigeon pea was much lower than other legumes such as lima beans, soy, and common beans (126). Hence, the higher release in amino–group residues of 40% PPF chapati is possibly due to increased digestibility of PPF protein and reduction in anti-nutritional factors caused by processing methods.

Protein digestibility is a major determinant of amino acid availability. Fast digestible proteins, such as whey protein, were directly related to an increase in protein absorption rate, which may lead to improvement in protein synthesis and oxidation (127). In the current study, a higher number of amino–group residues was shown for PPF, which may indicate higher protein digestibility than WWF. For this reason, it implies chances of higher absorption in the body, in turn suggesting increased bioavailability.

Sensory analysis

The sensory properties of food have long been considered as a major determinant of food selection (128). Thus, the consumer ratings are influenced by aroma, color, taste, texture, aftertaste, and overall acceptability; preferences, past experiences, and health problems (128). The hedonic test is considered to be an ideal and economical method to evaluate the influence of various factors such as ingredients or manufacturing (129). Therefore, hedonic assessment was used to evaluate the acceptance of whole wheat–pigeon pea composite chapati. A total of 6 parameters, including visual, taste, aroma, aftertaste, texture, and overall acceptability, were scored by panelists.

Even though not statistically significant, a slight reduction in visual scores was observed with increased substitution of PPF. This may be related to the high protein content in PPF. It has been reported that baked products become darker with increased levels of proteins because of the amino acids of the proteins that react with reducing sugars during cooking in the Milliard reaction (130). The current study also found that the mean aroma score of the chapati decreased as substitution of PPF increased. This may be due to the beany flavor of legume crops. Comparable results were reported by previous studies substituting various legume flours such as chickpea flour (131), cowpea flour (132), lima bean and sorghum flour (133); and soya flour (130) for the development of baked good with added value.

A significant reduction in texture, taste, aftertaste, and overall acceptability scores for 40% PPF chapati was seen. This may be because PPF has specific characteristics with beany and nutty taste which provides mouthfeel from itself after intake of chapati (102). In the current study, chapati's sensory textural score corresponded to the textural analysis. The finding might imply that an increase of hardness of PPF chapati seems to lower the sensory evaluation texture score. Tiwari et al. (2011) obtained similar results where biscuits made from a high addition of pigeon pea flour in cereals resulted in a harder texture than that of the control biscuits (134). Previous studies also indicated that bread or bakery products produced with partial substitution of soy flour (135) and chickpea flour (136) had decreased mean scores of taste, texture, and aftertaste parameters.

Furthermore, the results showed that the overall acceptability scores of the chapatis were significantly affected by types of consumers. Regular consumers gave significantly higher scores in all parameters for 20% PPF chapati as compared to the new consumers. The significantly lower score by new consumers may be due to the unfamiliarity with chapati. Available data suggested that lack of familiarity with new foods can affect expectations, influencing sensory experience and the overall likeability of the food product (137). Moreover, it has been reported that cultural differences in dietary experiences and food environments can influence sensory property preferences (138). These findings suggest that regular consumers are familiar with the product and more likely to give a positive scoring when compared to the new consumers.

Nonetheless, the substitution of PPF up to 20% showed no significant difference concerning the acceptability parameter or type of consumer when compared to the control chapati. Hence, 20% PPF substitution may be the optimum level of substitution to generate good results in the sensory acceptability of baked products.

CHAPTER VI

CONCLUSION

In conclusion, the pigeon pea seeds were turned into flour by heating, high-speed grinding, and sieving. The study found that PPF was slightly dull, yellow in color, and had lower moisture levels compared to WWF. Moreover, PPF could attenuate starch digestibility owing to its lower starch and higher SDS content. Additionally, the proximate analysis revealed two times higher protein content than WWF, corresponding to higher levels of protein digestibility. The higher ratio of PPF substitution in chapati exhibited increased hardness and demonstrated brighter with slight yellow color as compared to the control chapati. However, PPF substitution significantly decreased total starch, increased RS content, and alleviated glucose release from the flours, consequently lowering the predicted glycemic index. Chapati with PPF substitution also displayed a higher release of amino–group–containing residues, suggesting an increased protein digestibility and bioavailability of the product. Furthermore, the PPF chapati manifested a good sensory evaluation. As a result, the PPF is a good source of protein and reflects great nutritional properties, therefore, PPF may be used as an alternate ingredient to develop healthier foods, particularly plant-based products.

APPENDIX

Table i. Glucose release at each time interval (simulated gastro-intestinal digestion) of flour samples

	Time (minutes)								
Samples	10	20	30	40	60	90	120	150	180
WWF	35.91 ± 5.62^{a}	70.35 ± 5.78^{a}	92.10 ± 5.79^{a}	113.64 ± 9.31^{a}	151.35 ± 12.63^{a}	206.54 ± 22.10^{a}	249.61 ± 21.90^{a}	292.72 ± 25.08^{a}	326.15 ± 27.93^{a}
20% PPF	33.72 ± 3.17^{a}	63.66 ± 2.87^{ab}	83.17 ± 4.79^{a}	106.06 ± 3.12^{a}	147.57 ± 1.03^{a}	191.96 ± 3.40^{a}	232.00 ± 3.23^{a}	275.33 ± 6.67^{a}	300.89 ± 5.60^{a}
40% PPF	32.45 ± 2.84^{a}	58.46 ± 3.15^{ab}	78.50 ± 2.88^{a}	96.21 ± 5.45^{a}	131.72 ± 2.29^{a}	181.33 ± 5.68^{a}	225.11 ± 9.26^{a}	260.63 ± 11.78^{a}	287.47 ± 2.71^{a}
PPF	30.50 ± 1.73^{a}	56.56 ± 1.47^{b}	79.48 ± 2.14^{a}	97.06 ± 4.32^{a}	130.89 ± 9.79^{a}	177.16 ± 12.87^{a}	224.45 ± 17.74^{a}	256.79 ± 16.69^{a}	288.52 ± 17.41^{a}

Data expressed as Mean±Standard Error of Mean (SEM).

$^{a\text{-}c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p \leq 0.05$ based on one-way ANOVA and Duncan’s multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution.

Table ii. Glucose release at each time interval (simulated gastro-intestinal digestion) of chapati samples

	Time (minutes)								
Samples	**10**	**20**	**30**	**40**	**60**	**90**	**120**	**150**	**180**
WWF	26.25±6.29^{a}	43.99±8.01^{a}	61.14±11.62^{a}	76.28±13.16^{a}	101.33±17.51^{a}	138.70±26.95^{a}	167.19±37.36^{a}	194.96±44.44^{a}	199.12±47.14^{a}
20% PPF	15.26±2.17^{a}	30.58±2.13^{ab}	44.66±4.63^{a}	55.92±6.33^{ab}	74.51±7.45^{ab}	96.63±8.26^{ab}	111.28±8.88^{ab}	119.55±10.40^{ab}	128.68±11.59^{ab}
40% PPF	13.21±1.78^{a}	26.20±3.21^{b}	36.15±2.60^{a}	44.12±2.25^{b}	59.30±2.79^{b}	70.74±3.33^{b}	76.70±4.82^{b}	86.31±5.32^{b}	94.88±4.65^{b}

Data expressed as Mean±Standard Error of Mean (SEM).

$^{a\text{-}c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p \leq 0.05$ based on one-way ANOVA and Duncan’s multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution.

Table iii. Amino-group containing residues content of flour samples

Time	WWF	20% PPF	40% PPF	PPF
Gastric	17.72±0.18^{a}	25.46±0.92^{ab}	27.75±0.78^{ab}	32.88±5.88^{b}
0	25.68±1.01^{a}	40.52±1.90^{ab}	46.75±1.97^{ab}	61.58±14.49^{b}
10	27.42±1.07^{a}	39.72±1.86^{ab}	47.28±1.33^{ab}	60.51±12.90^{b}
15	28.99±0.21^{a}	41.07±1.72^{ab}	48.95±0.62^{ab}	61.96±13.60^{b}
20	31.23±0.58^{a}	42.50±1.39^{a}	48.38±1.20^{ab}	67.84±14.63^{b}
30	30.33±0.70^{a}	41.92±2.79^{a}	50.75±0.74^{ab}	69.34±14.37^{b}
40	32.10±0.16^{a}	45.45±2.31^{a}	54.25±0.79^{ab}	76.23±16.57^{b}
45	31.12±0.16^{a}	44.25±2.52^{a}	54.72±2.24^{ab}	81.77±18.34^{b}
60	34.36±0.54^{a}	45.55±2.95^{a}	58.10±1.69^{ab}	81.86±17.17^{b}
90	36.06±0.97^{a}	50.01±3.74^{a}	59.99±1.03^{ab}	90.42±20.55^{b}
120	38.75±1.78^{a}	50.39±2.58^{a}	63.27±0.30^{ab}	94.94±20.21^{b}
150	40.92±1.96^{a}	51.37±1.87^{a}	64.77±1.25^{ab}	94.87±18.23^{b}
180	41.61±1.26^{a}	54.62±2.82^{a}	70.50±2.07^{ab}	100.98±21.18^{b}

Data expressed as Mean±Standard Error of Mean (SEM).

$^{a\text{-}c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p \leq 0.05$ based on one-way ANOVA and Duncan's multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution.

Table iv. Amino–group containing residues content of chapati sample

Time	WWF	20% PPF	40% PPF
Gastric	18.98±0.47^{a}	22.15±0.66^{b}	25.46±1.23^{c}
0	22.99±0.34^{a}	23.89±1.67^{b}	27.94±0.40^{b}
10	27.16±2.64^{a}	26.02±2.24^{ab}	28.87±0.64^{b}
15	27.97±3.27^{a}	26.35±0.84^{a}	29.27±0.57^{b}
20	27.86±2.56^{a}	26.26±0.56^{a}	30.31±0.84^{b}
30	28.32±3.41^{a}	27.76±1.70^{a}	31.25±1.40^{a}
40	30.12±3.58^{a}	29.56±1.86^{a}	32.28±1.90^{a}
45	31.55±5.41^{a}	29.15±2.05^{ab}	33.78±1.66^{b}
60	32.80±4.53^{a}	30.14±2.36^{a}	34.20±2.25^{a}
90	35.61±5.95^{a}	32.05±2.52^{a}	34.84±1.13^{a}
120	36.90±5.76^{a}	35.01±1.99^{ab}	38.02±1.00^{b}
150	38.89±6.16^{a}	36.14±2.56^{a}	38.94±0.87^{a}
180	41.59±4.63^{a}	38.90±2.34^{a}	41.35±0.27^{a}

Data expressed as Mean±Standard Error of Mean (SEM).

$^{a-c}$Different superscript alphabets on the same column denote statistically significant difference in the mean values at $p \leq 0.05$ based on one-way ANOVA and Duncan’s multiple range post hoc analysis (n=3).

WWF, Whole Wheat Flour; PPF, Pigeon Pea Flour; 20% PPF, 20% pigeon pea substitution; 40% PPF, 40% pigeon pea substitution.

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