DIETARY FIBER-RICH POWDER FROM DRAGON FRUIT PEEL AND ITS EFFECTS ON NUTRIENT DIGESTION, INTESTINAL FUNCTION AND FOOD APPLICATION

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ผงที่อุดมไปด้วยใยอาหารจากเปลือกแก้วมังกร และผลกระทบต่อการย่อยสารอาหาร,การทำงานของลำไส้ และการประยุกต์ใช้ในอาหาร

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ศิริวรรณ จำเริญวิทยากุล : ผงที่อุดมไปด้วยใยอาหารจากเปลือกแก้วมังกร และผลกระทบต่อการย่อย
สารอาหาร,การทำงานของลำไส้ และการประยุกต์ใช้ในอาหาร. (DIETARY FIBER-RICH POWDER FROM
DRAGON FRUIT PEEL AND ITS EFFECTS ON NUTRIENT DIGESTION, INTESTINAL FUNCTION AND
FOOD APPLICATION) อ.ที่ปรึกษาหลัก : ศ. ดร.สิริชัย อดิศักดิ์วัฒนา, อ.ที่ปรึกษาร่วม : ดร.มาฮินดา อเบย์
วาเดนา,ผศ. ดร.ถาวรีย์ ถิละเวช

อาหารตะวันตกอุดมไปด้วยคาร์โบไฮเดรตเชิงเดี่ยวและไขมันที่ไม่ดีต่อสุขภาพ รวมถึงมีปริมาณใยอาหารต่ำ
ซึ่งเพิ่มความเสี่ยงของการเกิดความผิดปกติทางเมแทบอลิกและการทำงานของผนังลำไส้ การศึกษานี้ได้พัฒนาผงที่อุดมไป
ด้วยใยอาหารจากเปลือกแก้วมังกรเพื่อเป็นวัตถุดิบสำหรับอาหารเพื่อสุขภาพ ผงเปลือกแก้วมังกรที่ได้มีปริมาณใยอาหารสูง
(65.2%) โดยเฉพาะใยอาหารชนิดเซลลูโลสและเพคติน รวมถึงมีสารพฤกษเคมีซึ่งเป็นสารออกฤทธิ์ทางชีวภาพ เช่น
สารประกอบฟีนอลิกและเบตาไซยานิน โดยการศึกษานี้มีจุดประสงค์เพื่อศึกษาผลของผงเปลือกแก้วมังกรต่อการย่อยแป้ง
ในหลอดทดลอง การประยุกต์ใช้ในผลิตภัณฑ์เบเกอรี่ และผลกระทบต่อการทำงานของผนังลำไส้และจุลินทรีย์ในลำไส้ใน
หนูที่ได้รับอาหารที่มีไขมันและน้ำตาลฟรุกโตสสูง การศึกษาพบว่าการเติมผงเปลือกแก้วมังกรลงในแป้งประกอบอาหาร
เปลี่ยนแปลงอุณหภูมิการเกิดเจลาติไนเซชัน ค่าความหนืดสูงสุด และอุณหภูมิที่เกิดการเปลี่ยนแปลงค่าความหนืดของแป้ง
อย่างมีนัยสำคัญทางสถิติ ซึ่งสอดคล้องกับความสามารถในกรย่อยแป้งที่ลดลง การเติมผงเปลือกแก้วมังกรลงในคุกกี้
แป้งข้าวสาลี (ร้อยละ 1-5) ลดค่าดัชนีการย่อยและค่าดัชนีน้ำตาลของผลิตภัณฑ์คุกกี้ ซึ่งบ่งชี้ถึงความสามารถในการลด
การย่อยแป้งและระดับน้ำตาลในเลือด นอกจากนี้การเติมผงเปลือกแก้วมังกรยังเพิ่มปริมาณของสารออกฤทธิ์ทางชีวภาพ
ซึ่งสัมพันธ์กับความสามารในการต้านอนุมูลอิสระที่สูงขึ้นในคุกกี้ นำไปสู่การลดปริมาณสารพิษที่เกิดจากการปรุง
ประกอบอาหารที่ความร้อนสูงได้แก่ สารมาโลนไดอัลดีไฮด์ (ผลิตภัณฑ์จากปฏิกิริยาออกซิเดชันของไขมัน) และผลิตภัณฑ์
สุดท้ายจากปฏิกิริยาไกลเคชั่น นอกเหนือจากผลกระทบต่อการย่อยแป้งแล้ว ผงเปลือกแก้วมังกรยังช่วยบรรเทาความ
ผิดปกติของการทำงานของผนังสำไส้หนู การเสริมผงเปลือกแก้วมังกรในอาหาร (ร้อยละ 5) ยับยั้งการเหนี่ยวนำให้เกิด
ภาวะลำไส้รั่วจากการบริโภคอาหารที่มีไขมันและน้ำตาลฟรุกโตสสูง โดยลดระดับโปรตีนขนส่งสารไลโพโพลีแซคคาไรด์ใน
เลือด และกดการแสดงออกของยีนที่ควบคุมโปรตีนตัวรับ Toll like receptor 4 พร้อมกับเพิ่มการแสดงออกการแสดง
ของยีนที่ควบคุมโปรตีน tight junction ในเนื้อเยื่อลำไส้ใหญ่หลัง 12 สัปดาห์ของการศึกษา ผงเปลือกแก้วมังกรยัง
ส่งเสริมการเพิ่มจำนวนของแบคทีเรียที่สร้างกรดไขมันสายสั้น สอดคล้องกับปริมาณกรดไขมันสายสั้นที่เพิ่มขึ้นในของเหลว
จากลำไส้ใหญ่ ผลการศึกษาทั้งหมดแสดงให้เห็นถึงคุณค่าของผงที่อุดมไปด้วยใยอาหารจากเปลือกแก้วมังกรในการเป็นวัตถุ
ดิบสำหรับการประยุกต์ใช้ในอาหารที่มีแป้งเป็นส่วนประกอบเพื่อควบคุมระดับน้ำตาลในเลือด รวมถึงเป็นวัตถุดิบที่มี
คุณสมบัติในการบรรเทาความผิดปกติของการทำงานของผนังลำไส้ได้

สาขาวิชา	อาหารและโภชนาการ	ลายมือชื่อนิสิต ....
ปีการศึกษา	2565	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

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KEYWORD: Dragon fruit peel/ Dietary fiber/ In vitro digestion/ Food application/ Gut health

Siriwan Chumroenvidhayakul : DIETARY FIBER-RICH POWDER FROM DRAGON FRUIT PEEL AND ITS EFFECTS ON NUTRIENT DIGESTION, INTESTINAL FUNCTION AND FOOD APPLICATION. Advisor: Prof. SIRICHAI ADISAKWATTANA, Ph.D. Co-advisor: Prof. Mahinda Abeywardena, Ph.D.,Asst. Prof. Thavaree Thilavech, Ph.D.

The Western diet, which is rich in refined carbohydrates and unhealthy fats but low in dietary fiber, has been linked to an increased risk of metabolic disorders and gut barrier dysfunction. In the present study, the dietary fiber-rich powder from dragon fruit (*Hylocereus undatus*) peel (DFP) was developed as a functional food ingredient. DFP contains high dietary fiber (65.2%), primarily cellulose and pectin, along with bioactive phytochemicals such as phenolic compounds and betacyanins. This study aimed to investigate the effect of DFP on in vitro starch digestibility, its application in bakery products, and its impact on intestinal permeability and gut microbiota in rats fed a high-fat, high-fructose (HFHFr) diet. The incorporation of DFP in flours significantly altered the gelatinization enthalpy, peak viscosity, and pasting temperature, which correlated with a reduction in starch digestibility. Adding DFP (1-5% *w/w*) to wheat cookies resulted in remarkably lower hydrolysis index and predicted glycemic index, indicating reduced digestibility of starch and a lower impact on blood sugar levels. Moreover, DFP markedly increased the amount of bioactive compounds, which correlated with improved antioxidant capacity in the cookies. It also led to reductions in heat-induced toxicants, including, malondialdehyde (a lipid peroxidation product) and dietary advanced glycation end products. In addition to its effects on starch digestibility, DFP showed promising results in mitigating gut barrier dysfunction in rats. Supplementation of the diet with DFP (5% *w/w*) suppressed HFHFr diet-induced intestinal barrier dysfunction by decreasing serum lipopolysaccharide-binding protein levels and mRNA expression of Toll like receptor 4 in colonic tissue, along with an up-regulation of colonic tight junction protein expression after 12 weeks of the study ($p < 0.05$). For cecal microbiota, DFP significantly promoted the proliferation of SCFA-producing bacteria, consistent with the increased concentration of total SCFA in cecal contents. Taken together, dietary-rich powder from dragon fruit peel can be considered a valuable ingredient in flour-based food applications for regulating blood glucose, and a modulating compound for alleviating intestinal barrier dysfunction.

Field of Study:	Food and Nutrition	Student's Signature ....
Academic Year:	2022	Advisor's Signature ....
		Co-advisor's Signature ....
		Co-advisor's Signature ....

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Lastly, I would be remiss if I didn't mention my family, especially my father and mother. I am truly fortunate to have all of you in my life. Your unwavering belief in me has kept my spirits high and provided constant motivation throughout this journey. I am profoundly indebted to each and every one of you. Thank you from the bottom of my heart.

Siriwan Chumroenvidhayakul

TABLE OF CONTENTS

LIST OF TABLES

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 Background and linking of the study

Non-communicable diseases (NCDs), including obesity, type 2 diabetes, cardiovascular diseases, and cancers, are the leading causes of illness and death worldwide, posing a significant public health concern (Habib & Saha, 2010). According to the World Health Organization's report in 2018, NCDs accounted for 41 million global deaths (WHO, 2018). It has been established that lifestyle factors, particularly diet, play a crucial role in NCD development. The consumption of a Western diet, characterized by excessive intake of fat and refined carbohydrates, along with a lack of dietary fiber, emerges as a primary catalyst for metabolic disturbances associated with inflammation, both systemically and in the gastrointestinal tract (Liang, Saunders, & Sanossian, 2023; Liu et al., 2021). Recent studies indicate that overconsumption of high-carbohydrate and high-fat diets not only leads to dyslipidemia, insulin resistance, impaired glucose tolerance, and hypertension, but also induces gut dysbiosis (Zinöcker & Lindseth, 2018). Moreover, modern carbohydrate diets cooked at high temperatures with dry heat promotes heat-generated toxicants (such as malondialdehyde (MDA)), and dietary advanced glycation end products (known as dAGEs) from oxidative and Maillard browning reaction (Zhang, Wang, & Fu, 2020). Recent studies have shown that the consumption of dietary oxidized fat and AGE-rich

food significantly increases their circulation and accumulation in human serum and tissue (Eder, Keller, Hirche, & Brandsch, 2003; Mastrocola et al., 2013). This elevation contributes to the promotion of oxidative stress and inflammation, which are related to the risk of diabetes and atherosclerosis (Eder, Keller, Hirche, & Brandsch, 2003; Mastrocola et al., 2013). Additionally, high consumption of fat and refined carbohydrate, especially fructose diet, impairs intestinal bacteria homeostasis and disrupts intestinal epithelial barrier integrity (Chen et al., 2022; Sellmann et al., 2015). For example, a Western diet contains high levels of fat or fructose, which can lead to a loss of gut microbial diversity. Particularly, it reduces the proportion of bacteria that produce short-chain fatty acids (SCFAs) while significantly increasing the proportion of gram-negative Proteobacteria (Chen et al., 2022; Do, Lee, Oh, Kim, & Park, 2018). Diet-induced gut dysbiosis consequently results in higher levels of gram-negative bacterial endotoxin, known as lipopolysaccharides (LPS), which can disrupt the integrity of the intestinal barrier, leading to reduced expression of tight junction (TJ) proteins, such as zonula occludens-1 (ZO-1), occludin, and claudins-1. Accumulating evidence has shown that increased intestinal permeability promotes the leakage of LPS into the circulation. The binding to LSP to toll-like receptor 4 (TLR4) triggers activation of the inflammatory pathway, which contributes to systemic low-grade inflammation, insulin resistance, and hypertension in the host (Chen et al., 2022; Sellmann et al., 2015).

Interestingly, an increased intake of dietary fiber combined with bioactive compounds has demonstrated beneficial effects in improving metabolic abnormalities

and gut function (Chaouch & Benvenuti, 2020; Tavares et al., 2022). The World Health

Organization (WHO), the Food and Agriculture Organization (FAO), and the Scientific

Advisory Committee on Nutrition (SACN) recommend a daily consumption of 25–30 g

of total dietary fiber or five portions of fruits and vegetables to combat obesity,

metabolic diseases, and support gut health (O'Grady, O'Connor, & Shanahan, 2019).

Scientific evidence revealed that dietary fiber and phytochemical compounds can

influence nutrient digestibility (Zhang, Sun, & Ai, 2022). For instance, incorporating

dietary fiber into starch leads to molecular interactions that influence the

gelatinization properties of starch, thereby reducing its digestibility within the food

matrix (Zhang et al., 2021). In addition, dietary fibers have been revealed to increase

the viscosity of the digesta, forming physical barriers that restrict enzyme diffusion to

starch substrates, thereby slowing down the process of amylolysis (Qi, Al-Ghazzewi, &

Tester, 2018; Zhang et al., 2022). This increased viscosity prolongs the transit time in

the digestive system, leading to delayed gastric emptying, heightened satiety, and

ultimately a reduction in the consumption of starchy foods (Qi, Al-Ghazzewi, & Tester,

2018; Zhang et al., 2022). Previous observational studies have indicated that higher

intake of dietary fiber from fruits (18-22 g/day) is inversely associated with body mass

index and insulin resistance, while positively promoting improved gut microbiota

diversity and reducing the risk of developing type 2 diabetes in populations at high

risk for the disease (Ylönen et al., 2003; Jiang et al., 2020). A study by Demirci, et al.

in 2019 also revealed a significant correlation between daily intake of total dietary

fiber and reduced levels of advanced glycation end products (AGEs), oxidative stress, and inflammation in chronic kidney disease patients (Demirci, Tutal, Eminsoy, Kulah, & Sezer, 2019). Additionally, the study conducted by Jiang, et al. in 2016 demonstrated that dietary fibers derived from apple pomace not only inhibit weight gain and fat accumulation but also modulate gut microbiota composition by increasing the production of SCFAs and attenuating LPS-induced inflammation in rat models with diet-induced obesity (Jiang et al., 2016). It is noteworthy that soluble dietary fibers exhibit superior physiological properties and fermentability compared to insoluble dietary fibers (Cui et al., 2019). Although the health benefits of consuming fruits, particularly their pulps, are well-known (Cui et al., 2019), it is essential to recognize that significant amounts of dietary fibers are also present in fruit peels (Tavares et al., 2022), which generally discard as waste and pose a significant environmental challenge. Therefore, the development of novel functional ingredients from fruit waste presents an important and ongoing challenge.

Dragon fruit (*Hylocereus undatus*) is a tropical fruit crop with significant economic value and widespread consumption in Asia. It is recognized for its rich content of vitamins, minerals, dietary fibers, and phytochemical compounds (Jimenez-Garcia et al., 2022). Typically, only the pulps of dragon fruit are consumed, while the dragon fruit peel is discarded as waste (Jiang et al., 2021). It is worth noting that dragon fruit peel is a rich source of nutrition, particularly dietary fiber (69.3%), which includes pectin and cellulose, and bioactive phytochemicals such as phenolic compounds

and betacyanins (Bakar, ShuC., Muhammad, Hashim, & Noranizan, 2011; Jiang et al., 2021; Le, 2022). Betacyanins have been reported to possess antioxidant, anti-inflammatory, and lipid peroxidation-inhibiting properties (Jiang et al., 2021; Le, 2022). The consumption of pure betacyanin extract from dragon fruit peel resulted in decreased body weight gain, improved glucose intolerance and insulin resistance, and ameliorated inflammatory status in high-fat diet-fed mice (Song, Chu, Xu, Xu, & Zheng, 2016). Remarkably, betacyanins have also been found to modulate gut microbiota by reducing the Firmicutes-to-Bacteroidetes ratio and increasing the abundance of *Akkermansia* (Song, Chu, Yan, et al., 2016). Hence, the utilization of dragon fruit peel as a food ingredient holds promise due to its demonstrated biological activities. Various studies have explored the application of dragon fruit peel in food products such as chicken nuggets, beef sausages, noodles, bread, and ice cream, revealing increased dietary fiber content, antioxidant activity, total phenolic compounds, and total betacyanin levels (Jiang et al., 2021). While dragon fruit peel has shown positive effects in various biological aspects, its specific impact on inhibiting carbohydrate digestion, heat-generated toxicants, and modulation of gut health in high- fat, high-fructose fed rats remain unclear.

Therefore, this study aims to prepare dragon fruit peel powder (DFP) and investigate the its physicochemical properties and biological activities on inhibition of carbohydrate digestion *in vitro*. With regard to food application, DFP powder was applied on baking cookies, particularly in the study of physicochemical properties,

biological activities, and sensory acceptance. Finally, the effect of DFP on metabolic markers, intestinal permeability, and gut microbiota in high- fat, high-fructose-induced obese rats was investigated.

1.2 Objectives of the study

- To prepare dragon fruit peel (*Hylocereus undatus*) powder (DFP) and study its physicochemical, and biological properties.

- To examine the effect of DFP on *in vitro* starch digestibility, gelatinization, and pasting properties with various types of flour (potato, glutinous rice, rice, and wheat flour).

- To investigate the effect of DFP incorporated in cookies on physicochemical properties, functional properties, and sensory acceptance.

- To study the effect of DFP on intestinal epithelial permeability, gut microbiota composition, and SCFA production in high-fat, high-fructose diet fed rats.
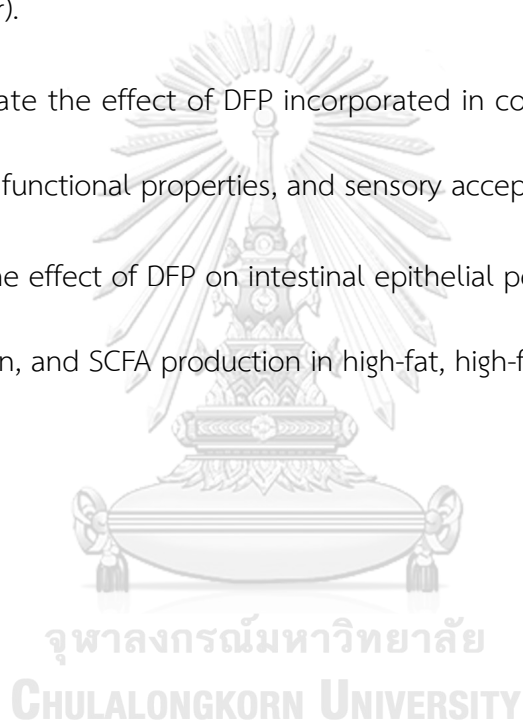
1.3 Hypotheses of the study

- DFP has high content in the total dietary fiber, betacyanin content, phenolic compound and demonstrate antioxidant activity. In addition, it has higher hydration properties and lower oil holding capacity when compared to carboxymethyl cellulose.

- DFP may alter the thermal properties and pasting properties of cooking flours, which may impact the rate of starch digestibility in simulated gastrointestinal system

- The addition of DFP into cookies may not alter physicochemical properties and sensory acceptability. DFP may increase the betacyanin content, phenolic compound and antioxidant activities in cookies, while potentially reducing the release of glucose resulting from starch digestion. Additionally, DFP may exhibit inhibitory effects on heat-induced toxicants present in the diet, when compared to the control cookies.

- The supplementation of DFP may contribute to enhancing the composition of short-chain fatty acids and gut microbiota, potentially mitigating damage to the intestinal barrier and reducing the leakage of lipopolysaccharide (LPS) into the bloodstream in rats fed a high-fat, high-fructose diet.

1.4 Conceptual framework
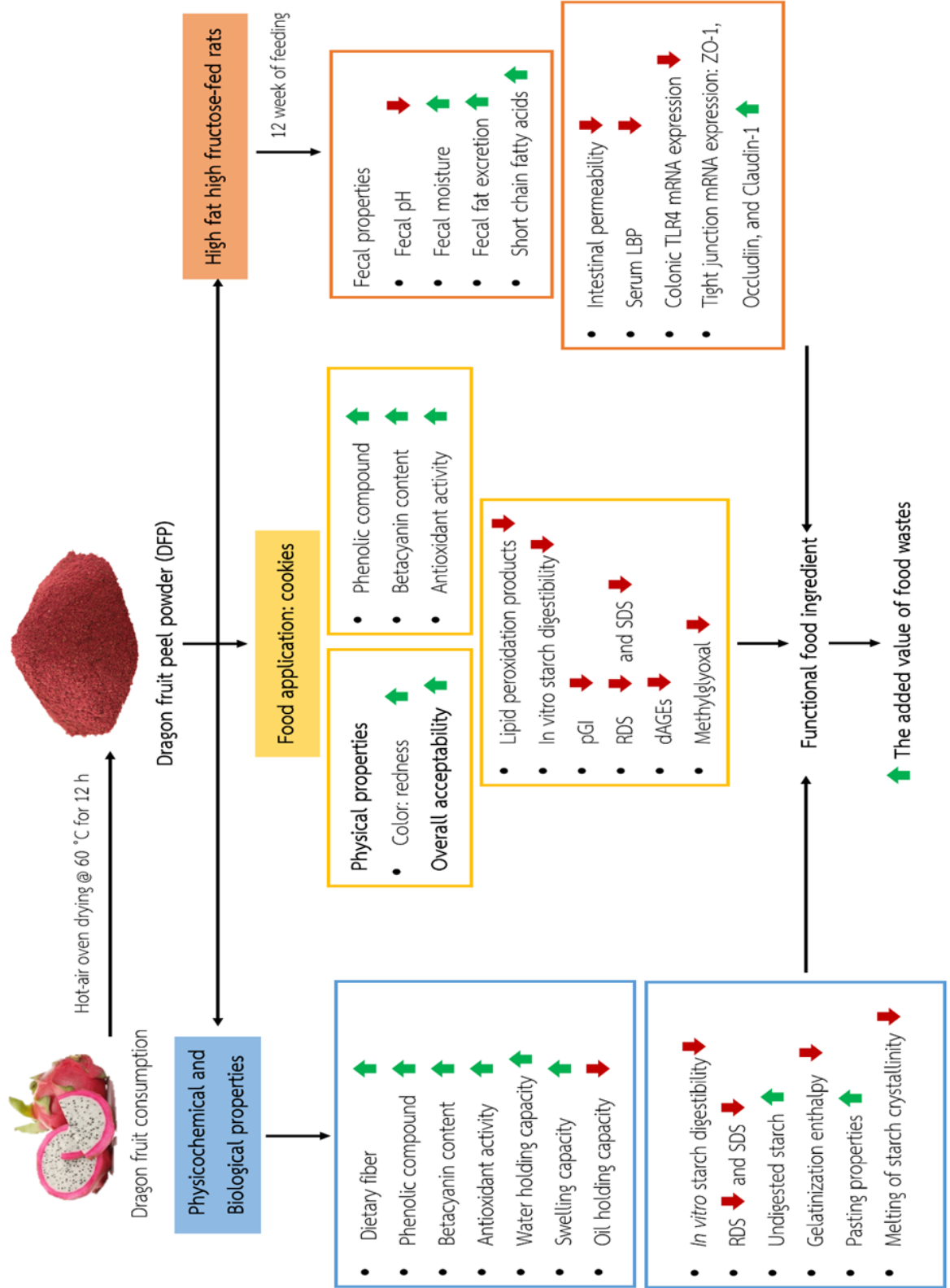
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CHAPTER II

INVESTIGATING THE IMPACT OF DRAGON FRUIT PEEL WASTE ON STARCH DIGESTIBILITY, PASTING, AND THERMAL PROPERTIES OF FLOURS USED IN ASIA

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2.2 Article status

2.3 Abstract

As a by-product of dragon fruit consumption, dragon fruit peel (DFP) was developed into powder as a natural ingredient. Nevertheless, the effect of DFP on the physicochemical properties of flours used in Asian food processing and cooking remains unknown. In this study, starch digestibility, thermal, pasting, and physicochemical properties of DFP and flours (potato, rice, glutinous rice, and wheat) were characterized. It was found that DFP contained 65.2% dietary fiber together with phenolic compounds, betacyanins, and antioxidant activity. The results demonstrated that DFP (from 125 to 500 mg) reduced starch digestibility of flours, rapidly digestible starch, and slowly digestible starch, along with an increased proportion of undigested starch. A marked increase in phenolic compounds, betacyanins, and antioxidant activity occurred when DFP and flour were incubated for 180 min under simulated gastrointestinal digestion. The results indicate that bioactive compounds in DFP were highly bioaccessible and remained intact after digestion. Moreover, DFP exerted a significantly lower gelatinization enthalpy of flours with increasing peak viscosity and setback with decreasing pasting temperature. FTIR confirmed the decreased ratio at 1047/1022 cm^{-1}, indicating the disruption of short-range orders of starch and DFP. These findings would expand the scope of DFP food applications and provide a knowledge basis for developing DFP flour-based products.

Keywords: dragon fruit peel; dietary fiber; in vitro starch digestion; physicochemical properties

2.4 Introduction

Carbohydrates are the essential macronutrients that provide energy to the body, supporting functions and physical activity. Long-term carbohydrate overconsumption has been associated with increased blood glucose and visceral fat storage, promoting insulin resistance and inflammation, and raising the risk of metabolic syndrome, type 2 diabetes, and cardiovascular diseases (CVDs) (Dehghan et al., 2017; Kwon, Lee, & Lee, 2018). Starch, a polysaccharide composed of glucose monomers, accounts for about 60% of the carbohydrates consumed by humans. In general, starches from wheat, rice, glutinous rice, and potatoes are the most popular ingredients used for culinary applications. Nevertheless, different types of flour provide unique physicochemical characteristics for food products and desserts, such as thickening, gelling, stabilizing, and moisture retention (Schmiele, Sampaio, & Pedrosa Silva Clerici, 2019). In addition to physicochemical properties, starches notably consist of different amylose to amylopectin ratios, affecting starch digestibility in the gastrointestinal tract (Agama-Acevedo, Flores-Silva, & Bello-Perez, 2019). In clinical studies, the consumption of high-amylose starch has been shown to reduce postprandial glycemic and insulinemic responses in healthy men and women compared to conventional starch (Behall & Hallfrisch, 2002; Belobrajdic et al., 2019). Moreover, the reduced hydrolysis rate of starch delays carbohydrate digestibility, leading to the suppression of postprandial glycemic response (Wee & Henry, 2020).

Substituting or incorporating dietary fibers and phytochemical compounds into starches has recently been viewed as a practical approach for glycemic control by inhibiting the activity of carbohydrate digestive enzymes and interacting with their granules to modify swelling grade, gelatinization, and pasting properties (Gómez & Martinez, 2018; Wee & Henry, 2020). For instance, apple peel containing 58.44% of dietary fibers effectively suppressed starch digestibility and decreased the glycemic index of wheat flour during simulated gastrointestinal digestion (Bae, Jun, Lee, & Lee, 2016). In addition, the incorporation of anthocyanin-rich extract from *Clitoria ternatea* flower into different flours such as potato, cassava, rice, corn, wheat, and glutinous rice resulted in a significant decrease in starch hydrolysis and glycemic index (Chusaket al., 2018).

White pulp Dragon fruit *(Hylocereus undatus)*, an economically important tropical fruit crop, is a low glycemic index food with a high concentration of vitamins, minerals, phytochemical compounds, and dietary fibers (Ibrahim, Mohamed, Khedr, Zayed, & El-Kholy, 2018). Furthermore, it has several biological properties, including antioxidant and anti-diabetic activity (Jiang et al., 2021). In Thailand, the pulp of white pulp dragon fruit is generally served on a patient menu in hospitals due to its nutritional benefits and inexpensive cost (Poolsup, Suksomboon, & Paw, 2017). Unfortunately, it may contribute to environmental issues by creating vast amounts of peel waste, approximately 22% of the whole fruit (Bakar, ShuC., Muhammad, Hashim, & Noranizan, 2011). To utilize this waste, dragon fruit peel (DFP) has become a

potential ingredient in developing functional foods because it comprises soluble and insoluble fibers up to 69.3% of dry weight (Bakar, ShuC., Muhammad, Hashim, & Noranizan, 2011). In recent decades, DFP has been used as an ingredient to improve the nutritional value of food products with high consumer acceptability and palatability, such as noodles, steamed bread, and cookies (Jiang et al., 2021). Although DFP is a promising food ingredient for incorporation into various types of starch and flour, its effects on the physicochemical and functional properties of flour remain unclear. Therefore, it is of great significance to explore the interaction between DFP and flours used in Asian food processing and cooking. The objective of this study was to investigate the physicochemical and functional properties of DFP received from hospital food waste. Moreover, the effect of DFP on in vitro starch digestibility, pasting, and gelatinization with various types of flour (potato, glutinous rice, rice, and wheat flour) used in Asia was further explored. The research conclusions would provide new insights into the use of DFP in developing innovative flour-based functional products.

2.5 Methods

2.5.1   Materials

The dragon fruit peel was obtained from Ramathibodi Hospitals, Bangkok, Thailand. Commercial potato, glutinous rice, rice, and wheat flour were purchased from a supermarket. Folin-Ciocalteu reagent, betanin, 2,4,6-tripyridyl-s-triazine (TPTZ), pepsin from porcine gastric mucosa powder, α-amylase Type VI-B from porcine

pancreas, and pancreatin from porcine pancreas were obtained from Sigma-Aldrich

Chemical (St. Louis, MO, USA). Amyloglucosidase from *Aspergillus niger* was purchased

from Roche Diagnostics (Indianapolis, IN, USA).

2.5.2   Dragon fruit peel powder (DFP) preparation

The fresh dragon fruit peel was cleaned to remove impurities and dried at 60

°C for 12 h using a hot air oven. Next, the dry material was milled (DXM-500, DX-FILL,

Samut Prakan, Thailand) and sifted through No. 40 sieves to obtain the powder.

Finally, DFP was stored in polyethylene foil bags and kept at −20 °C

2.5.3   Proximate analysis of DFP

The proximate composition of DFP, including moisture, ash, protein, total fat,

total sugar, and total dietary fiber, was conducted according to the Association of

Official Agricultural Chemists (AOAC) method (2016).

2.5.4   Determination of total phenolic content (TPC), total betacyanin

content (TBC), and antioxidant activity

The TPC, TBC, and ferric reducing antioxidant power (FRAP) of DFP were

determined according to previous studies (Phrueksanan, Yibchok-anun, &

Adisakwattana, 2014; Suantawee et al., 2015). The total phenolic content of DFP was

determined by the Folin–Ciocalteu method. DFP solution (0.01 g/mL, 50 µL) was

mixed with 50 µL of a 10-fold dilution of Folin–Ciocalteu's reagent and incubated for

5 min. Then, 50 µL of 10% (w/v) Na_2CO_3 was added to the solution and further

incubated for 60 min at room temperature. The absorbance was read at 750 nm. The results were expressed as mg of gallic acid equivalents (GAE) per gram of DFP powder.

Total betacyanin content in DFP powder was determined according to the previously described method by Tze et al. (2012). The DFP solution (10% *w/v* in distilled water) was sonicated for 1 h at room temperature. Then, the solution was centrifuged at 1904× *g* at 4 °C for 15 min. The supernatant was filtered through Whatman No. 1 filter paper. The sample solution was measured at 538 nm. The total betacyanin content was expressed as g of betanin equivalent per gram of DFP powder.

To make the fresh reagent for FRAP assay, 0.3 M acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution, 40 mM HCl, and 20 mM $FeCl_3$ were mixed in the following proportions: 10:1:1 (Benzie & Strain, 1996). DFP solution (10% *w/v* in distilled water, 10 μL) was mixed with 90 μL of FRAP reagent and incubated for 30 min at room temperature. The absorbance was read at 595 nm. The FRAP value of DFP was expressed in mmol $FeSO_4$ equivalent per gram of DFP powder.

2.5.5   Chemical properties of DFP pectin

The pectin content in DFP powder was extracted according to a previous study (Nguyen & Pirak, 2019). Five grams of DFP powder was mixed with 150 mL of distilled water and adjusted to pH 2.0. The solution was incubated at 75 °C for 1 h at 150 rpm. The solution was filtered and precipitated with an equal volume of 96%

(*v/v*) ethanol for 1 h. Then, the precipitated pectin was dried at 60 °C for 5 h. The extracted DFP pectin was stored at room temperature for further analysis.

The equivalent weight (EW) of DFP pectin was calculated according to the equation below:

$$EW \text{ (g/mol)} = \frac{\text{weight of sample (g)} \times 1000}{\text{Amount of alkali (mL)} \times \text{Normality of alkali}}$$

EW (g/mol) = weight of sample (g) × 1000/mL of alkali × Normality of alkali

The methoxyl content was calculated according to the equation below:

$$MeO \text{ (\%)} = \frac{\text{Amount of alkali (mL)} \times \text{Normality of alkali} \times 31 \times 100}{\text{Weight of sample (g)}}$$

where 31 is the methoxy group molecular weight.

The degree of esterification (DE) was determined by mixing DFP pectin powder (50 mg) with 10 mL of isopropanol (65%, *v/v*) and phenol red indicator. The solution was titrated with 0.1 N NaOH solution (A) to pink color. Then, a solution was added with 30 mL of 0.1 N NaOH and kept for 30 min, followed by 30 mL of 0.1 N HCl. The solution was further titrated with 0.1 N NaOH (B) to pH 7.5. DE was calculated by the following formula:

$$DE \text{ (\%)} = (B/A+B) \times 100$$

Total anhydrouronic acid (AUA) content in DFP pectin was calculated to determine the pectin purity by the following formula:

AUA (%) = ((176 × (0.1 × Z) × 100)/(W × 1000)) + ((176 × (0.1 × y) × 100)/(W × 1000))

where molecular unit of AUA (1 U) = 176 g; Z = volume of NaOH from equivalent weight determination; y = volume of NaOH from methoxyl content determination; W = weight (g) of sample.

2.5.6   Physicochemical properties of DFP

To determine the functional properties of DFP, oil holding capacity (OHC), water holding capacity (WHC), and swelling capacity (SC) were conducted following the previous study compared with sodium carboxymethyl cellulose (CMC) which is a commonly used food additive in the food industry (Siew Lian & Chong, 2015).

2.5.6.1   Oil holding capacity (OHC)

First, 250 mg of sample was mixed with 10 mL of rice bran oil. The solution was mixed for 1 min and left for 1 h at room temperature. Then, the sample was centrifuged at 1904× g for 5 min at room temperature. After removal of the supernatant, the sediment was weighed to calculate the OHC following the equation:

OHC (g of oil/g of sample) = weight of sediment (g) - weight of sample(g)

2.5.6.2   Water holding capacity (WHC)

Briefly, 250 mg of sample was placed in a centrifuge tube, and 10 mL of distilled water was added. The solution was mixed for 1 min and left for 1 h at room temperature. Then, the sample was centrifuged at 1904× g for 5 min. After removal of the supernatant, the sediment was weighed to calculate WHC using the following equation:

WHC (g of water/ g of sample) = weight of sediment (g) - weight of sample (g)

2.5.6.3   Swelling capacity (SC)

Two grams of DFP were weighed in a calibrated cylinder, and the initial volume (A) was recorded. Then, 100 mL of distilled water was added and left for 20 h at room temperature. The final volume (B) was calculated by using the following equation:

$$SC \text{ (mL of water/g of sample)} = (A - B)/\text{sample weight (g)}$$

2.5.7   In vitro starch digestion and starch fractions

The impact of DFP with potato (P), glutinous rice (G), rice (R), or wheat (W) flour on in vitro starch digestion and total starch content was performed as described in the previous studies (Goñi, Garcia-Alonso, & Saura-Calixto, 1997; Yousif, Nhepera, & Johnson, 2012). Prior to digestion, 500 mg of various types of flour, including potato flour, rice flour, glutinous rice flour, and wheat flour, were mixed with 5 mL of distilled water and heated for gelatinization. Then, 125, 250, and 500 mg of DFP were added to the gelatinized starch. The samples were then mixed with 1 mL of artificial saliva solution (250 U/mL porcine amylase in 0.2 M carbonate buffer, pH 7) and 5 mL of pepsin suspension (1 mg/mL) in 0.02 M HCl (pH 2). The mixtures were incubated for 1 h at 37 °C in a water bath shaker (100 rpm), followed by neutralization with 5 mL of 0.02 M NaOH and 25 mL of 0.2 M sodium acetate buffer (pH 6). The intestinal digestion was initiated by adding 5 mL of the enzyme mixtures (pancreatin (2 mg/mL) and amyloglucosidase (28 U/mL) to 0.2 M acetate buffer, pH 6. Next, the mixtures were further incubated at 37 °C, 100 rpm, and collected at 0, 20, 30, 60, 90, 120, and

180 min. The digesta fluid was immediately placed in an ice bath and centrifuged at 10,845× g for 15 min at 4 °C, then filtered through a 0.22 μm nylon filter to stop the reaction. The concentration of glucose was determined by Glucose LiquiColor® (HUMAN GmbH, Wiesbaden, Germany). The percentage hydrolysis index (HI) was calculated by dividing the area under the hydrolysis curve of each sample by the corresponding area of standard glucose.

Total starch content was performed by mixing 50 mg of samples with 6 mL of 2 M KOH and incubating at room temperature for 1 h. Then, 3 mL of 0.4 M sodium acetate buffer was added, and the pH was adjusted to 4.75 before adding 60 μL of amyloglucosidase (3260 U/mL) and incubating for 45 min at 60 °C in a water bath shaker (100 rpm). The mixture was heated at 100 °C and further centrifuged at 18,327× g, for 5 min, at 4 °C. The concentration of glucose was determined and converted into the starch fraction by multiplying with 0.9. The total starch (TS) was calculated from the described equations:

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

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

$$Undigested\ starch\ (\%) = (TS - (RDS + SDS))/TS \times 100$$

where G_0 = glucose content after 0 min of digestion; G_{20} = glucose content after 20 min of digestion; G_{120} = glucose content after 120 min of digestion; TS = total starch content.

2.5.8   Determination of total phenolic content (TPC), total betacyanin

content (TBC), and antioxidant activity during *in vitro* digestion

To investigate the impact of in vitro digestion on active compounds and

antioxidant activity of DFP with potato (P), glutinous rice (G), rice (R), or wheat (W)

flour. The digesta fluid at 0, 20, 30, 60, 90, 120, and 180 min was measured for total

phenolic, betacyanin content, and FRAP according to the above-mentioned protocol.

2.5.9   Thermal, pasting properties and Fourier transform infrared (FT-IR)

spectroscopy

The thermal, pasting characteristics and FTIR spectra of each type of flour

incorporated with DFP were investigated. The DFP–flour ratio at 250:500 was chosen

for the experiments according to the significant reduction of starch digestibility.

2.5.9.1   Thermal properties

The gelatinization parameters, including onset temperature (T_o), peak

temperature (T_p), the temperature at the conclusion (T_c), and gelatinization enthalpy

(ΔH), were recorded to investigate the impact of DFP on starch chains dissociating

and the granules lost in all flours during gelatinization by differential scanning

calorimetry (Netzsch DSC 204F1 Phoenix, Selb, Germany). Three milligrams (dry basis)

of the sample were suspended with 10 µL of deionized distilled water and put into

the sample pan and hermetically sealed. Then, it was allowed to stand for 1 h at

room temperature for equilibration. The heating temperature was raised from 25 ℃

to 100 ℃ at a rate of 10 ℃/min. An empty aluminum pan was used as the reference.

2.5.9.2 *Pasting properties*

The pasting profile of flours, including peak viscosity (PV), trough, breakdown (BD), final viscosity (FV), setback, and pasting temperature with or without 250 mg of DFP were evaluated by using a Rapid Visco Analyzer (RVA 4500, Newport Scientific Instrument, Newport, MN, USA). Three grams of the sample was dissolved in 25 mL of DW. The solution was heated at a rate of 12 °C per min from room temperature to 95 °C. After holding the sample at 95 °C for 2–3 min, it was cooled to 50 °C at a rate of 12 °C/min.

2.5.9.3 *Fourier transform infrared (FT-IR) spectroscopy*

To examine the effect of DFP on starch-ordered structure in all flours, the FT-IR spectra of samples were recorded using a Nicolet™ iS™50 spectrometer equipped with a Smart iTR™ diamond ATR concave tip (Thermo Fisher Scientific, Waltham, MA, USA) according to the previous study (Xiao et al., 2021).

2.5.10 Statistical analysis

The results were expressed as means ± standard error of mean (SEM) with three replicated determinations for each treatment group (n = 3). The area under the curves (AUCs) was calculated using the trapezoid rule. Data were analyzed by independent sample t-tests or one-way ANOVA, followed by Duncan's post hoc test. The statistically significant difference was defined as $p < 0.05$ among treatments. Statistical analysis was performed using IBM SPSS version 22.0 (International Business Machines Corporation, Armonk, NY, USA).

2.6 Results and Discussion

2.6.1   Proximate compositions, bioactive compounds, and antioxidant

activity of DFP

In this study, DFP was mainly carbohydrates (70.85%), which consisted of

65.17% dietary fibers and 5.68% of available carbohydrates. The other components

were followed by 15.91% ash, 6.37% protein, and 5.81% moisture, while total fat was

the lowest component (1.06%). TPC and TBC in DFP were 454.79 ± 18.72 mg of gallic

acid equivalent/g powder and 335.34 ± 2.26 mg of betanin equivalent/g powder,

respectively. In terms of antioxidant activity, the FRAP value of DFP was 49.30 ± 0.10

mmol $FeSO_4$ equivalent/g powder. The findings, particularly dietary fibers, were

lowered, while TPC and TBC were higher than those reported in red dragon fruit peel

because of the species, cultivation, maturity, and preparation method (Bakar et al.,

2011; Phrueksanan et al., 2014). Previous research indicates that cellulose and lignin

were major fractions of insoluble dietary fiber in dragon fruit peel, while pectin and

mucilage were also detected as soluble dietary fiber (Bakar et al., 2011; Jalgaonkar,

Mahawar, Bibwe, & Kannaujia, 2020). Furthermore, 17 types of betacyanins, mainly

betanin, gallic acid, chlorogenic acid, syringic acid, and ferulic acid, were

phytochemical compounds obtained from DFP, which exhibited strong free radical

scavenging capacities toward FRAP, ABTS, and DPPH (Jiang et al., 2021).

2.6.2   Pectin and physicochemical properties of DFP

The yield of pectin from the extraction was 10.37% of DFP. As shown in Table 1,
the average EW of DFP pectin was 397.63 ± 1.51 g/mol, relating to the gel strength of
pectin. DFP pectin contained 5.22 ± 0.11% MeO and 46.90 ± 3.03% DE, classified as
low methoxyl pectin (DE ≤ 50%), whereas the total AUA content of DFP pectin was
79.45 ± 0.53%. Interestingly, DFP has a lower EW pectin level and a more significant
proportion of apple peel than MeO, DE, and AUA (Virk & Sogi, 2004). According to the
Food Chemicals Codex (2016), DFP may be advantageous for food applications
because it contains a high purity of pectin, which can form a gel in the absence of
sugar (IPPA, 2014; Siew Lian & Chong, 2015). Regarding physicochemical properties,
DFP had 1.67-fold and 1.74-fold higher WHC, and SC values compared to
carboxymethyl cellulose (CMC), respectively. It suggests that the higher hydration
properties of DFP may be attributed to the presence of insoluble fibers, which
improve the syneresis, texture, and viscosity of food products (Wang et al., 2015).
However, there were no significant differences in OHC levels between DFP and CMC.

Table 1 Pectin chemical property and physicochemical property of dragon fruit peel powder (DFP).

Experiments	CMC	DFP
Pectin chemical property		
EW (g/mol)	N.A.	397.63 ± 1.51
MeO (%)	N.A.	5.22 ± 0.11
DE (%)	N.A.	46.90 ± 3.03
AUA (%)	N.A.	79.45 ± 0.53
Physicochemical property		
OHC (g of oil/g of sample)	$1.63 ± 0.06^a$	$1.93 ± 0.03^a$
WHC (g of water/g of sample)	$5.64 ± 0.16^a$	$9.44 ± 0.19^b$
SC (mL of water/g of sample)	$2.63 ± 0.06^a$	$4.60 ± 0.03^b$

Data are expressed as mean ± SEM, n = 3. Means with different superscripts are significantly different ($p < 0.05$). EW—equivalent weight; MeO—methoxyl content; DE—degree of esterification; AUA—total anhydrouronic acid; OHC—oil holding capacity; WHC—water holding capacity; SC—swelling capacity; CMC—sodium carboxymethyl cellulose; N.A.—not analyzed.

2.6.3   Effect of DFP on *in vitro* starch digestion, hydrolysis index, and starch fraction

The amount of glucose released from all types of flour at different concentrations of DFP was reported as the area under the curves (AUC) during simulated gastrointestinal digestion (Table 2). The results showed that the AUC glucose of the mixture of DFP and each type of flour was lower than that of the control, indicating that the addition of DFP (500 mg) remarkably suppressed the starch digestibility. The greatest suppressing effect of DFP was observed when incorporated with potato flour (38.5%), followed by glutinous rice flour (33.5%), rice flour (24.4%), and wheat flour (25.5%). In addition, the percentage of hydrolysis index (HI) of potato, rice, glutinous rice, and wheat flour significantly decreased when incorporated with DFP (125–500 mg) in a concentration-dependent manner (Figure 1). According to the proximate analysis, dietary fibers in DFP, a major component, may play a role in interfering with the starch digestion process through trapping starch granules, increasing viscosity, and diminishing enzyme accessibility (Qi, Al-Ghazzewi, & Tester, 2018). Moreover, betacyanins in DFP, especially betanin, could inhibit carbohydrate digestive enzymes, including α-amylase and α-glucosidase, as supported by a previous study of betacyanin-rich beetroot juice (Oboh, Obayiuwana, Aihie, Iyayi, & Udoh, 2021). The interactions of phenolic compounds with starch granules cause the formation of amylose single helices or complexes through hydrogen bonds, leading to a reduction in starch digestibility and HI (Sun & Miao, 2020). Bae et al. (2016) reported that the incorporation of apple peel dietary fibers (mainly insoluble

dietary fibers) into wheat flour decreased the starch hydrolysis, corresponding to the reduction of RDS and SDS, whereas the undigestible starch content increased. These results are consistent with our findings that the addition of DFP (500 mg) significantly decreased RDS (3.1–13.9%) and SDS (6.1–13.6%) of all types of flour (Figure 2). The percentage of undigested starch increased in the flour added with DFP (500 mg) by 20.6% for potato flour, 8.9% for glutinous rice flour, 21.2% for rice flour, and 5.2% for wheat flour. From this nutritional point of view, the decrease in RDS and SDS, concomitant with the increase in undigested starch, is considered an effective way to control postprandial glycemic and insulinemic responses, leading to a lower risk of chronic degenerative illnesses such as type 2 diabetes and obesity (Magallanes-Cruz, Flores-Silva, & Bello-Perez, 2017).
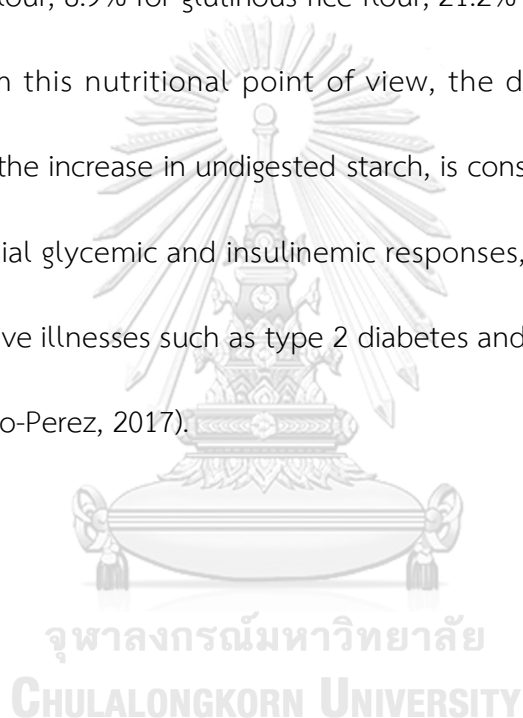
Table 2 The effect of the addition of dragon fruit peel powder at 125 (DFP125), 250 (DFP250), and 500 (DFP500) mg on the area under the curve (AUC) of released glucose (AUC$_{Glucose}$), total phenolic content (AUC$_{TPC}$), total betanin content (AUC$_{TBC}$), and ferric reducing antioxidant power (AUC$_{FRAP}$) of 500 mg of potato flour (P), glutinous rice flour (G), rice flour (R), and wheat flour (W) during *in vitro* starch digestion.

Experiments	AUC$_{Glucose}$	AUC$_{TPC}$	AUC$_{TBC}$	AUC$_{FRAP}$
P	8900.6 ± 82.5 [a]	208.1 ± 9.0 [a]	N.D.	241.4 ± 13.8 [a]
P + DFP125	8815.9 ± 46.6 [a]	334 ± 10.1 [b]	4250.3 ± 28.8 [a]	858.8 ± 14.6 [b]
P + DFP250	8520.9 ± 62.6 [b]	394.9 ± 4.2 [c]	8878.2 ± 28.7 [b]	1350.0 ± 19.1 [c]
P + DFP500	5474.2 ± 34.0 [c]	643.6 ± 16.5 [d]	14,545.4 ± 32.6 [c]	2146.2 ± 20.8 [d]
G	7551.1 ± 111.5 [a]	236.3 ± 3.2 [a]	N.D.	307.4 ± 15.3 [a]
G + DFP125	5597.8 ± 88.7 [b]	349.6 ± 2.5 [b]	4045.1 ± 6.6 [a]	1199.4 ± 50.7 [b]
G + DFP250	5405.5 ± 78.6 [b]	466.1 ± 1.6 [c]	7464.8 ± 18.9 [b]	1990.9 ± 50.4 [c]
G + DFP500	5020.2 ± 78.0 [c]	699.6 ± 4.1 [d]	15,363.8 ± 16.3 [c]	3447.7 ± 29.5 [d]
R	8261.6 ± 27.3 [a]	209.6 ± 10.0 [a]	N.D.	155.7 ± 27.5 [a]
R + DFP125	7989.2 ± 47.1 [b]	303.7 ± 2.3 [b]	3705.3 ± 20.8 [a]	1069.5 ± 11.1 [b]
R + DFP250	7179.2 ± 46.8 [c]	414.8 ± 3.2 [c]	6984 ± 10.0 [b]	1887.2 ± 20.7 [c]
R + DFP500	6244.2 ± 15.0 [d]	606.4 ± 4.6 [d]	13,490 ± 26.7 [c]	3270.9 ± 48.1 [d]
W	7684.8 ± 42.8 [a]	320.6 ± 2.7 [a]	N.D.	370.0 ± 41.8 [a]
W + DFP125	6884.4 ± 28.1 [b]	392.3 ± 2.5 [b]	4011.6 ± 15.0 [a]	1218.4 ± 14.3 [b]
W + DFP250	6001.2 ± 19.0 [c]	481.4 ± 6.0 [c]	8024.5 ± 13.5 [b]	2007.7 ± 18.1 [c]
W + DFP500	5478.8 ± 87.2 [d]	632.7 ± 5.7 [d]	15,818.4 ± 7.4 [c]	3217.8 ± 45.7 [d]

The results are expressed as mean ± SEM, n = 3. Means with different superscripts are significantly different ($p < 0.05$). AUC$_{glucose}$—g of glucose/100 g of sample × min; AUC$_{TPC}$—mg of gallic acid equivalent/total volume of digesta × min; AUC$_{TBC}$—mg of betanin equivalent/total volume of digesta × min; AUC$_{FRAP}$—mmol FeSO$_4$/total volume of digesta × min; N.D.—not detected.
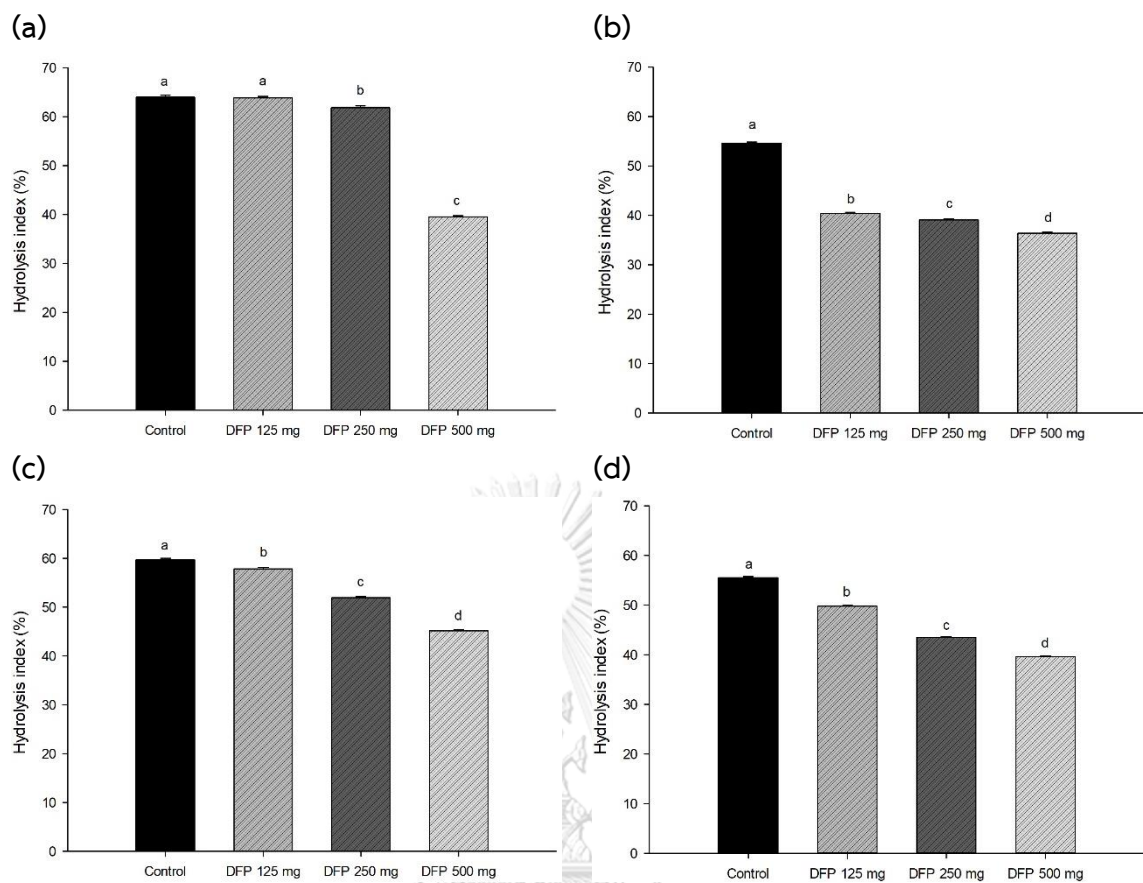
Figure 1 The effect of DFP addition on the percentage of hydrolysis index of (a) potato flour, (b) glutinous rice flour, (c) rice flour, and (d) wheat flour in combination with the different concentrations of DFP (125, 250, and 500 mg).

The results are expressed as mean ± SEM, n = 3. Means with different superscripts are significantly different ($p < 0.05$).
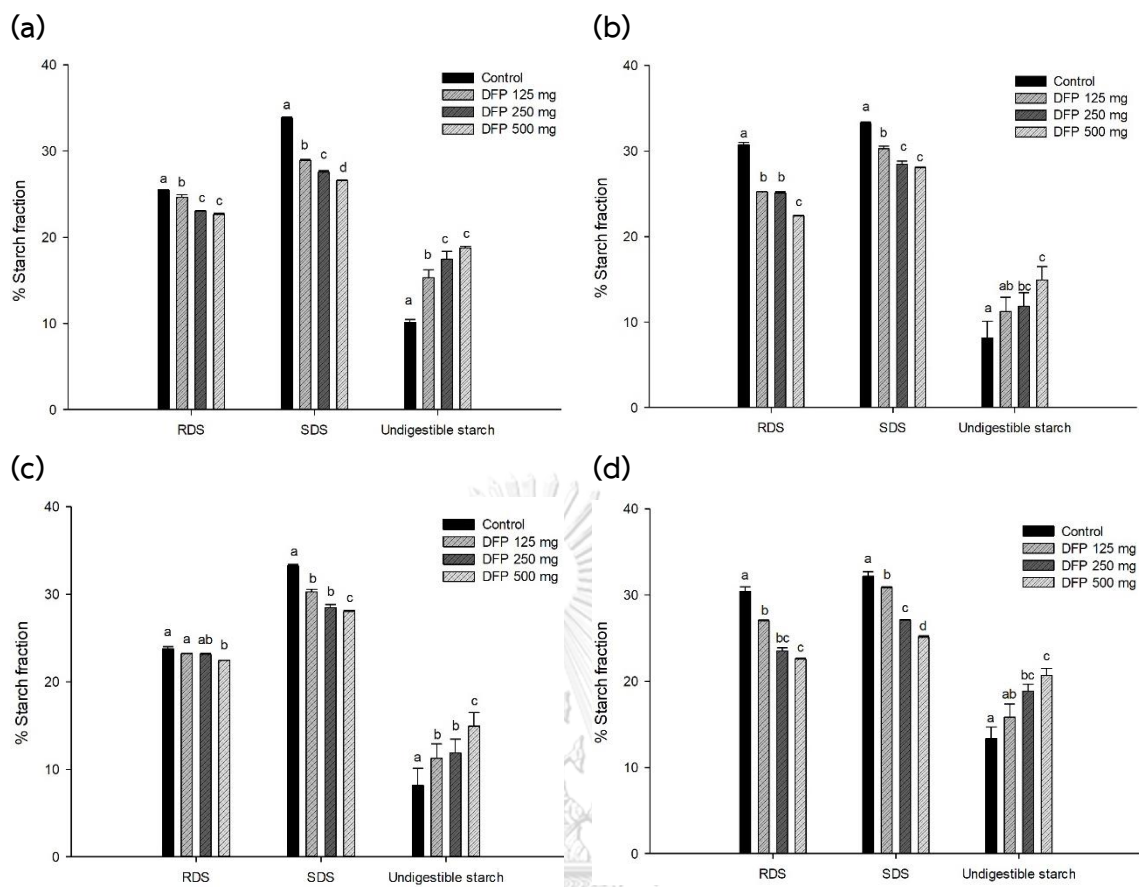
Figure 2 The effect of DFP addition on the percentage of starch fraction of (a) potato flour, (b) glutinous rice flour, (c) rice flour, and (d) wheat flour in combination with the different concentrations of DFP (125, 250, and 500 mg).

The results are expressed as mean ± SEM, n = 3. Means with different superscripts are significantly different ($p < 0.05$). RDS—rapidly digestible starch; SDS—slowly digestible starch.

2.6.4   Effect of DFP on TPC, TBC, and antioxidant activity during *in vitro* digestion

The effects of DFP on TPC, TBC, and antioxidant activity of flours are shown in Figure 3, Figure 4 and Figure 5. The results showed that TPC, TBC, and antioxidant activity (FRAP) were detected in all flours and remained unchanged in intestinal digestion (0–180 min). Interestingly, DFP exhibited a significant increase in antioxidant activity and the release of TPC and TBC when added to flour. As presented in Table 2, the addition of DFP (125–500 mg) to different flours enhanced the increase in the AUC_{TPC} and AUC_{TBC} during simulated digestion in a concentration-dependent manner. Consistent with TPC and TBC, the antioxidant activity of flours presented by the AUC_{FRAP} was significantly higher in the presence of DFP when compared to the flour control.

In general, the food matrix containing various types of starch significantly influences the bioaccessibility and bioavailability of bioactive compounds and their biological activity. Our results indicate that bioactive compounds (total phenolic compounds and betacyanins) in DFP are highly bioaccessible and remained intact after simulated starch digestion, contributing to the antioxidant activity. This finding is consistent with the previous study indicating that betacyanins from red dragon fruit were retained after simulated gastrointestinal digestion and demonstrated their antioxidant activity (Choo, Ong, Lim, Tan, & Ho, 2019). Next, we investigated the correlation between the effects of DFP on the reduction of glucose released from flour, TPC, TBC, and antioxidant activity after simulated digestion (as shown in Figure

3, Figure 4 and Figure 5). As shown in Table 3, the $AUC_{Glucose}$ showed a significantly negative correlation with the AUC_{TPC}, AUC_{TBC}, and AUC_{FRAP} (r = −0.786, −0.688, and −0.755, respectively). Interestingly, the AUC_{TPC} and AUC_{TBC} were significantly positively correlated with the AUC_{FRAP}. These results support the notion that phenolic compounds and betanin released during *in vitro* simulated digestion may play a vital role in antioxidant activity and suppressing starch digestibility (Abedimanesh et al., 2021; Sun & Miao, 2020).
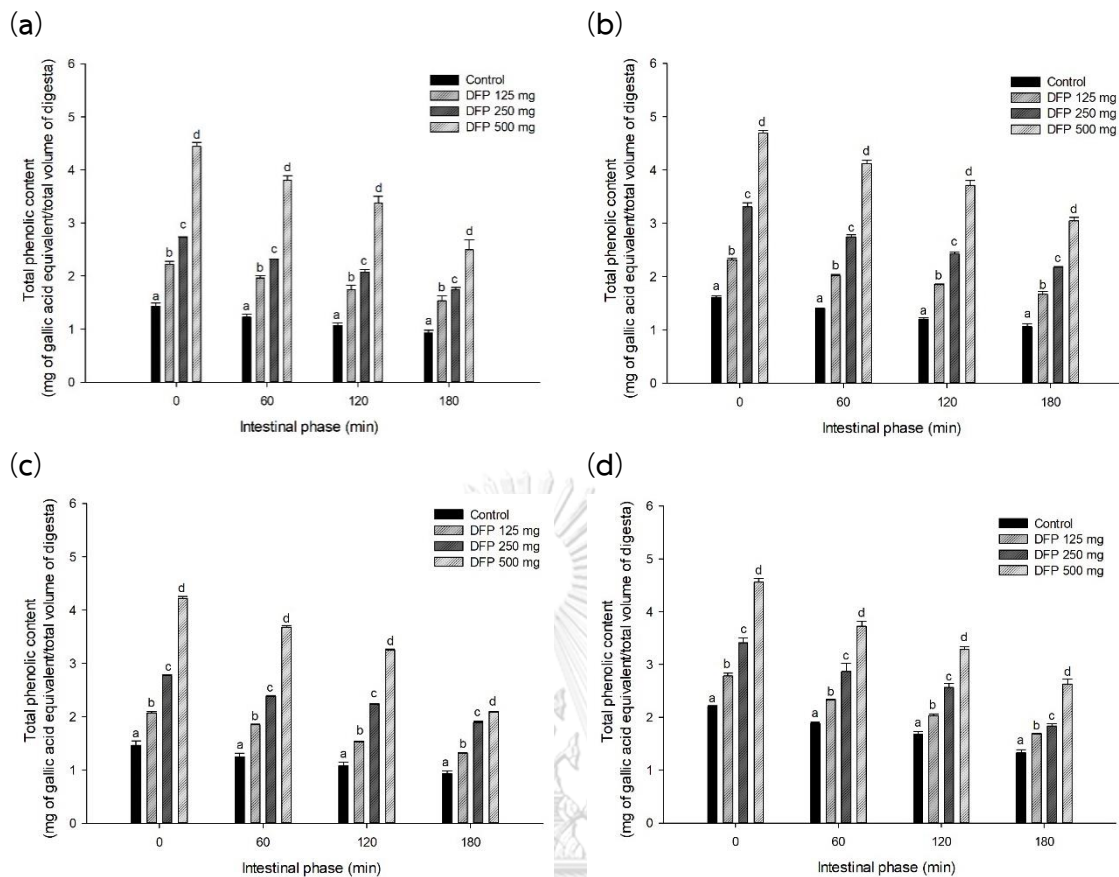
Figure 3 The release of total phenolic content (TPC) of (a) potato flour, (b) glutinous rice flour, (c) rice flour, and (d) wheat flour in combination with the different concentrations of DFP (125, 250, and 500 mg) during *in vitro* digestion. The results are expressed as mean ± SEM, n = 3. Means with different superscripts are significantly different ($p < 0.05$).
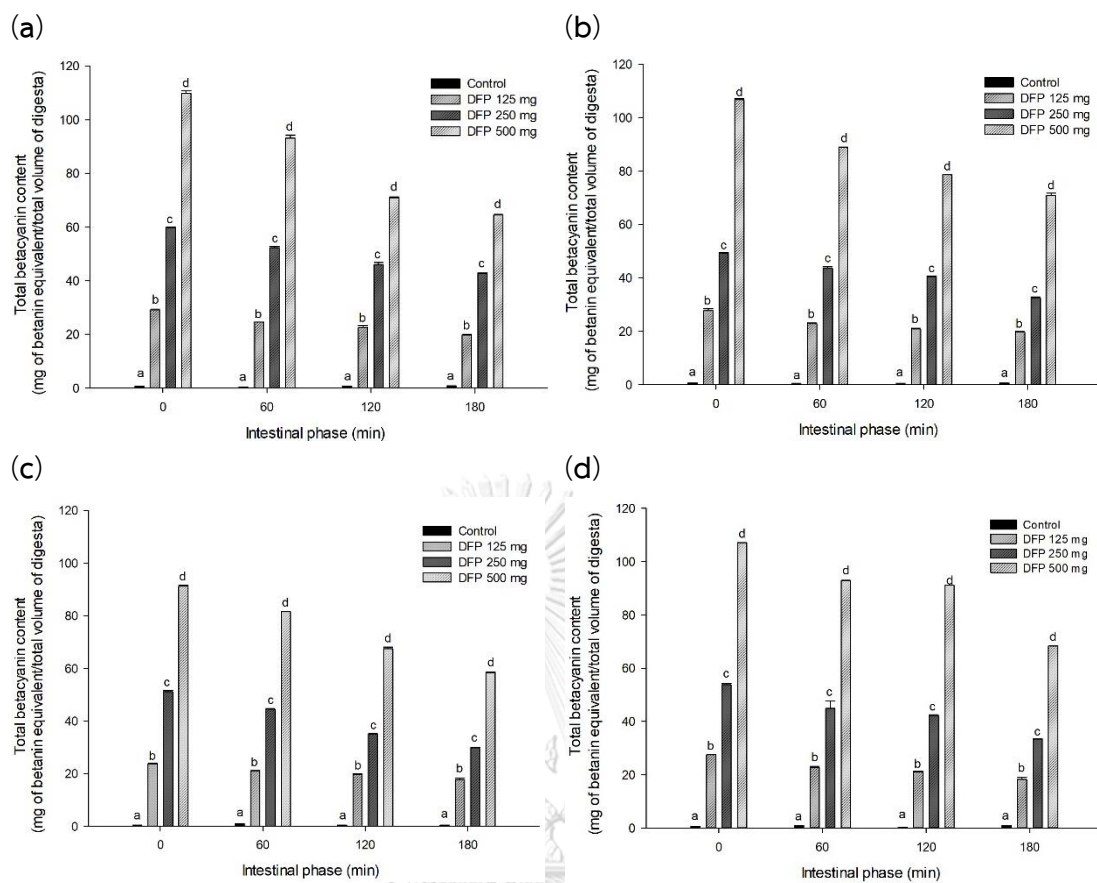
Figure 4 The release of total betacyanin content (TBC) of (a) potato flour, (b) glutinous rice flour, (c) rice flour, and (d) wheat flour in combination with the different concentrations of DFP (125, 250, and 500 mg) during *in vitro* digestion.

The results are expressed as mean ± SEM, n = 3. Means with different superscripts are significantly different (*p* < 0.05).
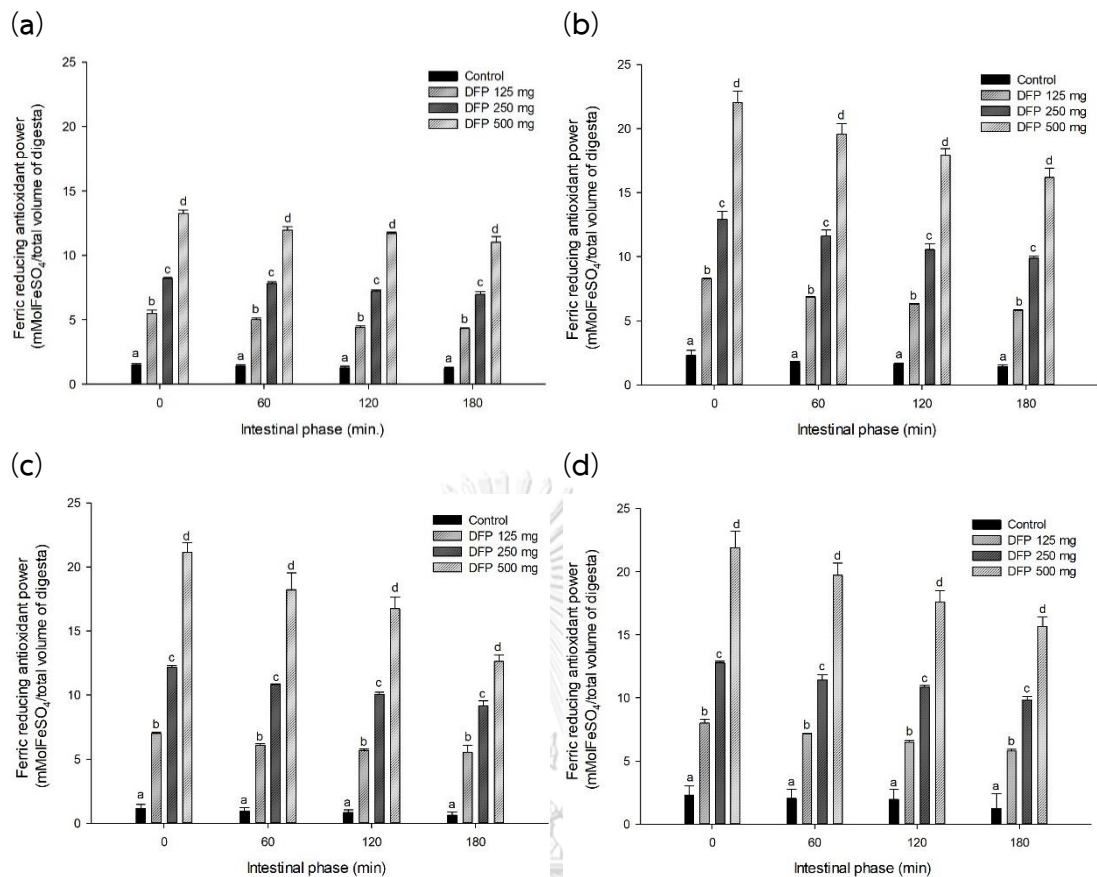
Figure 5 The ferric reducing antioxidant power (FRAP) of (a) potato flour, (b) glutinous

rice flour, (c) rice flour, and (d) wheat flour in combination with the different

concentrations of DFP (125, 250, and 500 mg) during *in vitro* digestion.

The results are expressed as mean ± SEM, n = 3. Means with different superscripts

are significantly different (p < 0.05).

Table 3 Pearson correlation coefficients between dragon fruit peel powder (DFP) and the area under the curve (AUC) of released glucose ($AUC_{Glucose}$), total phenolic content (AUC_{TPC}), total betanin content (AUC_{TBC}), and ferric reducing antioxidant power (AUC_{FRAP}) during *in vitro* starch digestion.

	$AUC_{Glucose}$	AUC_{TPC}	AUC_{TBC}	AUC_{FRAP}
$AUC_{Glucose}$	1	−0.786 *	−0.688 *	−0.755 *
AUC_{TPC}		1	0.965 *	0.948 *
AUC_{TBC}			1	0.948 *
AUC_{FRAP}				1

* Significant correlation at $p < 0.01$. Correlations were done using the average value for each treatment, n = 3, in a mixture of various types of flour (potato, glutinous rice, rice, and wheat flour).

2.6.5   Effect of DFP and various types of flour on thermal properties

The thermal parameters of DFP (250 mg) with different flours are summarized in Table 4. The onset temperature (T_o) and peak temperature (T_p) of potato, glutinous rice, and wheat flour mixed with DFP were significantly increased, except for rice flour. Furthermore, the conclusion temperature (T_c) of flours mixed with DFP also increased significantly. The gelatinization enthalpy (ΔH) of potato, glutinous rice, rice, and wheat flour was lowered by 2.38, 1.19, 2.22, and 1.88 times, respectively, when DFP was added. The gelatinization enthalpy is an essential thermal energy that causes the swelling, crystallite melting, and solubilization of starch granules (S. Wang & Copeland, 2013). Soluble dietary fibers have been shown to reduce gelatinization enthalpy, resulting in the limitation of the susceptibility of starch digestibility (Sevilmis & Sensoy, 2022). In this study, DFP demonstrates a high-water holding capacity which competes for available water with starch granules and disrupts the melting crystalline structures of starch granules. Consequently, this effect causes interference with starch granule swelling and increases the amount of ungelatinized starch (Zhao et al., 2021).

The pasting properties of flour incorporated with DFP are shown in Table 5. The pasting properties of potato, glutinous rice, rice, and wheat flour were significantly altered by adding DFP (250 mg). Moreover, DFP markedly increased the peak viscosity (PV), trough viscosity, final viscosity (FV), and breakdown viscosity (BD) of flours.

In agreement with a previous study, the high hydration property of DFP may compete to attach water with starch granules, causing the rupture of starch granules

and hindering the release of amylopectin, which is linked to interfering with the formation of starch gel and chain network (Yildiz et al., 2013). In addition, the setback viscosity of the flour incorporated with DFP was higher than that of the control flour. The results indicate that DFP alters starch's solubility, gel formation, and retrogradation, potentially reducing its structure and digestibility (S. Wang & Copeland, 2013).

Moreover, the pasting temperature of all flours was significantly decreased after DFP incorporation. These results suggest that dietary fibers in DFP interfere with flour's gelatinization and heat stability (Phimolsiripol, Siripatrawan, & Henry, 2011).

The interaction between various types of flour and DFP observed by the Fourier transform infrared spectrometer (FT-IR) is displayed in Figure 6. The essential characteristic peaks of flour were characterized in this study, including stretching vibrations of the O-H bond (3200–3300 cm^{-1}), the C-H bond (2900–3000 cm^{-1}), the C=O bond, and water absorption in the amorphous region (1640 cm^{-1}). Following the addition of DFP (250 mg), the characteristic peaks in all flours were shifted to lower wavenumbers, indicating the interaction between starch and DFP. These interactions were also observed when mixing phenolic compounds, proanthocyanidins, and soluble dietary fiber with flour, causing a reduction in starch digestibility (Xiao et al., 2021; Zhang, Zeng, Wang, He, & Wang, 2020). In general, the ratio of the bands at 1047, 1022, and 1000 cm^{-1} represents crystalline and amorphous structures in starch, which refer to amylose and amylopectin content and affect the starch digestibility.

For example, higher amylose content and a lower proportion of amylopectin in rice starches have been associated with reduced susceptibility to enzymatic hydrolysis, providing higher resistance to enzymatic digestion (Sasaki et al., 2009; Warren, Gidley, & Flanagan, 2016). In the present study (Table 5), adding DFP into flour decreased the ratio of 1047/1022 cm^{-1} while increasing the ratio of 1022/1000 cm^{-1}. The results suggest that phytochemical compounds and dietary fibers in DFP may interfere with the melting of the crystalline region by strengthening the amylose chain (amorphous part) and reducing hydrogen bonds, leading to slow digestibility flours (Chai, Wang, & Zhang, 2013; Xiong, Li, Shi, & Ye, 2017). However, further study is needed to clarify the impact of DFP on amylose content in all flours.
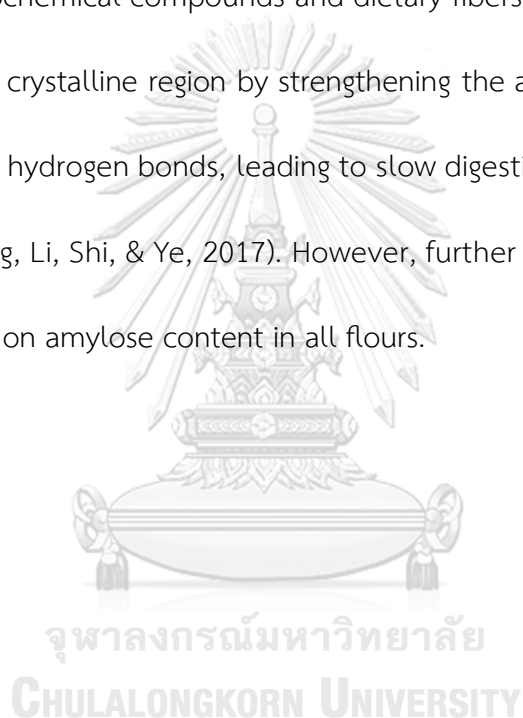
Table 4 The effect of dragon fruit peel powder (DFP) on thermal properties of potato flour (P), glutinous rice flour (G), rice flour (R), and wheat flour (W).

Experiments	Thermal Properties			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
P	64.85 ± 0.35	71.75 ± 0.55	79.95 ± 0.45	11.81 ± 0.10
P + DFP250	68.90 ± 0.10 *	76.75 ± 0.15 *	83.65 ± 0.65 *	4.96 ± 0.13 *
G	63.50 ± 1.30	69.10 ± 0.40	74.15 ± 0.45	5.47 ± 1.52
G + DFP250	66.80 ± 0.40 *	74.50 ± 0.10 *	79.30 ± 0.50 *	4.60 ± 1.58
R	67.15 ± 2.95	74.75 ± 2.35	82.05 ± 0.25	8.02 ± 0.17
R + DFP250	66.70 ± 0.00	72.50 ± 0.50	86.10 ± 0.50 *	3.61 ± 0.96 *
W	57.35 ± 0.25	63.70 ± 0.30	69.60 ± 0.30	5.93 ± 0.18
W + DFP250	61.55 ± 0.05 *	68.00 ± 1.00 *	72.50 ± 1.40 *	3.15 ± 0.27 *

The results are expressed as mean ± SEM, n = 3. * $p < 0.05$ when compared to the control flour without DFP. T_o—onset temperature; T_p—peak temperature; T_c—conclusion temperature; ΔH—enthalpy gelatinization.

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Table 5 The effect of dragon fruit peel powder (DFP) on pasting properties and FTIR ratio of potato flour (P), glutinous rice flour (G), rice flour (R). and wheat flour (W).

Experiments	Thermal Properties						FTIR Ratio	
	PV (RVU)	Trough (RVU)	BD (RVU)	FV (RVU)	Setback (RVU)	PT (°C)	1047/1022 cm−1	1022/1000 cm−1
P	267.08 ± 1.25	87.20 ± 0.13	179.88 ± 1.13	128.71 ± 0.04	41.50 ± 0.08	71.83 ± 0.08	1.107	1.024
P + DFP250	573.17 ± 1.42 *	287.42 ± 1.50 *	285.75 ± 0.08 *	395.79 ± 4.04 *	108.38 ± 5.55 *	70.50 ± 0.40 *	1.103	1.042
G	387.13 ± 2.83	254.75 ± 1.58	132.38 ± 0.79	318.88 ± 1.05	64.13 ± 0.55	71.78 ± 0.02	1.151	0.890
G + DFP250	521.77 ± 0.01 *	403.96 ± 2.21 *	117.79 ± 2.21 *	584.25 ± 2.17 *	180.30 ± 4.38 *	66.48 ± 0.48 *	1.084	1.174
R	156.13 ± 0.21	149.21 ± 0.13	6.92 ± 0.09	259.25 ± 0.58	110.04 ± 0.46	90.05 ± 0.35	1.167	0.905
R + DFP250	456.09 ± 2.84 *	433.08 ± 3.50 *	23.00 ± 0.67 *	604.25 ± 1.67 *	171.17 ± 1.83 *	70.53 ± 0.38 *	1.122	1.159
W	108.25 ± 0.92	66.63 ± 0.70	41.63 ± 0.20	151.88 ± 0.21	85.25 ± 0.92	88.83 ± 0.02	1.140	1.005
W + DFP250	456.50 ± 1.00 *	373.42 ± 2.34 *	83.09 ± 1.34 *	553.96 ± 2.04 *	180.54 ± 0.29 *	58.85 ± 0.45 *	1.078	1.044

The results are expressed as mean ± SEM, n = 3. * $p < 0.05$ when compared to the plain flour. PV—peak viscosity; BD—breakdown value; FV—final viscosity; PT—pasting temperature; RVU—rapid visco unit
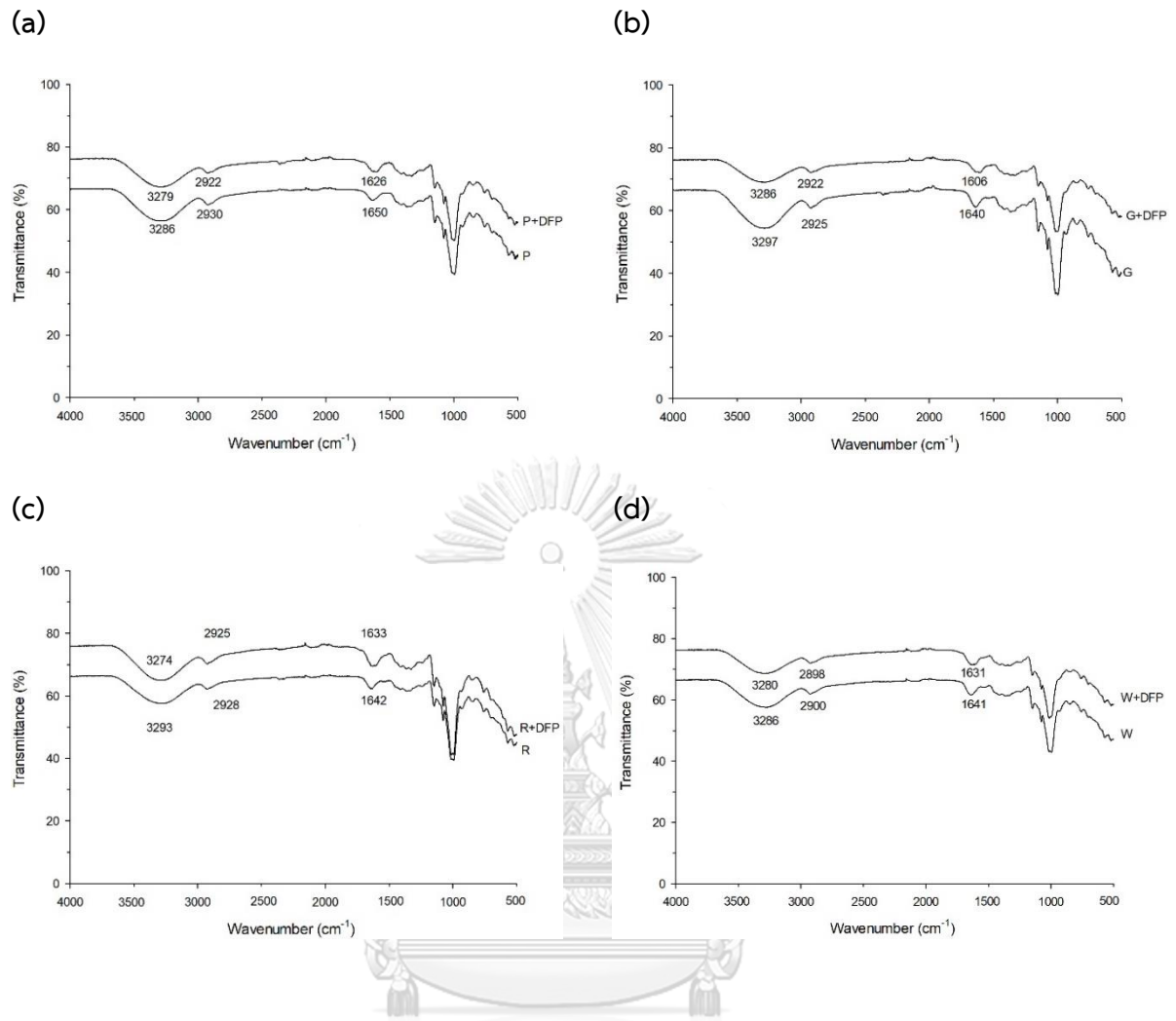
Figure 6 Fourier transform infrared spectrometer (FT-IR) spectra of (a) potato flour,

(b) glutinous rice flour, (c) rice flour, and (d) wheat flour in combination with 250 mg

the dragon fruit peel powder (DFP).

2.7 Conclusions

DFP powder made from the by-product of white pulp dragon fruit consumption contains dietary fibers together with phytochemical compounds and antioxidant activity. DFP significantly decreased starch digestibility of flour with an increase in undigested starch and higher antioxidant activity. In addition, it altered flours' gelatinization enthalpy and pasting properties through interference with crystalline and amorphous structures in starch. These findings would expand the scope of DFP applications and provide a knowledge base of DFP waste for developing flour-based products.
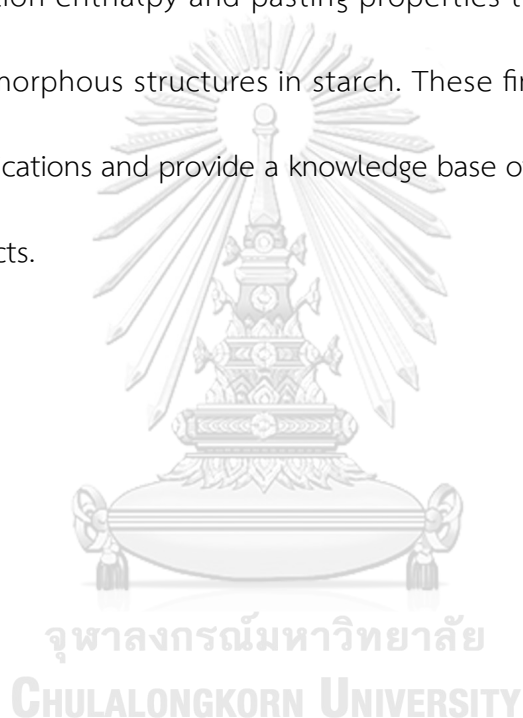
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CHAPTER III

DRAGON FRUIT PEEL WASTE (*HYLOCEREUS UNDATUS*) AS A POTENTIAL INGREDIENT FOR REDUCING LIPID PEROXIDATION, DIETARY ADVANCED GLYCATION END PRODUCTS, AND STARCH DIGESTIBILITY IN COOKIES

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3.2 Article status

3.3 Abstract

Excessive consumption of cookies has been linked to harmful health outcomes owing to the presence of refined carbohydrates and heat-induced toxicants including end products of lipid peroxidation and dietary advanced glycation end products (dAGEs). To address this issue, this study explores the addition of dragon fruit peel powder (DFP), which is rich in phytochemicals and dietary fibers, to cookies as a potential solution to mitigate their adverse effects. The results indicate that adding DFP at 1%, 2%, and 5% *w/w* of raw cookie dough significantly improves the total phenolic and betacyanin contents and antioxidant activity, as evidenced by increased ferric reducing antioxidant power. DFP incorporation also led to reductions in malondialdehyde and dAGEs ($p < 0.05$). Furthermore, the starch digestibility, hydrolysis index, and predicted glycemic index were all reduced in the presence of DFP, with the latter estimate being due to the higher content of undigested starch. Incorporating DFP in cookies resulted in significant changes in their physical properties, including texture and color. However, sensory evaluation indicates that the overall acceptability of the cookies was not negatively impacted by the addition of up to 2% DFP, suggesting that it is a viable option for enhancing the nutritional value of cookies without compromising their palatability. These findings suggest that DFP is a

sustainable and healthier ingredient that can improve the antioxidant capacity of cookies while also mitigating the harmful effects of heat-induced toxins.

Keywords: dragon fruit peel; cookie; starch digestion; advanced glycation end products.
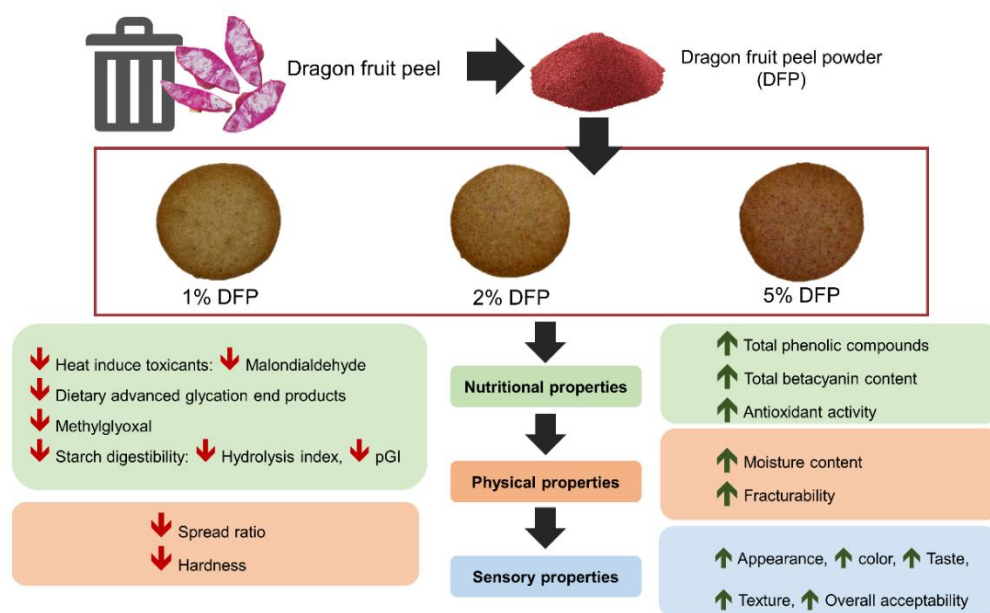
Figure 7 Graphical Abstract

3.4 Introduction

Bakery products, including cookies, are a popular food choice worldwide because of their accessibility and delicious taste. Nevertheless, their nutritional value has been extensively researched, revealing certain concerns (Arribas-Lorenzo & Morales, 2010; Carnell, Benson, Gibson, Mais, & Warkentin, 2017). Moreover, their high content of refined carbohydrates, simple sugars, and fat, combined with low dietary fiber content, poses a significant risk to human health (Ning et al., 2021). In addition, cookies can undergo non-enzymatic reactions and oxidation during baking, which can

lead to the formation of glycated molecules (Kuzan, 2021). These molecules can then undergo further chemical transformations, resulting in the production of toxic lipid peroxidation products (such as malondialdehyde (MDA)), and dietary advanced glycation end products (known as dAGEs) (Arribas-Lorenzo & Morales, 2010; Kuzan, 2021; Nowotny, Schröter, Schreiner, & Grune, 2018). Remarkably, consuming cookies with a high glycemic index and heat-generated toxicants has been linked to the development of hyperglycemia and increased levels of circulating oxidized low-density lipoprotein, MDA, and AGEs in human serum and tissues (Eder, Keller, Hirche, & Brandsch, 2003; Nowotny et al., 2018). These harmful compounds can accelerate the progression of several chronic diseases such as inflammation, cancer, atherosclerosis, diabetes, and glomerulosclerosis (Eder et al., 2003; Nowotny et al., 2018; Sergi, Boulestin, Campbell, & Williams, 2021). To address this issue, minimizing the glycemic index and decreasing the formation of MDA and dAGEs in cookies can help alleviate their potential negative impact on human health.

In recent years, researchers have emphasized the significance of managing the glycemic index and mitigating the formation of heat-induced toxicants, such as lipid peroxidation products and dAGEs, in food formulations. One promising strategy to attain these objectives is by reformulating food products using dietary fibers and phytochemicals derived from plants, which possess potent antioxidant properties (Ning et al., 2021; Wang et al., 2022; Zhang, Chen, & Wang, 2014). For example, cookies fortified with apple flowers have been shown to effectively prevent the

formation of reactive dicarbonyl compounds, such as methylglyoxal (MGO), and fluorescent AGEs, owing to the presence of flavonoids that exhibit remarkable scavenging and metal-chelating capabilities. These findings provide a plausible explanation for the observed MGO-scavenging and antiglycative properties of the cookies in both fructose and glucose BSA models (Gao, Sun, Li, Zhou, & Wang, 2020; Song, Liu, Dong, Wang, & Zhang, 2021). In addition, the inclusion of dietary fibers and green tea polyphenols has been found to significantly reduce the levels of available glucose and acrylamide content in baked starchy food models (Torres, Dueik, Carré, & Bouchon, 2019). The mechanism behind the decrease in starch digestibility can be attributed to the modification of starch granule microstructure, induced by green tea polyphenols, whereas dietary fibers create a physical barrier that impedes the access of amylolytic enzymes (Dikeman, Murphy, & Fahey, 2006; Xiao et al., 2011). These findings highlight the potential of using natural ingredients as an alternative approach to enhance the nutritional value of cookies while mitigating their potentially harmful effects on human health.

Dragon fruit, scientifically named *Hylocereus undatus*, is a tropical fruit crop widely grown and consumed in Thailand. Although the flesh of the dragon fruit provides a significant source of vitamins, minerals, and dietary fibers, the peel, which accounts for 22% of the fruit's weight, is typically discarded after consumption, despite its potential nutritional and functional benefits (Bakar, ShuC., Muhammad, Hashim, & Noranizan, 2011). However, recent scientific studies have discovered that

dragon fruit peels contain significant amounts of bioactive compounds such as betacyanins, phenolic compounds, and dietary fibers. These compounds exhibit a range of biological properties, including antioxidant, antidiabetic, antihyperlipidemic, and anticancer activities (Cheok et al., 2018; Jiang et al., 2021). As a result of these benefits, dragon fruit peels are now considered a by-product with potential for use as a dietary fiber enhancer, natural colorant, and antioxidant in various food products. Interestingly, our prior investigation has established that the incorporation of dragon fruit peel powder (DFP) into various flour types led to a decline in starch digestibility, alteration of gelatinization enthalpy, and pasting properties, which were attributed to the presence of dietary fibers, phenolic compounds, and betacyanins (Chumroenvidhayakul, Thilavech, Abeywardena, & Adisakwattana, 2022). In line with these findings, the aim of the present study was to investigate the effect of incorporating DFP into wheat cookies on starch digestibility, predicted glycemic index (pGI), and heat-induced toxicants. Additionally, the study evaluated the antioxidant activity, physical characteristics, and sensory attributes of DFP cookies, expanding the potential applications of dragon fruit peel as a functional food ingredient. The outcomes of this study have important implications for utilizing fruit waste, particularly dragon fruit peel, as a more sustainable and health-promoting material in the development of novel and nutritionally improved food products.

3.5 Materials and Methods

3.5.1   Materials

Dragon fruit peels were sourced from Ramathibodi Hospital in Bangkok, Thailand. The cookie ingredients used were commercially available from a supermarket. The following reagents and chemicals were purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA): Folin–Ciocalteu reagent, betanin (betanidin 5-β-D-glucopyranoside), TPTZ (2,4,6-tripyridyl-s-triazine), 2,6-di-tert-butyl-4-methylphenol (BHT), 2-thiobarbituric acid (TBA), malondialdehyde tetrabutylammonium salt (MDA), pepsin from porcine gastric mucosa powder (250 U/mg), α-amylase type VI-B from porcine pancreas (15.8 U/mg), pancreatin from porcine pancreas, methylglyoxal (40% aqueous solution), 5-methylquinoxaline (5-MQ), and o-phenylenediamine (OPD). Amyloglucosidase from *Aspergillus niger* was purchased from Roche Diagnostics in Indianapolis, IN, USA.

3.5.2   Preparation of dragon fruit peel powder (DFP)

The DFP was prepared in accordance with a previously published method (Chumroenvidhayakul, Thilavech, Abeywardena, & Adisakwattana, 2022). Fresh dragon fruit peels were first carefully washed, and the epicarp was removed. The peels were subsequently dried in a hot air oven for 12 h at a temperature of 60 °C. Once dried, the peels were ground and sieved through a No. 40 sieve to obtain the DFP. The powder was then stored in polyethylene foil bags at a temperature of −20 °C until further use. The characteristics of the DFP used in this study were previously

described in our published research (Chumroenvidhayakul et al., 2022). The proximate composition of DFP consisted of 70.85% carbohydrates, including 65.17% dietary fibers and 5.68% available carbohydrates. The remaining components were 15.91% ash, 6.37% protein, 5.81% moisture, and 1.06% fat.

3.5.3 Preparation of cookies

To prepare the cookie dough, all ingredients (presented in Table 6) were mixed according to the recipe by Mudgil et al. (2017), with some modifications. The DFP was added to the dough at varying concentrations of 0% (control), 1%, 2%, and 5% (*w/w*) of the weight of raw cookie dough. The dough was subsequently baked at a temperature of 170 °C for a duration of 13 min. After baking, the cookies were allowed to cool to room temperature and stored under dry conditions at 25 °C in an aluminum foil bag for further analysis.

Table 6 Cookie recipes fortified with dragon fruit peel powder (DFP).

Ingredients (%)	Composition of cookies			
	Control	1% DFP	2% DFP	5% DFP
Wheat flour	34.2	34.2	34.2	34.2
Skim milk powder	5.9	5.9	5.9	5.9
Sugar	20.0	20.0	20.0	20.0
Egg	13.7	13.7	13.7	13.7
Vanilla flavor	0.2	0.2	0.2	0.2
Unsalted butter	25.1	25.1	25.1	25.1
Baking soda	0.9	0.9	0.9	0.9
DFP	0.0	1.0	2.0	5.0

3.5.4 Determination of phytochemical compounds and antioxidant activity

In order to evaluate the active compounds and antioxidant activity, one gram of ground cookie from each sample was extracted with 20 mL of distilled water and continuously stirred in a shaker at 100 rpm for 3 h. The resulting mixture was then centrifuged at 2000× g for 15 min at 4 °C to collect the supernatant, which was filtered through Whatman No. 1 filter paper and stored in a dark room. The extracted cookie solution was used to determine the total phenolic content (TPC), betacyanin content (TBC), antioxidant activity (ferric-reducing antioxidant power (FRAP)), and lipid oxidation.

The TPC of cookies was determined according to Adisakwattana et al. (2012). In brief, the extracted cookie solution (50 μL) was mixed with 50 μL of a 10-fold dilution

of Folin–Ciocalteu reagent and incubated at room temperature for 5 min. Then, 50 µL of 10% (*w/v*) Na_2CO_3 was added and further incubated for 60 min. The absorbance was read at 750 nm, and the results were expressed as mg gallic acid equivalent per 100 g cookie.

The TBC in cookies was determined according to the method previously described by Chumroenvidhayakul et al. (2022). The absorbance of the extracted cookie solution was measured at 538 nm, and the amount of TBC was calculated using a standard curve of betanin. The TBC was expressed as mg betanin equivalent per 100 g cookie.

To assess antioxidant activity, the FRAP assay was performed according to a previous publication (Benzie & Strain, 1996). Fresh FRAP reagent was prepared in the ratio of 10:1:1 of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$, respectively. Then, the cookie solution (10 µL) was mixed with 90 µL of FRAP reagent and incubated for 30 min at room temperature. The absorbance was read at 595 nm, and the FRAP value was expressed in µmol $FeSO_4$ equivalent per 100 g of cookie.

3.5.5   Determination of lipid peroxidation

The level of lipid peroxidation in cookies was measured according to a previous report (Chusak et al., 2018), with modification based on the formation of thiobarbituric acid reactive substances (TBARSs). Briefly, the extracted cookie solution (200 µL) was mixed with 200 µL of 10% (*w/v*) trichloroacetic acid and 30 µL of 50 mM BHT. The

mixture was centrifuged at 10,845× *g* at 4 °C for 15 min. The supernatant (200 µL) was

mixed with 0.67% (*w/v*) TBA. The mixture was heated at 95 °C for 10 min and allowed

to cool down at room temperature. The absorbance was read at a wavelength of 532

nm, and the results were calculated against the standard curve of MDA. The results

were expressed as nmol MDA equivalent per 1 g cookie.

3.5.6   Determination of released glucose under simulated gastrointestinal digestion

The glucose release from the cookie during simulated digestion was

determined following the protocol described by Chumroenvidhayakul et al. (2022).

Briefly, 500 mg of ground cookie or glucose (used as a reference compound) was

mixed with 1 mL of artificial saliva solution (250 U/mL porcine amylase in 0.2 M

carbonate buffer, pH 7) for 15–20 s, followed by the addition of 5 mL of pepsin

suspension (1 mg/mL) in 0.02 M HCl (pH 2). The mixture was incubated in a water

bath shaker (100 rpm) at 37 °C. After 1 h, the reaction was neutralized by adding 5

mL of 0.02 M NaOH and 25 mL of 0.2 M sodium acetate buffer (pH 6), and intestinal

digestion was initiated by adding 5 mL of the enzyme mixture containing pancreatin

(2 mg/mL) and amyloglucosidase (28 U/mL) in 0.2 M acetate buffer, pH 6. The mixture

was further incubated at 37 °C in a water bath shaker (100 rpm), and samples were

collected at 0, 20, 30, 60, 90, 120, and 180 min. The digesta fluid was immediately

heated at 100 °C and centrifuged (10,845× *g*, 4 °C for 15 min), and the glucose

concentration in the resulting supernatant was determined using a glucose oxidase kit

(HUMAN GmbH, Wiesbaden, Germany). The percentage hydrolysis index (HI) and the predicted glycemic index (pGI) were calculated using the following equations:

$$HI = \frac{\text{Area under the curve of released glucose from cookies}}{\text{Area under the curve of standard glucose (reference)}} \times 100$$

$$pGI = 39.71 + 0.549\ HI$$

3.5.7   Determination of starch fraction

The total starch content (TS) in cookies was determined following a previous method (Goñi, Garcia-Alonso, & Saura-Calixto, 1997), with slight modifications. In brief, 50 mg of cookie sample was mixed with 6 mL of 2 M KOH and incubated at room temperature for 1 h. The pH was then adjusted to 4.75 using concentrated HCl, and 3 mL of 0.4 M sodium acetate buffer was added. The mixture was further incubated at 60 °C for 45 min in a water bath shaker (100 rpm). After the mixture was heated to 100 °C, it was centrifuged at 10,845$\times$ g for 5 min. The glucose concentration was determined using a glucose oxidase kit. The total starch content was calculated by multiplying the glucose concentration by 0.9 and expressed as g/100 g sample. The rapidly digestible starch (RDS) was calculated as the difference between the glucose released at 20 min (G_{20}) and free glucose (G_0) after *in vitro* digestion, while the slowly digestible starch (SDS) was calculated as the difference between the amount of glucose measured at 120 min (G_{120}) and 20 min. The amount of resistant starch (RS) was defined as the amount of glucose that remained undigested after 120 min. The equations used for calculating starch content are presented below:

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

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

$$Undigested\ starch\ (\%) = (TS - (RDS + SDS))/TS \times 100$$

where G_0 = glucose content after 0 min of digestion; G_{20} = glucose content after 20 min of digestion; G_{120} = glucose content after 120 min of digestion; TS = total starch content.

3.5.8   Determination of dietary advanced glycation end products (dAGEs)

The fluorescent dAGEs in cookies were determined in accordance with a previous method (Gao et al., 2020). Ground cookies weighing 250 mg were extracted using 4.75 mL of a buffer solution consisting of 0.05% Tween-20, 1% SDS, 5% β-mercaptoethanol, and 50 mM Tris-HCl at pH 7.4. The mixture was incubated at room temperature with shaking at 100 rpm for 12 h. Following extraction, the sample was centrifuged at 1904$\times$ g for 5 min, and the supernatant was analyzed using a spectrofluorometer (Perkin Elmer, Waltham, MA, USA) at excitation and emission wavelengths of 355 nm and 460 nm, respectively.

3.5.9   Determination of methylglyoxal (MGO) content

The MGO content in cookies was conducted following the method by Thilavech et al. (2016), with some modifications. Briefly, five grams of ground cookies was extracted by sonication in 50 mL of 50% (v/v) methanol for 1 h. After centrifugation at 3808$\times$ g for 15 min, the supernatant was filtered through Whatman No. 1 filter paper. The filtrate was then subjected to concentration by rotary

evaporation (Buchi, Switzerland) at 55 °C, and the extracted sample was redissolved in 1.5 mL of 50% (v/v) methanol in distilled water. Next, 900 µL of the extracted sample was mixed with 300 µL OPD (20 mM) and incubated at 37 °C for 24 h. The solution was then centrifuged at 10,845x g at 4 °C for 15 min. The determination was carried out by high-performance liquid chromatography (HPLC) using an LC-10 AD pump, an SPD-10A UV-VIS detector, and an Inersil-ODS3V C18 column (150 × 4.6 mm i.d.; 5 µm particle size) as the stationary phase. An isocratic program was conducted with 50% (v/v) methanol in distilled water as the mobile phase and a constant flow rate of 1 mL/min. The injection volume was 10 µL, and the absorbance was recorded at 315 nm. The total running time was 14 min, and the internal standard used was 5-methylquinoxaline in methanol (0.06%, v/v). The amount of MGO was calculated by comparing it to the standard curve of MGO.

3.5.10 Physical properties of cookies

Prior to the color measurement, the colorimeter (ColorFlex 45/0-HunterLab, Hunter Associates Laboratory, Inc., Reston, VA, USA) was calibrated using black and white standards. The color values were expressed using the CIE color scales, specifically L^* for lightness (where 0 represents black and 100 represents white), a^* for the green–red axis (where $-a^*$ denotes greenness and $+a^*$ denotes redness), and b^* for the blue–yellow axis (where $-b^*$ denotes blueness and $+b^*$ denotes yellowness) (Bukolt et al., 2019).

The spread ratio, diameter, and thickness of the cookies were measured using the method described by Mudgil and Barak (2017). To measure the diameter, six cookies were arranged edge to edge, and the average diameter was calculated. The thickness was determined by stacking six cookies, and the average thickness was calculated using a caliper. The spread ratio of the cookies was calculated using the following equation:

Spread ratio = Average of diameter (mm)/Average of thickness (mm)

The moisture content of cookies was determined following the previous study (Laganà, Giuffrè, De Bruno, & Poiana, 2022), using an infrared moisture analyzer (FD-610 Kett Electric Laboratory, Tokyo, Japan). Briefly, five grams of ground cookies were accurately weighed and placed on an aluminum dish with a cover before being dried. The processing temperature for moisture analysis was set at 140 °C for 5 min. The results were reported as the percentage of moisture content in the cookies.

The textural profile of cookies, including hardness and fracturability, was determined using a texture analyzer in compression mode with an HDP/3PB probe (Lloyd Instruments/Ametek TA1 Texture Analyzer, AMETEK (GB) Ltd., West Sussex, UK), as described in previous studies (Bukolt et al., 2019). The hardness of the cookies, indicated by the maximum peak force required for breakage, and the fracturability, determined by the distance (mm) of the first significant break peak in the texture profile analysis curve, were analyzed. The compression test was conducted with a

return to start cycle, a pretest speed of 1.0 mm/s, a test speed of 2.0 mm/s, a post-test speed of 10 mm/s, and a distance of 5.0 mm.

3.5.11 Sensory analysis

The sensory evaluation of the cookies involved 50 untrained panelists, who were both male and female, aged between 18 and 50 years, and did not have any sensory impairments or food allergies. These panelists were regular consumers of bakery products. The study received approval from the Office of the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board (COE.No.MU-DT/PY-IRB 2023/015.0404 Project No. 2023/PY042) and was conducted in accordance with the laboratory's ethical guidelines, with each panelist providing written informed consent. To prevent bias, the samples were assigned unique 3-digit codes, and their serving orders were randomized using software. Mouth rinsing with plain water was performed between samples. The sensory attributes of the cookies, including appearance, color, aroma, taste, texture, and overall acceptability, were evaluated using a 9-point hedonic scale, with 1 indicating "extremely dislike", 5 indicating "neither like nor dislike", and 9 indicating "like extremely".

3.5.12 Statistical analysis

All experiments were conducted in triplicate, unless otherwise stated. Statistical analysis was performed using one-way analysis of variance (ANOVA), and the post hoc comparison was made using Duncan's test. The area under the curve (AUC) was calculated using the trapezoidal rule. The significance level for all analyses

was set at $p < 0.05$. The statistical software used for the analysis was IBM SPSS version 22.0 (International Business Machines Corporation, Armonk, NY, USA).

3.6 Results and Discussion

Based on our preliminary investigation, we found that the viscosity of the cookie dough significantly increased when the concentration of DFP was increased beyond 5% (*w/w*). This increase in viscosity impeded the even spreading of the dough, leading to unevenness and lumpiness in the baked cookies due to inadequate heat penetration. As a result, we determined that the optimal range for incorporating DFP into cookies was between 1% and 5% (*w/w*). Within this range, the dough retained the desired consistency, which enabled it to be evenly spread, resulting in uniformly baked cookies with minimal lumpiness. The appearance of cookies produced from DFP is shown in Figure 8. As demonstrated in Table 7, the addition of DFP in varying concentrations to cookies resulted in a concentration-dependent increase in both TPC and TBC. The TPC increased from 173.48 mg GAE/100 g in the control cookie to 500.88 mg GAE/100 g in the 5% DFP cookie, while the TBC increased from 0.11 mg BE/100 g in the control cookie to 8035.71 mg BE/100 g in the 5% DFP cookie. Correspondingly, FRAP values for cookies containing 1%, 2%, and 5% DFP were 1.19-, 1.60-, and 2.16-fold higher than that of the control, respectively, indicating an increase in antioxidant activity. These results suggest that the addition of DFP to cookies can significantly enhance their phytochemical content and antioxidant capacity. The observed increase in phytochemical compounds and antioxidant

capacity in cookies may be ascribed to the high levels of phytochemicals found in DFP, notably betanin, gallic acid, chlorogenic acid, syringic acid, coumaric acid, and ferulic acid. These compounds are well known for their potent antioxidant properties and have been reported to exhibit a wide range of health benefits, including anti-inflammatory, antimicrobial, and anticancer effects (Jiang et al., 2021).

These findings are consistent with previous reports demonstrating that the addition of DFP to various food products, including mantou, noodles, bread, and cookies, can increase betacyanin and polyphenol content, as well as antioxidant capacity (Jiang et al., 2021; Chumroenvidhayakul et al., 2022).

Cookies enriched with DFP at varying concentrations (1%, 2%, and 5%) exhibited notable reductions in the production of MDA (Table 7), a reactive aldehyde generated as a result of lipid peroxidation. Specifically, we observed dose-dependent reductions in MDA production, with the highest concentration of DFP leading to a remarkable 42.3% decrease in MDA levels. Notably, dietary fats exposed to high temperatures are known to generate high levels of oxidized compounds, including MDA. The accumulation of such compounds can increase lipid peroxidation products and lipophilic carbonyl compounds in blood and tissues, leading to reduced protein functionality, elevated production of reactive metabolites, oxidative modification of LDL, inflammation, and the initiation of atherosclerotic lesions and endothelial dysfunction (Staprans, Hardman, Pan, & Feingold, 1999; Kanner, 2007). Our findings suggest that the incorporation of DFP, which contains phytochemical compounds and

dietary fibers, can effectively impede the formation of lipid peroxidation and its toxic by-products in baked products, potentially mitigating adverse health outcomes. The underlying mechanisms of DFP's lipid-peroxidation inhibitory effects may involve the increased binding of free water content and the scavenging of free radicals. For instance, betanin, a pigment present in DFP, has been reported to inhibit lipid peroxidation in meat, possibly by reducing the concentration of reactive oxygen species (Vieira Teixeira da Silva et al., 2019). Additionally, the high hydration properties of the dietary fibers present in DFP allow them to form a pseudoplastic barrier by binding with free water in the food system (Liu, Fang, Luo, Ding, & Liu, 2019; Yemenicioğlu, Farris, Turkyilmaz, & Gulec, 2020; Chumroenvidhayakul et al., 2022). This can slow down the movement of free water molecules and reduce the extent of lipid peroxidation (Liu et al., 2019; Q. Zhang, Wang, & Fu, 2020). In addition to the potential health benefits, incorporating DFP into cookies may also prolong their shelf life by preventing fat-induced rancidity, thus improving the overall quality of the product.

Control **1% DFP** **2% DFP** **5% DFP**
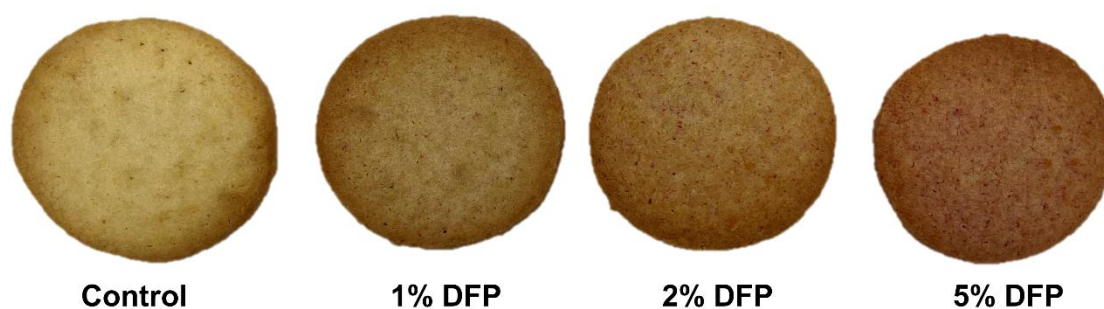
Figure 8 Photographs of cookies made with raw cookie dough containing dragon fruit peel at 1–5% (*w/w*).

Table 7 Effects of adding dragon fruit peel powder (DFP) to raw cookie dough on total phenolic content (TPC), total betacyanin content (TBC), ferric-reducing antioxidant power (FRAP) activity, lipid peroxidation, and predicted glycemic index (pGI).

Experiments	TPC (mg GAE/100 g cookie)	TBC (mg BE/100 g cookie)	FRAP (μmol $FeSO_4$ equivalent/100 g cookie)	Lipid peroxidation (μmol MDA equivalent/100 g cookie)	pGI
Control	173.48 ± 2.45^a	0.11 ± 0.02^a	752.75 ± 8.47^a	6.38 ± 0.15^a	53.68 ± 0.1^a
1% DFP	239.77 ± 8.32^b	$4{,}519.48 \pm 29.99^b$	894.87 ± 3.60^b	4.90 ± 0.06^b	52.53 ± 0.3^b
2% DFP	263.37 ± 2.87^c	$6{,}087.66 \pm 75.42^c$	$1{,}207.24 \pm 10.91^c$	3.94 ± 0.08^c	51.89 ± 0.2^c
5% DFP	500.88 ± 3.45^d	$8{,}035.71 \pm 37.26^d$	$1{,}624.44 \pm 12.80^d$	2.66 ± 0.02^d	51.04 ± 0.2^d

The values are presented as means ± standard error of the mean (SEM), n=3. Means with a different superscript indicate a significant difference ($p < 0.05$). GAE—gallic acid equivalent; BE—betanin equivalent; MDA— malondialdehyde.

MGO is a highly reactive compound that can be generated in food during various stages of processing, cooking, and storage. This occurs when the amino group of proteins reacts with the carbonyl group of reducing sugars in the presence of heat. These reactions result in the structural modification of proteins and the formation of dAGEs, which are formed through a series of intricate mechanisms such as caramelization, the Maillard reaction, and lipid peroxidation (Han, Tan, Wang, Yang, & Tan, 2015; Nowotny et al., 2018). Our study findings revealed the promising potential of DFP in reducing the levels of MGO (Figure 9A) and dAGEs (Figure 9B) in cookies. By adding DFP at varying concentrations ranging from 1% to 5%, we observed a substantial reduction in MGO content, with the 5% DFP group exhibiting the most significant decrease of up to 52.1%, followed by the 2% and 1% DFP groups. Moreover, we observed a noteworthy inhibitory effect of DFP against dAGE formation in cookies, with the 5% DFP group demonstrating the highest efficacy in reducing dAGE formation by up to 36.9%, followed by the 2% and 1% DFP groups.

The results indicate that the incorporation of DFP into cookies may be a promising approach to reduce the formation of harmful compounds associated with thermal food processing and storage. The ability of DFP to inhibit the formation of MGO and dAGEs is attributed to the presence of its natural bioactive compounds, such as phenolics, betacyanins, and pectin (Passos et al., 2018; Song et al., 2021). Emerging scientific evidence suggests that phenolic compounds and betanin are capable of directly trapping intermediate glycation reactive compounds, such as

MGO, and reducing the formation of AGE products, such as Nε-(carboxymethyl) lysine (Mirmiran, Houshialsadat, Gaeini, Bahadoran, & Azizi, 2020; Song et al., 2021). Pectin, on the other hand, has been shown to effectively inhibit the formation of AGEs in a BSA-galactose system (Passos et al., 2018). Furthermore, DFP is also able to inhibit the formation of MGO and dAGEs by capturing reactive dicarbonyl compounds, scavenging free radicals, and chelating metal ions in heat-induced non-enzymatic browning reactions (Song et al., 2021). Notably, dietary fibers naturally present in DFP are known to increase the availability of water and dilute reactants in the aqueous phase, ultimately reducing the formation of dAGEs (Chaouch, Hafsa, Rihouey, Le Cerf, & Majdoub, 2016; Passos et al., 2018). The dietary fibers also interfere with non-enzymatic browning reactions by interacting with amino acids, which helps prevent the formation of dAGEs during baking (Chaouch et al., 2016; Liu et al., 2019; Q. Zhang et al., 2020).

(A)

(B)
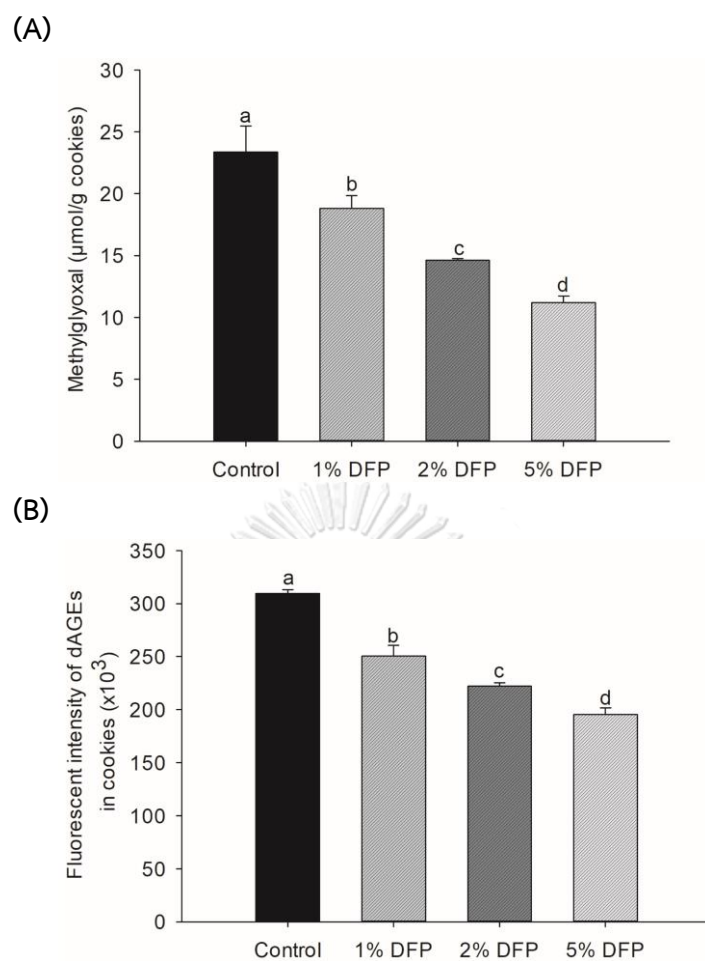
Figure 9 The effect of dragon fruit peel powder (DFP) used to fortify raw cookie dough on (A) the level of methylglyoxal and (B) dietary advanced glycation products (dAGEs).

The results are presented as mean ± SEM, n = 3. Statistically significant differences in mean values are denoted by different letters, with a significance level set at $p < 0.05$.

Incorporating 5% DFP into cookies resulted in a remarkable reduction in glucose released during simulated digestion (Figure 10A), as indicated by a significant decrease in the area under the glucose release curve compared to the control cookie ($p <$ 0.05). This decrease in starch digestibility rate, which was 18.9%, is consistent with our earlier study demonstrating the suppressive effect of DFP on starch digestibility when added to wheat flour (Chumroenvidhayakul et al., 2022). The underlying mechanism for this effect is believed to be due to the presence of dietary fibers and phytochemicals in DFP, which alter starch properties by interfering with the formation of starch gel and chains, increasing onset temperature, decreasing gelatinization enthalpy, and disrupting the dissolution of the crystalline structures of starch granules (Chumroenvidhayakul et al., 2022). Dietary fibers have been reported to alter the starch system by trapping starch granules and increasing viscosity, which reduces enzyme accessibility to starch (Repin, Cui, & Goff, 2018). Furthermore, phenolic compounds in DFP may interact with starch granules and digestive enzymes, leading to limited starch digestion (Mirmiran et al., 2020; Zhu, 2015). To evaluate the nutritional properties of DFP cookies, we conducted an analysis of the hydrolysis index (HI) and predicted glycemic index (pGI), crucial indicators for estimating the physiological glycemic response in the *in vivo* system (Englyst, Kingman, & Cummings, 1992; Lal, Singh, Sharma, Singh, & Kumar, 2021). With the addition of 1%, 2%, and 5% of DFP, the HI percentage of DFP cookies was lower than that of the control by 8.6%, 12.9%, and 19.0%, respectively (Figure 10B). Remarkably, DFP significantly reduced

the glycemic index in cookies by decreasing pGI from 53.68 in the control to 52.53 in

1% DFP, 51.89 in 2% DFP, and 51.04 in 5% DFP (Table 7). These findings indicate that

DFP can be employed to prepare functional cookies, resulting in reduced starch

digestibility rates and low-glycemic-index foods that can become a healthier bakery

option for consumers. From a nutritional standpoint, dietary starches are classified

into three primary fractions based on their *in vitro* digestibility: rapidly digestible

starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al.,

1992). RS, or undigested starch, is particularly beneficial to health as it remains

undigested after 120 min, thus controlling the postprandial glycemic response

(Bojarczuk, Skąpska, Mousavi Khaneghah, & Marszałek, 2022). Significantly, the addition

of DFP to cookies increased the amount of undigested starch while decreasing the

RDS and SDS compared to cookies without DFP (Figure 10C). A similar outcome was

reported in a previous study where cookies treated with passion fruit peel flour

containing high levels of dietary fibers and bioactive compounds demonstrated a

lower hydrolysis percentage and reduced RDS content (Ning et al., 2021). Research

has shown that consuming a diet containing a reduced fraction of SDS and an

increased proportion of undigested starch can yield several health benefits, such as

improved glycemic control and insulin sensitivity (Bojarczuk et al., 2022; G. Zhang &

Hamaker, 2009). Consequently, the incorporation of DFP in cookies can substantially

lower the rate of starch digestibility and augment the content of resistant starch,

thereby producing low-glycemic-index foods that hold promise as potential health-

promoting dietary options. Further investigations are required to assess the acute effects of DFP cookies on postprandial glycemic response in human subjects.

(A)

(B)

(C)
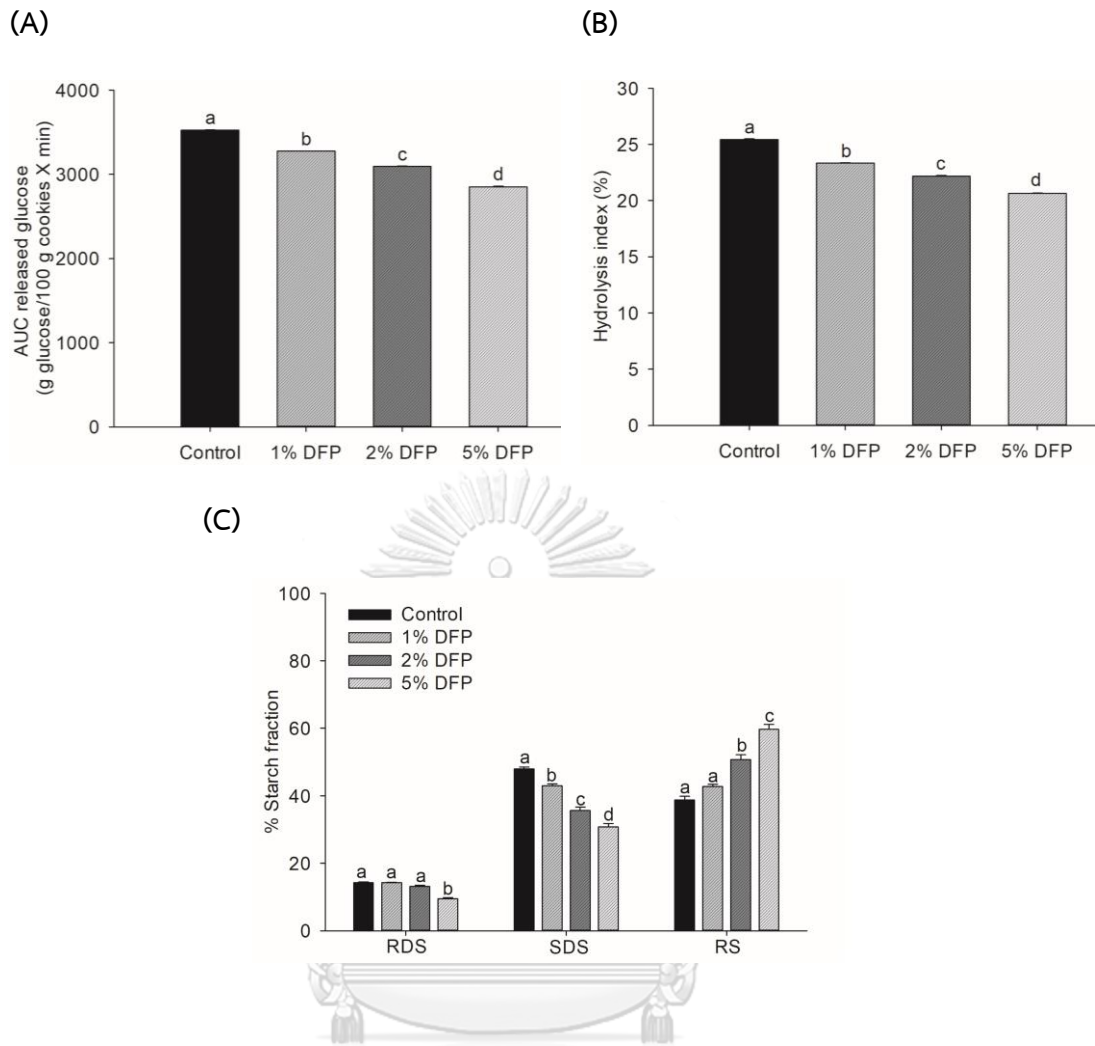
Figure 10 The area under the curve (AUC) of glucose release (A), hydrolysis index (B),

and starch fractions (C) of cookies fortified with dragon fruit peel powder (DFP) in raw

cookies dough during *in vitro* digestion.

The results are presented as mean ± SEM, n = 3. Statistically significant differences in

mean values are denoted by different letters, with a significance level set at $p < 0.05$.

RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: Resistant starch.

Table 8 provides a comprehensive overview of the physical characteristics of cookies supplemented with DFP. The addition of DFP had a significant influence on the cookies' color, spread ratio, moisture content, and texture profiles. The results indicated a noteworthy increase in the redness (a*) of the cookies, while the yellowness (b*) declined significantly ($p < 0.05$). This effect can be attributed to the presence of betacyanin pigments found in the red peels of the DFP (Chumroenvidhayakul et al., 2022; Ho & Abdul Latif, 2016). Interestingly, the incorporation of 2% or 5% DFP resulted in a statistically significant reduction in the spread ratio of cookies.
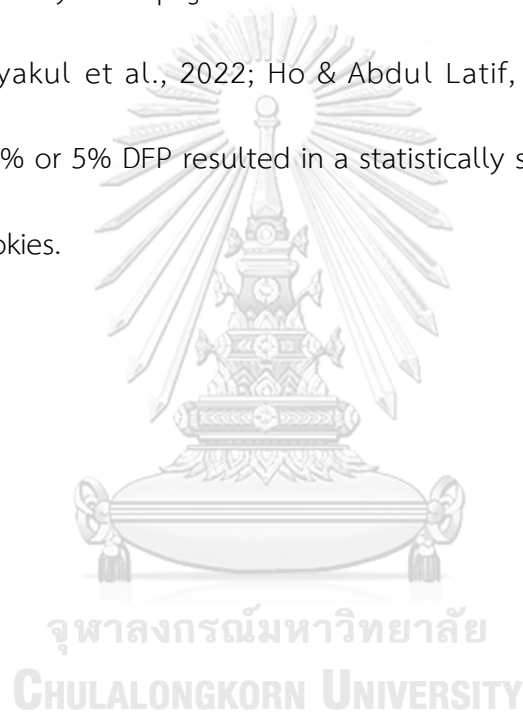
Table 8 The physical characteristics of cookies fortified with dragon fruit peel powder (DFP).

Experiments	Control	1% DFP	2% DFP	5% DFP
Color				
$L*$	47.86 ± 4.31^a	53.48 ± 3.00^a	53.27 ± 0.29^a	48.78 ± 0.83^a
$a*$	6.03 ± 0.20^a	7.24 ± 0.49^b	8.26 ± 0.23^{ab}	9.10 ± 0.25^c
$b*$	36.04 ± 0.32^a	34.73 ± 0.44^{ab}	32.66 ± 2.19^{ab}	31.60 ± 0.56^b
Spread ratio	9.10 ± 0.25^a	8.26 ± 0.23^{ab}	7.24 ± 0.49^b	6.03 ± 0.20^c
Moisture (%)	5.13 ± 0.02^a	5.07 ± 0.03^a	5.40 ± 0.05^b	5.80 ± 0.05^c
Texture profiles				
Hardness (N)	34.09 ± 1.93^a	25.28 ± 1.93^b	19.83 ± 1.19^c	17.66 ± 1.27^c
Fracturability (mm)	1.01 ± 0.06^a	1.05 ± 0.10^a	2.04 ± 0.20^b	2.26 ± 0.16^b

The values are presented as means ± standard error of the mean (SEM), n=3. Means with a different superscript in each row indicate a significant difference ($p < 0.05$).

This outcome can be attributed to the pronounced water-binding capacity of DFP, which led to an increase in the thickness and a decrease in the diameter of the cookies (Chumroenvidhayakul et al., 2022). We suggest that the incorporation of DFP into the dough mixture resulted in the absorption of water, which elevated the dough's viscosity and consistency. Consequently, the insufficient sugar dissolves in the dough system, leading to a decrease in the diameter of dough expansion but an increase in cookie thickness during baking (Becker, Damiani, de Melo, Borges, & de Barros Vilas Boas, 2014; Mancebo, Rodríguez, Martínez, & Gómez, 2018). This finding aligns with previous studies, which have reported that high hydration properties of ingredients tend to reduce the width and spread factor of cookies (Mancebo et al., 2018). Moreover, the moisture content of the cookies significantly increased when 2% or more DFP was added to the formulation (Palaniappan et al., 2015). With respect to the texture profile analysis, the hardness of the cookies decreased significantly, while the fracturability increased with an increase in the DFP level. This phenomenon can be attributed to gluten dilution and the high-water absorption capacity of DFP, which hinder gluten development upon the addition of dietary fibers (Mancebo et al., 2018; Palaniappan et al., 2015). These findings provide insight into the potential of DFP as a functional ingredient in bakery products, offering not only desirable color attributes but also improving texture and moisture profiles.

The sensory acceptability of cookie characteristics, including appearance, color, odor, taste, texture, hardness, and overall acceptance, were evaluated for cookies

containing DFP, as shown in Table 9. The acceptability scores for appearance, odor, texture, and hardness were not significantly different from those of the control group, except for the 5% DFP addition. Regarding color and taste, the 2% DFP addition had the highest score, while the 5% DFP addition had the lowest score. These findings could be attributed to the darker color and slightly sour, as well as bitter taste derived from DFP, as previously reported (Bakar et al., 2011; Ho & Abdul Latif, 2016). In this study, the overall acceptability score of DFP-treated cookies was greater than 7, which is indicative of high sensory acceptance (Everitt, 2009). Moreover, the highest acceptance score was observed for the 2% DFP addition, indicating the high acceptability of DFP addition in cookies. Therefore, it appears feasible to manufacture a functional cookie containing dietary fiber and phytochemicals with an acceptable sensory quality with the addition of DFP. However, further studies are necessary to clarify the impact of DFP on physical properties, sensory qualities, and acceptability during storage.

Table 9 Sensory evaluation of cookies fortified with dragon fruit peel powder (DFP).

Sensory characteristics	Control	1% DFP	2% DFP	5% DFP
Appearance	7.16 ± 0.17^a	7.12 ± 0.19^a	7.32 ± 0.16^b	6.80 ± 0.20^c
Color	7.38 ± 0.15^a	7.26 ± 0.17^a	7.48 ± 0.14^a	6.54 ± 0.18^b
Odor	7.34 ± 0.17^a	7.28 ± 0.19^a	7.22 ± 0.17^a	6.9 ± 0.21^a
Taste	7.36 ± 0.17^a	7.26 ± 0.10^{ab}	7.50 ± 0.19^a	6.92 ± 0.08^b
Texture	7.20 ± 0.21^a	7.36 ± 0.19^a	7.48 ± 0.17^b	7.02 ± 0.20^c
Hardness	7.92 ± 0.14^a	7.80 ± 0.18^{ab}	7.80 ± 0.15^{ab}	7.56 ± 0.15^b
Overall acceptability	7.60 ± 0.15^a	7.56 ± 0.15^a	7.74 ± 0.15^a	7.16 ± 0.16^b

The values are presented as means ± standard error of the mean (SEM), n=50. Means

with a different superscript in each row indicate a significant difference ($p < 0.05$).

3.7 Conclusions

Incorporating dragon fruit peel powder (DFP) in raw cookie dough can enhance the nutritional and functional properties of cookies. The addition of DFP can increase the bioactive phytochemical content and antioxidant capacity of cookie products, decrease levels of heat-generated food toxicants, and reduce starch digestibility. Although cookies enriched with DFP showed alterations in their physical properties, incorporating up to 2% DFP did not affect the overall acceptability of the cookies. This innovative approach offers a promising opportunity for the food industry to increase the value of waste materials generated during fruit processing and create healthier food products with improved sustainability.

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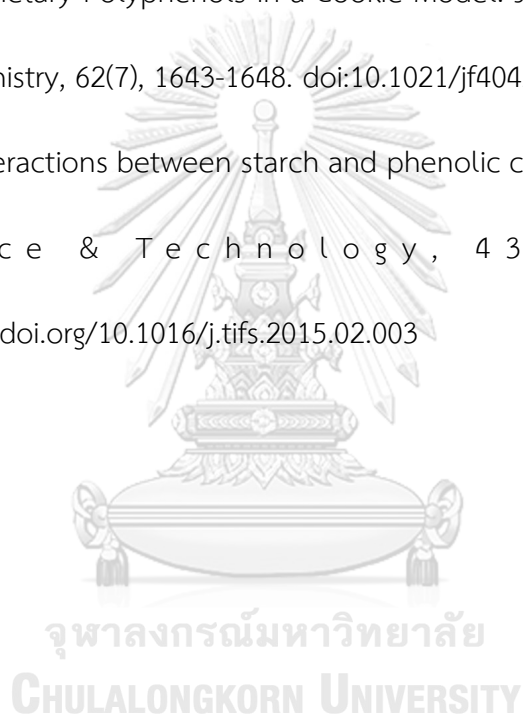
CHAPTER IV

MODULATION OF COLONIC BACTERIAL METABOLITES AND MUCOSAL BARRIER BY DRAGON FRUIT PEEL WASTE (*HYLOCEREUS UNDATUS*) IN A HIGH-FAT, HIGH-FRUCTOSE DIET-INDUCED RAT MODEL

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4.3 Abstract

Recent studies have shown that a Westernized diet high in fat and fructose (HFHFr) disrupts gut microbiota and damages the gut barrier, leading to increased intestinal permeability. However, dragon fruit peel powder (DFP), rich in dietary fiber and phytochemicals, may offer a solution. This study examined the effects of combining DFP with an HFHFr diet on fecal properties, gut barrier function, cecal microbial population, and short-chain fatty acid (SCFA) production in rats. Four groups were assigned: normal diet (C), C with 5% (*w/w*) DFP (C + DFP), HFHFr, and HFHFr + 5% (*w/w*) DFP (HFHFr + DFP) for 12 weeks. The findings demonstrated that HFHFr consumption significantly increased fecal pH while reducing fecal moisture. Conversely, supplementation with DFP elevated fecal moisture and lipid excretion while decreasing fecal pH. HFHFr feeding caused intestinal barrier dysfunction by increasing serum lipopolysaccharide-binding protein levels and mRNA expression of TLR4 in colonic tissue ($p<0.05$), which were markedly decreased by supplementing with 5% DFP ($p<0.05$). Additionally, the gene expression of tight junction-related proteins was also up-regulated in the DFP groups. For colonic microbiota, DFP affected the abundance of specific microbial groups, including Bacteroidetes, and the *Firmicutes/Bacteroidetes* ratio, and significantly promoted the proliferation of SCFA-producing bacteria, consistent with the increased concentration of total SCFA,

acetate, and propionate in cecal contents ($p<0.05$). These results indicate that DFP has the ability to modulate the composition of gut microbiota, promote colon barrier function in HFHFr-fed rats, and ultimately exert a positive influence on gut health.

Keywords: Dragon fruit peel, dietary fibers, gut microbiome, gut health

4.4 Introduction

The rapid advancements in modern civilization have profoundly influenced our lifestyle and dietary habits, with the Western diet emerging as a crucial aspect. This diet is widely recognized for its association with the progression of various metabolic disorders, including obesity, type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease (NAFLD) (Dunford, Popkin, & Ng, 2022; Martinez, Leone, & Chang, 2017). These adverse health effects can be attributed to the high concentration of saturated fats and refined sugars, particularly fructose, in the diet. Recent research has extensively demonstrated that a diet rich in saturated fat and refined sugar, while being low in fiber, can disrupt the composition of the gut microbiota (Horne et al., 2020; Martinez et al., 2017; Rahman et al., 2016). This disruption, known as gut dysbiosis, has been shown to impair gut barrier function and significantly increase the risk of developing various metabolic disorders (Horne et al., 2020; Martinez et al., 2017; Rahman et al., 2016). For example, studies have shown that a high-fructose diet (60% *w/w*) leads to a decrease in populations of beneficial bacteria, such as Bifidobacterium and Lactobacillus, in rats (Jegatheesan et al., 2016). Similarly, a high-fat diet has been found to increase the population of *Escherichia coli*, down-regulate

tight-junction genes (occludin and claudin-1), and up-regulate toll-like receptor 4 (TLR4) gene levels in rats, resulting in gut mucosal inflammation (Li et al., 2019). Additionally, high-fat, high-fructose feeding disrupts the intestinal barrier, alters gut bacteria composition, and increases inflammatory lipopolysaccharides (LPS) in the bloodstream, triggering hepatic inflammation (Rahman et al., 2016). It also disrupted the intestinal barrier and altered gut bacteria composition, decreasing *Bacteroidetes* and increasing *Proteobacteria* and *Firmicutes* in rats (Rahman et al., 2016).

Given the impact of diet on gut health, dietary fiber intake has emerged as one of the most effective interventions for improving the gut microenvironment and strengthening the gut barrier (Chaouch & Benvenuti, 2020). Dietary fiber, being indigestible and unabsorbed by humans, undergoes fermentation by the gut microbiota, producing beneficial metabolites such as short-chain fatty acids (SCFAs), which positively influence the gut microbiota and support gut barrier integrity (Liu et al., 2021). Phytochemicals, including phenolic compounds and betacyanin, have also shown potential in reducing leaked LPS levels and enhancing the expression of tight junction proteins, such as zonula occludens-1 (ZO-1), occludin, and claudin-1 (Bernardi et al., 2020; Khoshbin & Camilleri, 2020). Consequently, fruit by-products rich in dietary fibers and phytochemicals offer promising sources of bioactive compounds for improving gut health.

Dragon fruit (*Hylocereus undatus*) is a tropical fruit of high economic importance, cultivated commercially in several regions worldwide, with Thailand being a

prominent producer, owing to its exceptional nutritional value (Chumroenvidhayakul et al., 2022; Jiang et al., 2021). However, dragon fruit consumption generates a significant amount of waste, particularly in the form of dried dragon fruit peel powder (DFP), which mainly consists of dietary fiber (65%) and phytochemicals, including phenolic compounds and betacyanins (Chumroenvidhayakul et al., 2022). Previous studies have shown that a purified oligosaccharide extract from dragon fruit improves gastrointestinal motility disorders in rats (Khuituan et al., 2019; Pansai et al., 2020). It enhances colonic smooth muscle motility, increases fecal mass, and modulates the gut microbiota by promoting beneficial bacteria (*Bifidobacteria* and *Lactobacilli*) while reducing *Bacteroides* and *Clostridia*. This results in higher concentrations of SCFAs and a strengthened gut immune response (Pansai et al., 2020). Betacyanin extracted from red dragon fruit has also exhibited prebiotic properties by promoting the growth of *Bacteroidetes* at the phylum level and *Akkermansia* at the genus level, while decreasing the proportion of *Firmicutes* and significantly reducing the *Firmicutes*-to-*Bacteroidetes* (F/B) ratio in mouse models fed a high-fat diet (Song et al., 2016).While previous studies have offered valuable insights, there is still limited understanding regarding the specific effects of DFP on gut microbiota composition, SCFA production, intestinal epithelial permeability, and the leakage of LPS markers in rats with diet-induced dysbiosis resulting from a high-fat, high-fructose diet. Therefore, the objective of the current study is to investigate and shed light on the effects of DFP on these variables in rats exposed to a high-fat, high-fructose diet.

4.5 Materials and Methods

    4.5.1   Materials

    To prepare the dragon fruit peel powder (DFP), a previously published method was followed (Chumroenvidhayakul et al., 2022). Initially, fresh dragon fruit peels were washed, removed their epicarp, and dried in a hot air oven at 60 °C for 12 h. The dried peels were then ground, passed through No. 40 sieves, and stored in polyethylene foil bags at a temperature of -20°C. DFP primarily comprises carbohydrates (70.85%), of which 65.17% is dietary fiber and 5.68% is available carbohydrates. Other constituents included 15.91% ash, 6.37% protein, and 5.81% moisture, whereas total fat was the least abundant component (1.06%) (Chumroenvidhayakul et al., 2022).

    4.5.2   Animals

    Forty male Sprague-Dawley rats (250–350 g; 8 weeks of age), were sourced from the Animal Resource Centre. The Animal Ethics Committee of the University of Adelaide approved all experimental protocols related to animal experiments (Approval No.: s-2021-101). Additionally, all animal experiments were conducted in compliance with the 8th edition (2013) of the Australian National Health & Medical Research Council code of practice for the care and use of animals for scientific purposes.

4.5.3 Experimental design

Forty male Sprague-Dawley rats were housed in wire-bottomed cages with four rats per cage. They were provided with environmental enrichment and kept at a controlled temperature of 22 ± 2 °C and a lighting schedule of 12 hours light and 12 hours dark. The rats were acclimated to the facility for two weeks and had ad libitum access to water and the AIN93-M diet. After the acclimatization period, the experimental rats were randomized into one of four groups (n = 8) for 12 weeks: (1) control diet (C); (2) control diet with 5% (*w/w*) DFP (C + 5% DFP); (3) high-fat, high-fructose diet with 10% (*w/v*) fructose water (HFHFr: 20% fat and 60% fructose); and (4) HFHFr with 5% (*w/w*) DFP (HFHFr + 5% DFP). The composition of the experimental diets is shown in Table 10. Body weight was measured weekly, while feed intake was recorded daily throughout the trial period.

Table 10 Composition of experimental diets

Diet composition (%)	Experimental groups			
	C	C + DFP	HFHFr	HFHFr + DFP
Corn starch	53.2	51.8	0	0
Sucrose	10	10	0	0
Fructose	0	0	50	48.8
Casein	20	20	20	20
Sunflower seed oil	7	7	2.5	2.5
Lard	0	0	17.5	17.5
Mineral mixture	4	4	4	4
Vitamin mixture	1	1	1	1
Tert-Butylhydroquinone	0.0014	0.0014	0.0014	0.0014
Choline	0.25	0.25	0.25	0.25
Cellulose	5	1.7	5	1.7
DFP	0	5	0	5
Fructose in drinking water (*w/v*)	0	0	10	10

C: normal diet (AIN 93M); C + DFP: normal diet with 5% (*w/w*) dragon fruit peel powder; HFHFr: high-fat, high-fructose diet; HFHFr + DFP: high-fat, high-fructose diet with 5% (*w/w*) dragon fruit peel powder.

4.5.4   Sample collection

The study involved multiple procedures and measurements carried out at different time points. At week 12, fecal samples (without urine and feed contamination) were collected and analyzed for pH, moisture, and excreted fat content. At the end of the experiment, the animals were anesthetized with isoflurane and then euthanized by exsanguination via the abdominal aorta. Blood samples were taken using a sterile serum tube (non-additive or red top tube; pyrogen-free) and centrifuged at 3,500 ×g for 15 minutes at 4 °C to obtain serum. The obtained serum was used to determine the level of serum lipopolysaccharide binding protein (LBP). To analyze the gut microbiota and short-chain fatty acids (SCFAs), cecal digesta was immediately frozen using liquid nitrogen and stored at -80°C. Segments of the colon were collected, put into sterile tube, and mixed with RNAlater® solution (Sigma-Aldrich, Germany) for gene expression analysis.

4.5.5   Fecal properties analysis

The pH of the fecal sample was measured following the protocol of a previous study (Shang et al., 2017). 0.3 g of fecal sample was mixed with 3 mL of MilliQ water and thoroughly shaken for 1 h. The mixture was then centrifuged at 3,000 ×g for 10 min at room temperature, and the supernatant was collected to measure the pH value by a pH meter (SevenCompact S220 pH/Ion-meter, Mettler-Toledo Ltd., Greifensee, Switzerland).

The fecal moisture was measured using a freeze dryer, following a previously established method (Shannon, Conlon, & Hayes, 2022). Prior to freeze-drying, fresh samples were weighed accurately and stored at −80 °C for 48 h. The samples were then placed in a freeze dryer (Model Alpha 1-2 LDplus; Martin Christ, Osterode, Germany) that had been precooled to −50 °C, immediately sealed, and subjected to vacuum drying until a constant weight was obtained. The mass difference of the fecal samples was determined, and the water content percentage was calculated following the equation:

$$\text{Moisture content (\%)} = \frac{\text{Wet weight of the feces (g) - Dried weight of the feces (g)}}{\text{Wet weight of the feces (g)}} \times 100$$

Fecal lipid excretion was measured with some modifications to a previously established method [19]. The freeze-dried feces (1 g) were ground and extracted using a chloroform-methanol mixture (2:1, *v/v*) while stirring for 24 h. The chloroform phase was collected, and the fat was dried using an oven set at 105 °C until a constant weight was reached. The weight of the dried fat residue was determined and used to calculate the fecal lipid excretion following the equation:

$$\text{Lipid content (\%)} = \frac{\text{Wet weight of extracted fat (g)}}{\text{Wet weight of the feces (g)}} \times 100 \times 4$$

4.5.6   Short chain fatty acids (SCFA) analysis

To analyze SCFA concentrations in cecal digesta, the filtration method of gas chromatography was used, as modified from previous study (Shannon et al., 2022). Initially, the frozen cecal digesta (1 g) was mixed thoroughly with 3 mL of 1.68 mM

heptanoic acid as internal standard. The samples were then centrifuged at 2,095 ×g

for 10 min at 4 °C, and the supernatant was transferred to a pre-cooled tube after the

excess solids were removed by centrifugation at 15,400 ×g for 5 min at 4 °C. The

resulting 300 µL of supernatant was acidified with 10 µL of 1 M phosphoric acid in the

filter vials (Whatman PTFE 0.45 µm Mini-UniPrep tube, Cytiva, Marlborough, MA, USA).

The filter vial was pressed through a compressor and held at the bottom position for

30 sec to ensure gradually smooth motion of solution. The samples were then

loaded onto the GC (model 7890A; Agilent Technologies, Santa Clara, CA, USA)

equipped with a flame ionisation detector and capillary column (Zebron ZB-FFAP, 30

m × 0.53 mm × 1.0 µm, Phenomenex, Lane Cove, NSW, Australia). Helium was used

as the carrier gas, with an initial oven temperature of 90 °C held for 1 min and

increased at 20 °C/min to 190 °C held for 2.5 min. The injector and detector

temperature were 210 °C, and the gas flow and septum purge rates were at 7.7 and

3.0 mL/min, respectively. A series of standard SCFA mixtures with a concentration

gradient were prepared, including acetic acid (17.49 µmol/mL of final concentration),

propionic acid (9.93 µmol/mL of final concentration), isobutyric acid (1.64 µmol/mL

of final concentration), butyric acid (10.90 µmol/mL of final concentration), isovaleric

acid (2.71 µmol/mL of final concentration), valeric acid (2.74 µmol/mL of final

concentration), caproic acid (2.38 µmol/mL of final concentration), and heptanoic

acid (0.84 µmol/mL of final concentration). The final concentrations of fatty acids in

cecal digesta were then calculated in µmol/g cecal content by comparing their peak

areas with the standards curve.

4.5.7   Serum lipopolysaccharide-binding protein (LBP) analysis

Serum LBP was measured to assess LPS leakage in the systemic circulation

using commercially available kits according to the manufacturer's instructions

(RTDL00620, LBP Rat ELISA kit, Assay Genie, Dublin, Ireland).

4.5.8   Tissue RNA extraction and quantitative real-time polymerase chain

reaction (qPCR)

Total RNA was extracted from the colon tissue (40 mg) using bead beating and

the ReliaPrep™RNA tissue miniprep system kit (Promega, WI, USA) according to the

manufacturer's instructions. RNA concentration was determined using a

Nanodrop™2000 Spectrophotometer (Thermo Fisher Scientific, MA, USA) before

reverse transcription into cDNA using the GoScript™ reverse transcriptase (Promega,

WI, USA).

To determine mRNA expression levels, qPCR was performed using iTaq™

Universal SYBR® Green Supermix (Bio-Rad, CA, USA) and a CFX384 Touch™ Real-Time

PCR Detection system (Bio-Rad, CA, USA). The target genes, including zonula

occludens (ZO-1), occludin (Ocln), claudin-1 (Cldn-1), and Toll-like receptor 4 (TLR4),

were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a

reference gene. The primers were obtained from the PrimePCR™ PCR Primers (Bio-

Rad, CA, USA). The cycle profile consisted of polymerase activation at 95 °C for 2 min,

denaturation at 95 ℃ for 5 sec, annealing at 60 ℃ for 30 sec repeated for 40 cycles,

and extension at 65 ℃ for 5 sec. The PCR products were analyzed for amplicon

homogeneity by melting curve analysis (65-95 ℃; 0.5 ℃ increments, 5 sec/step). The

expression of housekeeping genes did not vary between diets (p = 0.81) in duplicates.

Each sample and standard were run simultaneously in duplicate, and the average of

each duplicate was used for statistical analysis. The results were presented as relative

differences of the threshold cycle number (ΔC_t) between each sample using the $2^{-\Delta\Delta Ct}$ method.

4.5.9 Bacterial 16S rRNA sequencing

The percentage relative abundance of bacteria at each taxonomic level, from

phylum to species, in cecal samples of experimental rats after 12 weeks of feeding

was determined by 16S rRNA gene sequencing using the Roche 454 platform at the

Australian Genome Research Facility (AGRF), University of Queensland, Brisbane, QLD,

Australia. PCR amplification of the V3-V4 region of bacterial samples was sequenced

on an Illumina MiSeq platform. Primer sequences are as follows: 341F forward primer

5′-CCTAYGGGRBGCASCAG-3′, and 806R reverse primer 5′-GGACTACNNGGGTATCTAAT-3′,

with an amplicon sequencing read length of 300 bp. The bioinformatic analysis

involved demultiplexing, quality control, Amplicon Sequence Variant (ASV) calling,

and taxonomic classification. Diversity profiling analyses were performed using

Quantitative Insights into Microbial Ecology 2 platform (QIIME 2 version 2019.7,

Caporaso Lab Pathogen and Microbiome Institute, Northern Arizona University,

Flagstaff, AZ, USA). The remaining high-quality reads were then used to identify chimeras, and the pooled high-quality reads were clustered into operational taxonomic units (OTUs). This study obtained approximately 24,000 OTUs per sample, and OTU taxonomic assignments were generated using the SILVA-132-99 classifier open reference database under a confidence threshold of 97% (Quast et al., 2013).

4.5.10 Statistical analysis

Data were expressed as means ± S.E.M. for each treatment group (n = 8). A two-way ANOVA with diet (fat/fructose) and treatment (DFP) as main effects was performed. The Least Significant Difference test was used to determine significant differences between means ($p < 0.05$). IBM SPSS 22.0 was used for statistical analyses, and GraphPad Prism 9.5 was used for graph generation. Relative abundances at the phylum and genus level were evaluated using a Shapiro-Wilk test for normality. Alpha and beta diversity analyses utilized the Shannon index and Bray-Curtis-PCoA plot, respectively, implemented in R studio Package. Core microbiota analyses focused on bacterial taxa present in >0.1% of all individuals and employed Kruskal-Wallis's rank testing. False Discovery Rate analysis by the Benjamini-Hochberg procedure was applied for multiple testing adjustments.

4.6 Results

4.6.1   Effects on the body and organ weights

Based on the data presented in Table 11, rats fed a HFHFr diet exhibited lower body weight gain compared to those on a normal diet. However, supplementation with DFP effectively mitigated the increase in body weight in both the normal diet and HFHFr groups. Additionally, rats fed the HFHFr diet showed a significant decrease in daily food intake compared to the normal diet group, while a decrease in food intake was observed in rats fed a normal diet with DFP supplementation.

Furthermore, the HFHFr diet stimulated a higher daily intake of fluids. Remarkably, the addition of DFP to the HFHFr diet led to a significant reduction in daily fluid intake compared to the HFHFr group without DFP supplementation.

Moreover, the total calorie intake was significantly higher in the HFHFr group compared to the normal diet group, indicating increased calorie consumption due to the high-fat, high-fructose diet. However, the inclusion of DFP resulted in a reduced total calorie intake in both the HFHFr and normal diet groups. Despite no change in the weight of cecal tissue among the various dietary treatments, significant differences were observed in the weight of cecal digesta (as shown in Table 11). The groups receiving DFP supplementation, namely the C + DFP and HFHFr + DFP groups, showed markedly higher weights of cecal digesta compared to the groups without DFP supplementation. These results suggest that DFP supplementation might influence the weight of cecal digesta.

Interestingly, the length of the small intestine in rats fed the HFHFr diet was significantly longer compared to the normal diet group. This finding indicates that the consumption of a high-fat, high-fructose diet induces intestinal adaptation, leading to an increase in small intestine length. However, DFP supplementation did not show any significant effect on the alteration of small intestine length.

4.6.2   Effects of DFP on fecal pH, moisture, and excreted fats

After 12 weeks of feeding, a significant increase in fecal pH was observed in the HFHFr group (Table 11). However, the rats that received a diet enriched with DFP exhibited a markedly lower fecal pH compared to those without DFP. This suggests that DFP supplementation may have an acidifying effect on the fecal environment.

The inclusion of DFP in both diet formulations resulted in a significant increase in fecal moisture content. Compared to the normal diet, the addition of DFP led to a 29.5% increase in fecal moisture content. Similarly, when compared to the HFHFr diet, the DFP supplementation caused a substantial 49.0% increase in fecal moisture content. This indicates that DFP may contribute to increased water content in the feces, potentially affecting stool consistency and hydration. Furthermore, incorporating DFP into the diet resulted in a substantial increase in fecal fat excretion for both dietary groups. Specifically, the fecal lipid excretion of the C + DFP and HFHFr + DFP groups was 1.2-fold and 1.9-fold greater, respectively, compared to those without DFP. This suggests that DFP supplementation may enhance the elimination of dietary fat through fecal excretion.

Table 11 The effect of dragon fruit peel (DFP) on body weight change, diet intake, and gut organ weight and length, and fecal properties in normal diet (C), and high-fat, high-fructose diet (HFHFr) diet-fed rats

Parameters	Groups				Significance of effects		
	C	C + DFP	HFHFr	HFHFr + DFP	Diet	DFP	Diet × DFP
Body weight gain (g)	329.8 ± 24.2	279.3 ± 18.9*	303.8 ± 11.4	248.5 ± 12.2#	NS	<0.01	NS
Food intake (g/rat/day)	31.2 ± 0.6	28.1 ± 0.3*	20.9 ± 0.5*	19.9 ± 0.4	<0.001	<0.001	<0.05
Fluid intake (mL/rat/day)	37.7 ± 1.1	33.5 ± 0.3	100.2 ± 3.0*	81.1 ± 3.5#	<0.001	<0.001	<0.01
Calorie intake (kcal/rat/ day)	123.5 ± 2.4	109.6 ± 1.3*	142.5 ± 2.3*	130.1 ± 2.6#	<0.001	<0.001	NS
Cecum tissue (g)	0.8 ± 0.04	0.9 ± 0.06	0.8 ± 0.03	0.8 ± 0.04	NS	NS	NS
Cecum digesta (g)	2.3 ± 0.2	2.8 ± 0.1	1.7 ± 0.2*	2.2 ± 0.2#	<0.001	<0.01	NS
Length of small intestine (mm)	132 ± 2.2	127 ± 3.1	139 ± 3.1*	136 ± 3.0	<0.01	NS	NS
Length of colon (mm)	22 ± 0.8	21 ± 0.7	22 ± 0.7	22 ± 0.7	NS	NS	NS
Fecal pH	7.6 ± 0.03	7.3 ± 0.06*	7.9 ± 0.06*	7.8 ± 0.01	<0.001	<0.01	NS
Fecal moisture (%)	56.3 ± 0.7	72.9 ± 0.2*	45.4 ± 0.4*	67.7 ± 0.7#	<0.001	<0.001	<0.001
Fecal lipid excretion (%)	3 ± 0.1	3.5 ± 0.1*	7.4 ± 0.1*	13.1 ± 0.1#	<0.001	<0.001	<0.001

The results are expressed as mean ± SEM (n = 8). Two-way ANOVA revealed effects of diet, DFP and their interaction; NS: non-significant differences. * $p < 0.05$ compared to C. # $p < 0.05$ compared to HFHFr.

4.6.3   Effects of DFP and on serum lipopolysaccharide-binding protein (LBP)

and colonic Toll-like receptor 4 (TLR4) gene expression

The concentration of serum LBP was measured to assess the primary transporter of circulating lipopolysaccharides (LPS), which is associated with metabolic endotoxemia. Figure 11A demonstrates that rats fed the HFHFr diet had a significant 3.7-fold increase in serum LBP concentration compared to rats on the regular diet. This suggests higher exposure to LPS and a greater risk of metabolic endotoxemia in the HFHFr group. However, the addition of DFP to the HFHFr diet effectively reversed the elevation of serum LBP levels, bringing them back to a similar level as that of rats fed the normal diet. This indicates that DFP supplementation can mitigate the increase in serum LBP induced by HFHFr feeding.

Additionally, Figure 11B shows the abundance of Toll-like receptor 4 (TLR4) mRNA expression in the colon. TLR4 is a key receptor involved in the recognition of LPS and triggering of the inflammatory response. Among the groups, the highest TLR4 expression was observed in the colon of rats fed the HFHFr diet, indicating increased inflammatory signaling. However, DFP supplementation significantly reduced TLR4 expression in the HFHFr group.

(A)

(B)

Figure 11 The effect of dragon fruit peel (DFP) on (A) serum lipopolysaccharide-binding protein (LBP), and (B) mRNA expression of Toll-like receptor 4 (TLR4) in the colonic tissue of rats.

The results are expressed as mean ± SEM (n = 8). Two-way ANOVA revealed effects of diet, DFP and their interaction; NS: non-significant differences. $^{*}p < 0.05$ compared to C. $^{#}p < 0.05$ compared to HFHFr.
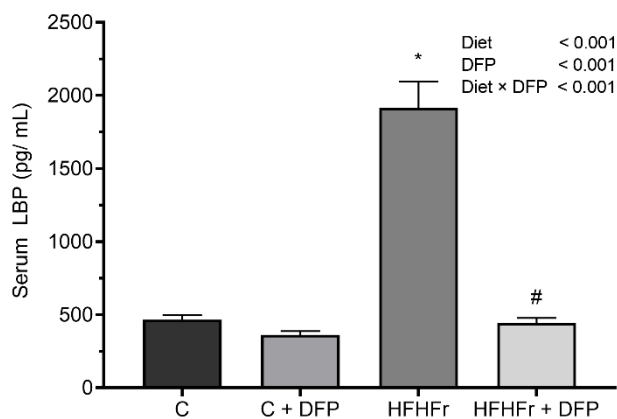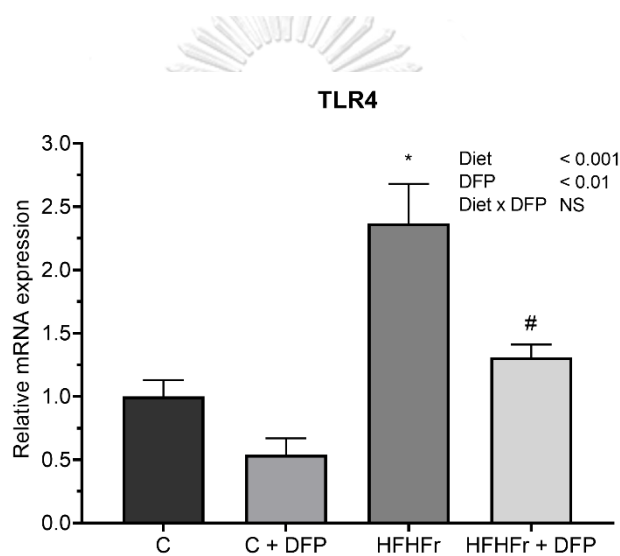
4.6.4   Effects of DFP on gene expression of tight junction proteins in colon

Figure 12 illustrates the mRNA expression levels of colonic tight junction proteins in rats, which are closely linked to intestinal barrier dysfunction and low-grade chronic inflammation when they are compromised. As expected, dietary modifications involving high fat and fructose were found to significantly decrease the mRNA expression of these tight junction genes, indicating compromised intestinal barrier integrity. However, treatment with DFP resulted in a significant recovery in the gene expression of ZO-1, occludin, and claudin-1 in the colon tissues of rats in the HFHFr + DFP group. This suggests that DFP supplementation can effectively mitigate the negative effects of HFHFr diet on the expression of tight junction proteins, thereby improving intestinal barrier function. Furthermore, supplementing DFP to the normal diet led to a remarkable upregulation of the mRNA levels claudin-1 compared to the normal diet group. This indicates that DFP supplementation in the context of a normal diet can enhance the expression of tight junction proteins, further strengthening the intestinal barrier.
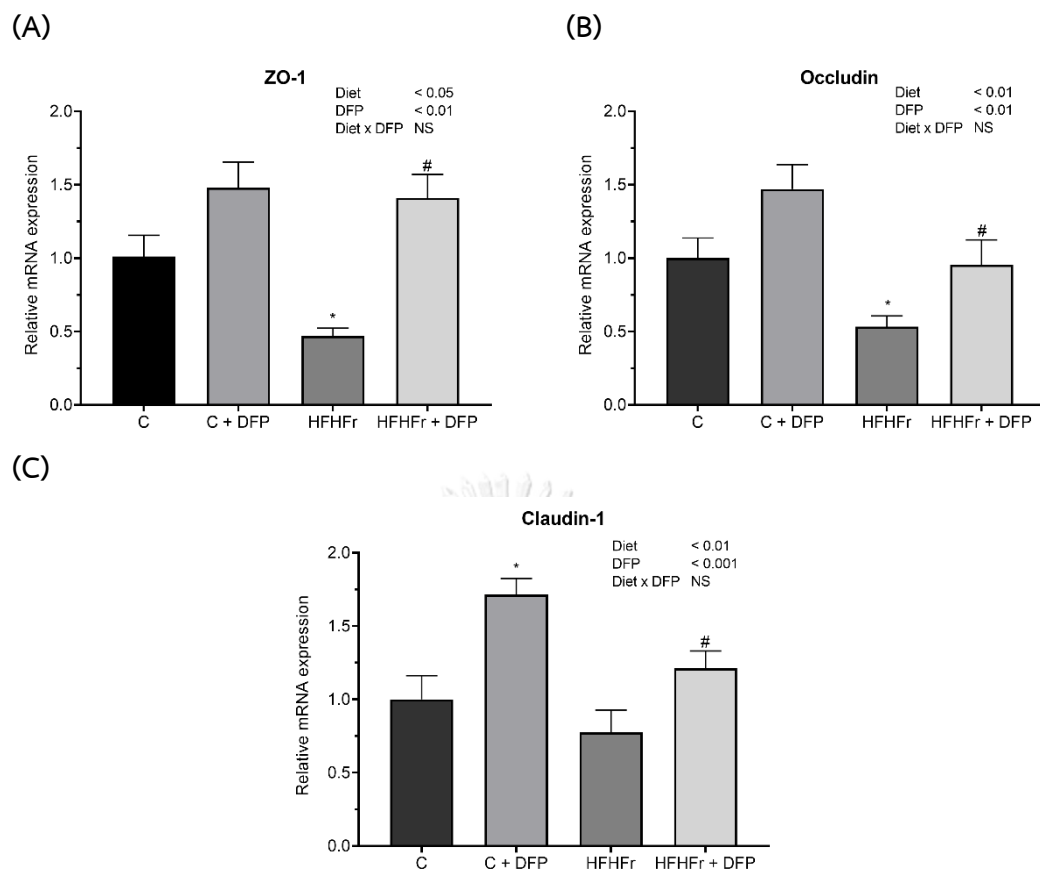
Figure 12 The effect of dragon fruit peel (DFP) on the mRNA expression of (A) Zonula

occludens-1 (ZO-1), (B) Occludin, and (C) Claudin-1 in the colonic tissue of normal

diet (C), and high-fat, high-fructose (HFHFr) diet-fed rats.

The results are expressed as mean ± SEM (n = 8). Two-way ANOVA revealed effects

of diet, DFP and their interaction; NS: non-significant differences. $*p < 0.05$ compared

to C. $^{\#}p < 0.05$ compared to HFHFr.

4.6.5   Effects of DFP on the cecal microbiome modulation

Figure 13 provides an overview of the gut microbiota composition at the phylum level and highlighting major species differences. It was discovered that the cecum is primarily populated by eight dominant phyla: *Firmicutes*, *Actinobacteria*, *Patescibacteria*, *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Cyanobacteria*, and *Deferribacteres*. While the analysis of alpha and beta diversity, as depicted in Figure 14, indicated that supplementation with DFP had no significant effect on the bacterial biodiversity of the cecal contents.

Although no significant differences were observed in the abundance of *Firmicutes* among the experimental groups, the rats fed the HFHFr diet exhibited a markedly higher abundance of *Bacteroidetes* compared to the C + DFP group (Figure 15). Furthermore, the ratio of *Firmicutes* to *Bacteroidetes* (F/B ratio) was significantly higher in the rats treated with DFP compared to those without DFP. This suggests that DFP may influence the relative abundance of these two phyla in the gut. In contrast, there was a noticeable decrease in the levels of *Actinobacteria* in rats fed the HFHFr diet ($p < 0.05$). Additionally, the levels of *Lentisphaerae* were significantly higher in the HFHFr group compared to the other groups, suggesting the presence of DFP may exert a moderating effect on the abundance of *Lentisphaerae* in HFHFr based diet.

This study also investigated the variations in the bacterial community at the genus level in rat cecal samples (Figure 16). Through high-throughput sequencing, a total of 55 bacterial genera were identified, with *Allobaculum, Blautia, Lachnospiraceae*_unclassified, *Lactobacillus*, and *Roseburia* being the most abundant across all experimental groups.

A comprehensive analysis was performed to assess the statistical significance of differences between groups at the genus level, revealing 14 species belonging to different genera. Figure 17 shows that the HFHFr group exhibited a significant increase in the abundance of potential pathogenic bacteria, including *Romboutsia, Dubosiella, Clostridium sensu stricto* 1, and *Peptostreptococcaceae* unclassified, in comparison to the other groups.

When comparing the four groups, both the C + DFP and HFHFr + DFP groups exhibited a similar pattern of gut microbiota changes (Figure 18). The abundance of beneficial bacteria, such as *Lachnospiraceae*_unclassified, *Christensenellaceae* R-7 group, *Lachnospiraceae* NK4A136 group, *Ruminococcus* 1, *Erysipelotrichaceae*_unclassified, *Ruminiclostridium* 9, *Ruminiclostridium* 5, and *Ruminiclostridium* 6 was observed in rats from both groups. However, an increase in the levels of *Eubacterium xylanophilum* group, *Coriobacteriaceae* UCG-002, and *Lachnospiraceae* UCG-001 was observed exclusively in the HFHFr + DFP diet fed rats.
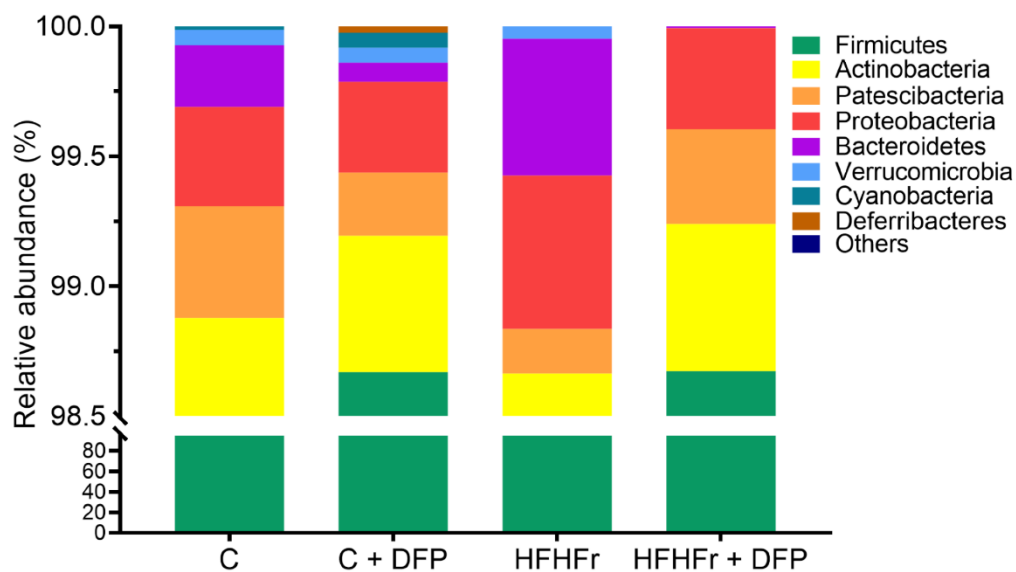
Figure 13 The impact of dragon fruit peel (DFP) on composition of the gut microbiota

at phylum level in normal diet (C), and high-fat, high-fructose (HFHFr) diet-fed rats.

The percentage of relative abundance for only the classified genera with a relative

abundance above the 0.01% cut-off level, while "Others" represents all genera with a

mean relative abundance less than 0.01%.

(A)
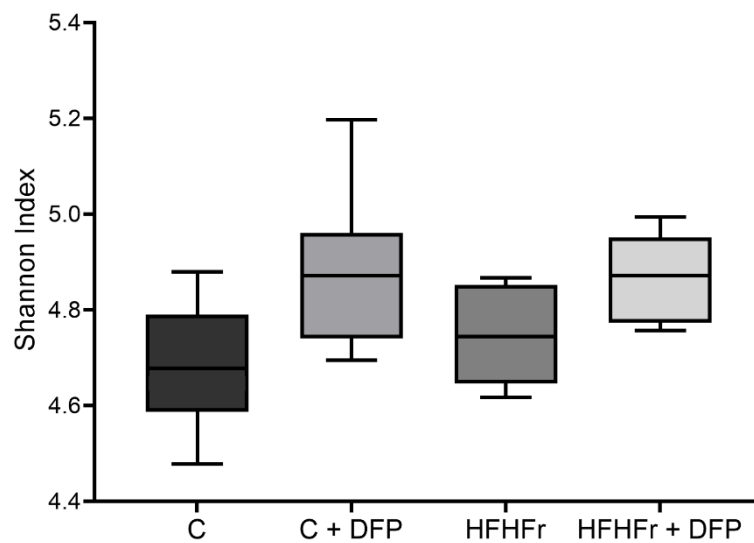
(B)
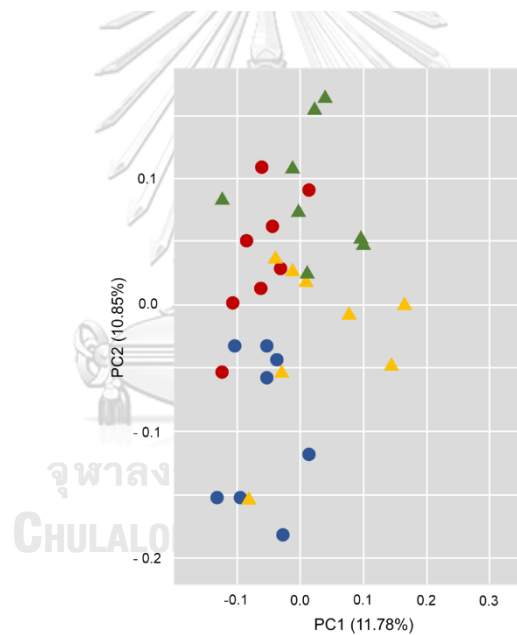
Figure 14 Effect of dragon fruit peel (DFP) on gut microbial community structure in rats fed normal diet (C) and high-fat, high-fructose diet (HFHFr) (A) Shannon index, and (B) Principal coordinates analysis (PCoA) plot of Bray-Curtis's distance.

The results are presented as means ± SEM, n = 8. ● C; ● C + DFP; ▲ HFHFr; ▲ HFHFr + DFP.
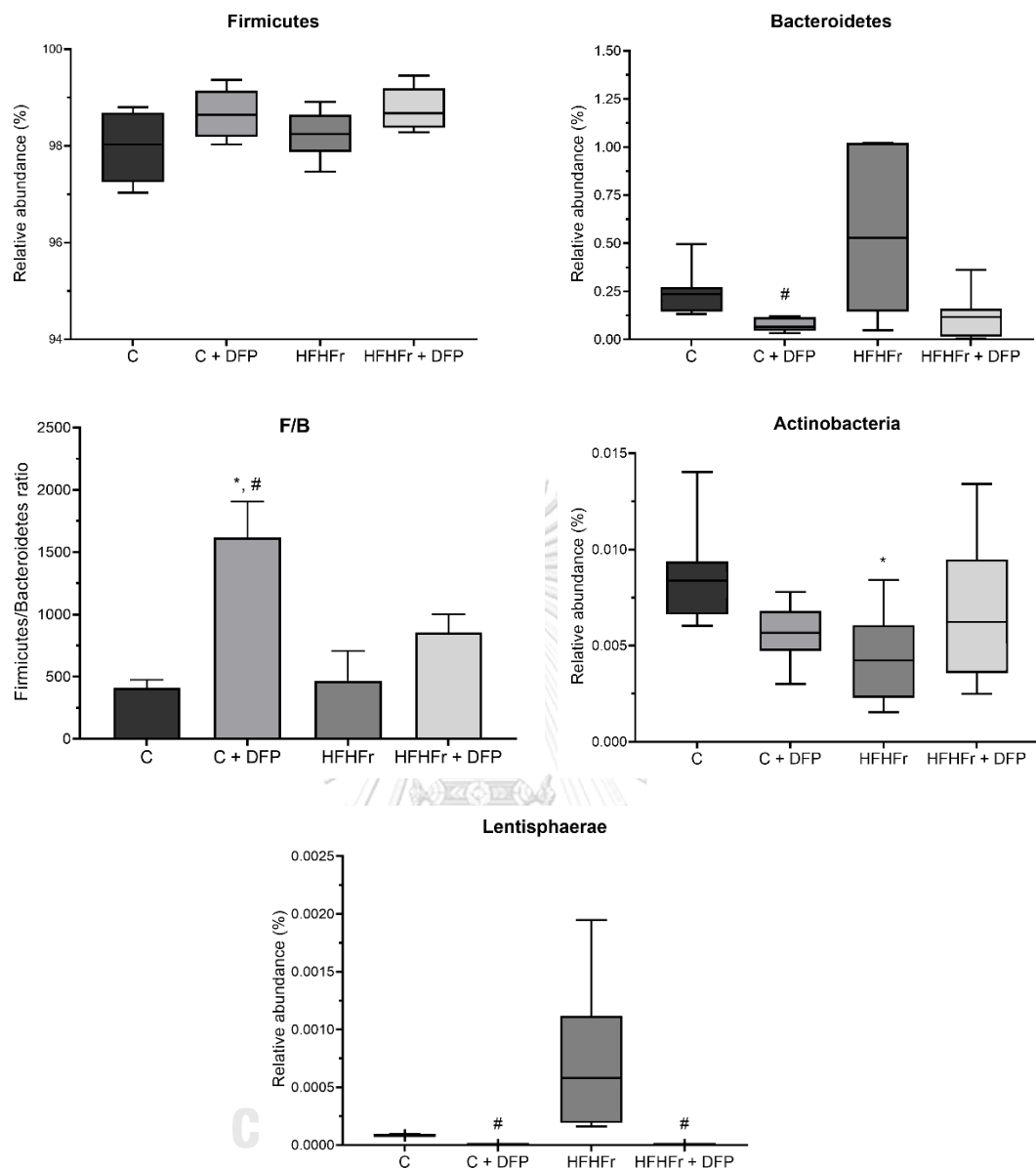
Figure 15 The species with major differences in the phylum, including *Firmicutes*, *Bacteroidetes*, *Firmicutes/Bacteroidetes* (F/B) ratio, *Actinobacteria*, and *Lentisphaerae*.

The data is presented as box plots, with horizontal lines indicating the mean values of each group (n = 8). Significance is denoted as follows: *$p < 0.05$ compared to C; # $p < 0.05$ compared to HFHFr.
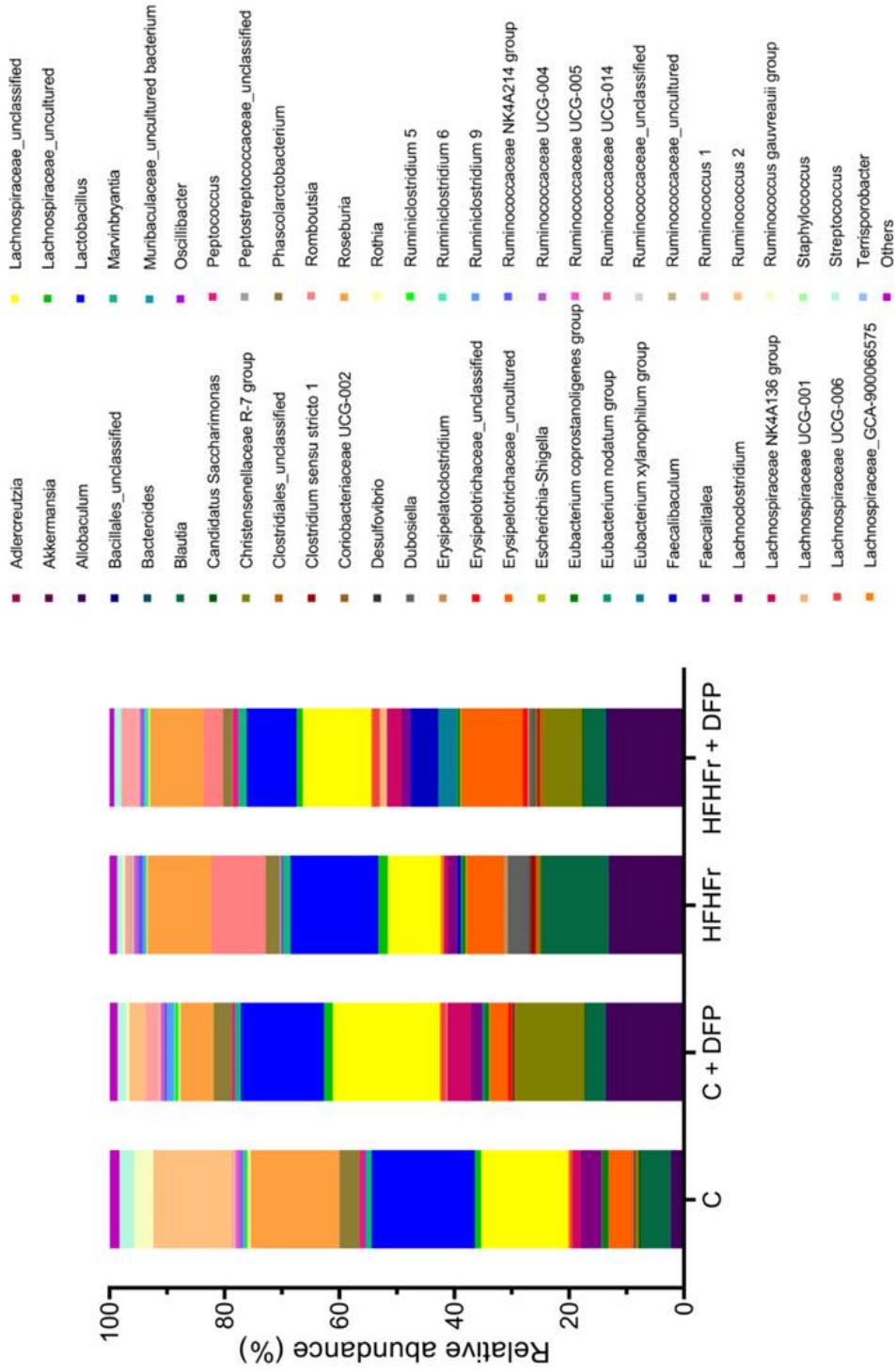
Figure 16 Effect of dragon fruit peel (DFP) on the percentage of relative abundance of gut microbiota composition at the genus level in normal diet (C), and high-fat, high-fructose (HFHFr) diet-fed rats.
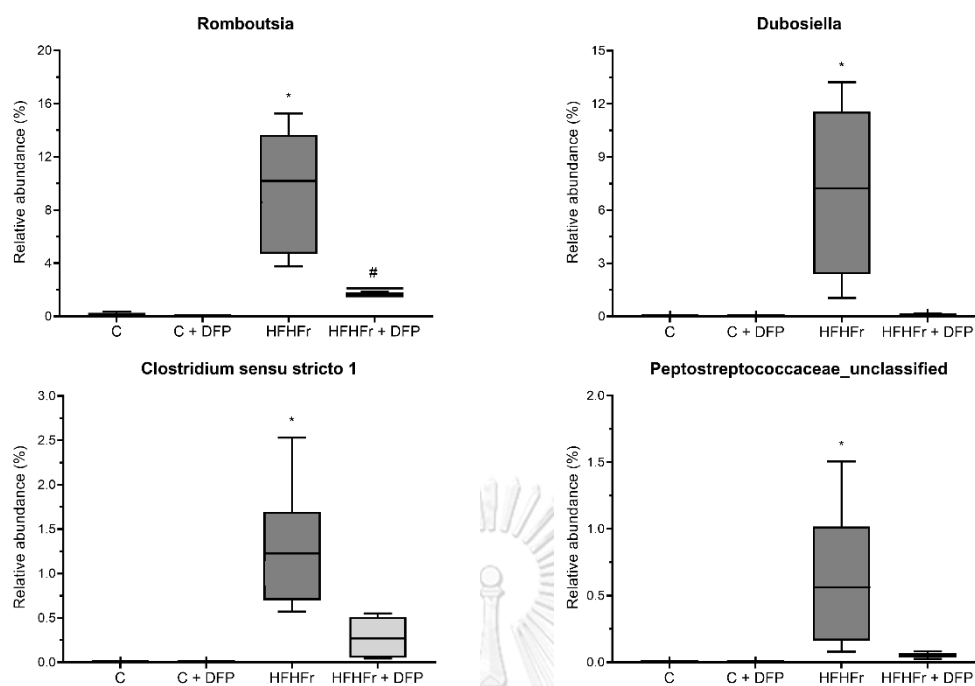
Figure 17 Box plots of significance microbiota composition in high-fat, high-fructose (HFHFr) diet-fed rats.

The data is presented as box plots, with horizontal lines indicating the mean values of each group (n = 8). Significance is denoted as follows: $^*p < 0.05$ compared to C; $^\#p < 0.05$ compared to HFHFr.
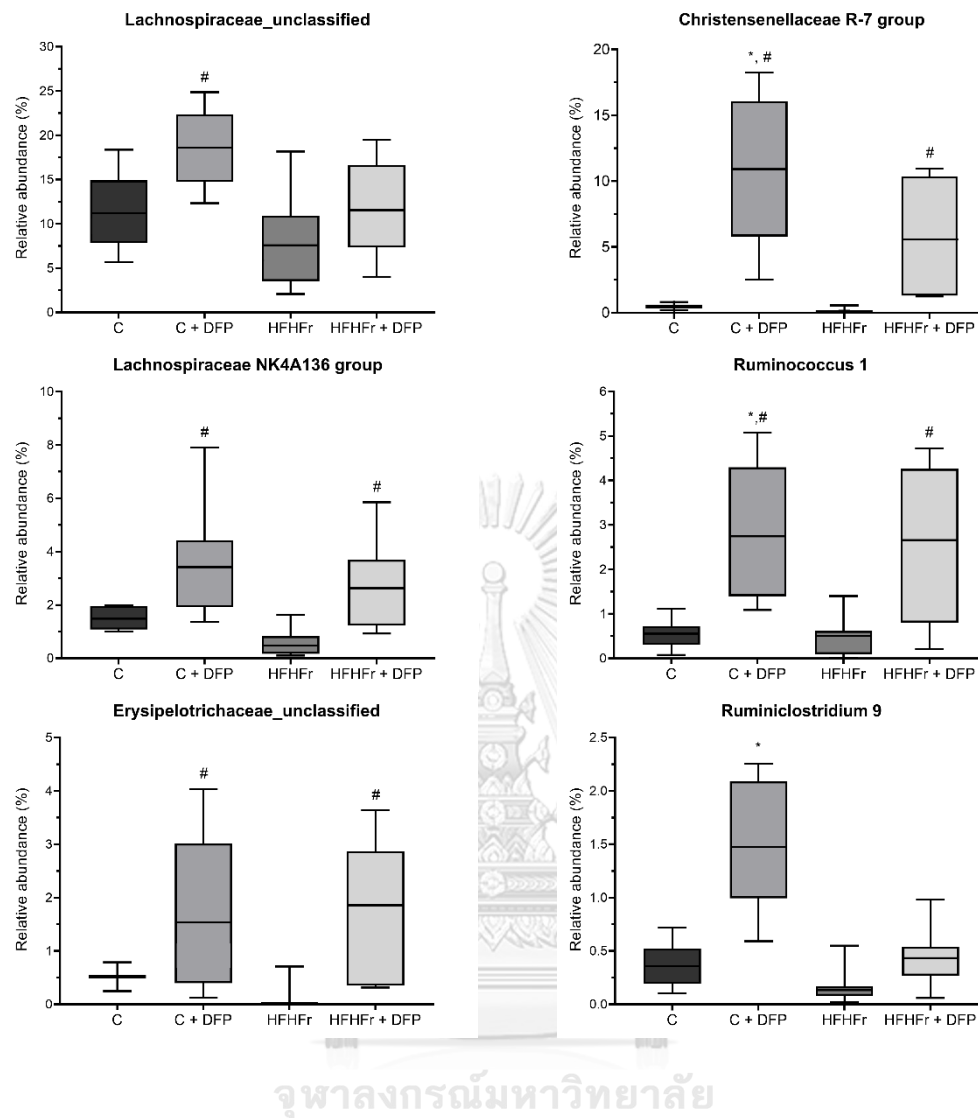
Figure 18 Box plots of significance microbiota composition in in normal diet (C), and

high-fat, high-fructose (HFHFr) diet- plus dragon fruit peel (DFP) fed rats.

The data is presented as box plots, with horizontal lines indicating the mean values

of each group (n = 8). Significance is denoted as follows: $^*p < 0.05$ compared to C; $^\#$

$p < 0.05$ compared to HFHFr.
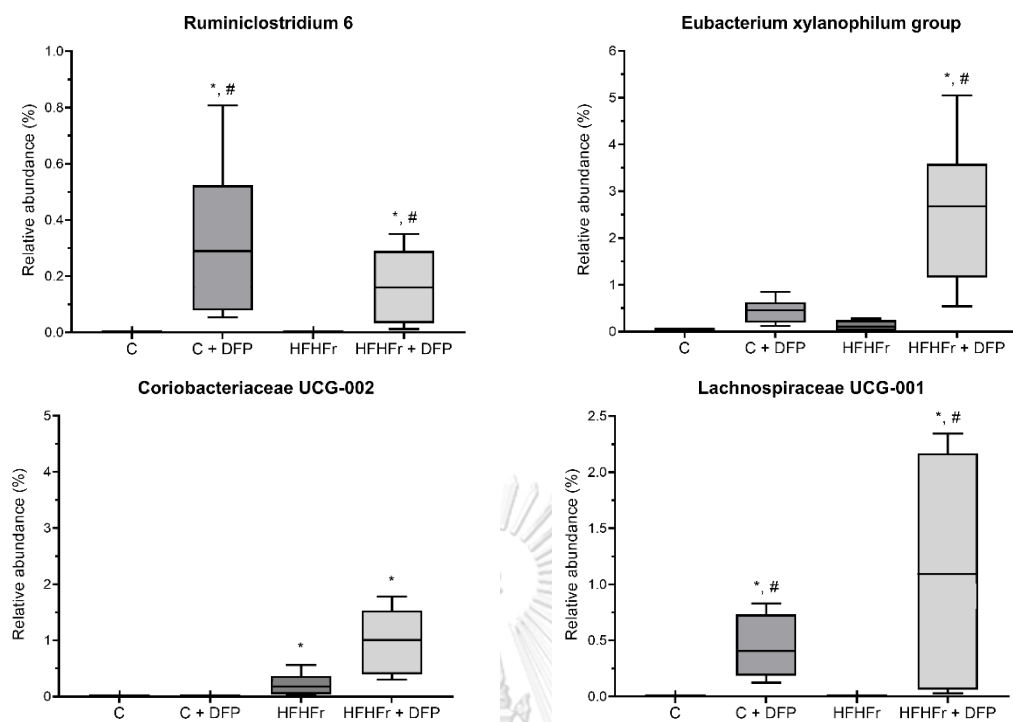
Figure 18 *Cont.*

4.6.6   Effects of DFP on the cecal short-chain fatty acids (SCFAs) composition

Table 12 illustrates the distribution of SCFAs in the cecal digesta among the different experimental groups. The C + DFP group displayed the highest proportions of individual SCFAs as well as total SCFAs. The results also found that Rats consumed the HFHFr diet exhibited significantly lower levels of total SCFAs, acetate, and isobutyrate in their cecum. The addition of DFP to the HFHFr diet resulted in a substantial improvement in total SCFA levels. Specifically, the HFHFr + DFP group demonstrated higher concentrations of acetate, propionate, and isobutyrate compared to the rats fed the HFHFr diet alone. This indicates that DFP supplementation in the normal diet enhances SCFA production in the cecum. Additionally, it significantly mitigates the adverse impacts of the HFHFr diet-induced decline in total SCFAs.

Table 12 The effect of dragon fruit peel (DFP) on cecal short chain fatty acids (SCFAs) concentration in normal diet (C), and high-fat, high-fructose diet (HFHFr) diet-fed rats

SCFA concentration (μmol/g cecal digesta)	Groups				Significance of effects		
	C	C + DFP	HFHFr	HFHFr + DFP	Diet	DFP	Diet × DFP
Acetate	45.52 ± 1.44	77.50 ± 2.62*	39.22 ± 1.52*	68.43 ± 2.56#	<0.001	<0.001	NS
Propionate	11.47 ± 0.47	14.52 ± 0.74*	8.98 ± 0.46*	11.88 ± 0.67#	<0.001	<0.001	NS
Butyrate	10.95 ± 0.33	16.52 ± 0.86*	10.25 ± 0.45	11.25 ± 1.32	<0.001	<0.001	<0.01
Iso-butyrate	1.30 ± 0.08	1.71 ± 0.07*	1.06 ± 0.04*	1.55 ± 0.04#	<0.01	<0.001	NS
Total SCFA	71.60 ± 1.85	110.95 ± 5.19*	64.17 ± 2.34*	101.27 ± 3.22#	<0.05	<0.001	NS

The results are expressed as mean ± SEM (n = 8). Two-way ANOVA revealed effects of diet, DFP and their interaction; NS: non-significant differences. * $p < 0.05$ compared to C. # $p < 0.05$ compared to HFHFr.

4.6.7   Correlation analysis between gut microbiota and cecal SCFA

To further investigate the intestinal bacteria strains involved in SCFA production

and their metabolites, Spearman's rank correlation analysis was conducted between

the relative abundances of the microbial community and SCFAs (Figure 19). The

results revealed that 30 bacterial strains exhibited significant associations with at least

one of the evaluated cecal SCFAs. The concentrations of total SCFAs and acetate

showed positive correlations with the abundance of the *Christensenellaceae* R-7

group (r = 0.686 and 0.712), *Erysipelotrichaceae* unclassified (r = 0.576 and 0.528),

Eubacterium xylanophilum group (r = 0.604 and 0.625), *Lachnospiraceae* NK4A136

group (r = 0.576 and 0.606), *Lachnospiraceae* UCG-001 (r = 0.644 and 0.616),

Ruminiclostridium 6 (r = 0.633 and 0.680), and *Ruminococcus* 1 (r = 0.528 and 0.545).

Furthermore, *Dubosiella* and *Ruminococcaceae* UCG-005 displayed moderate-to-

strong negative correlations. Almost all bacterial strains significantly associated with

acetate excretion also exhibited associations with propionate or butyrate excretion,

with *Lactobacillus* presenting a moderate positive correlation with propionate (r =

0.472). Furthermore, isobutyrate demonstrated a strongly significant positive

correlation (*p* < 0.001) with *Peptococcus* (r = 0.532), *Ruminiclostridium* 5 (r = 0.542),

and *Ruminococcus* 2 (r = 0.513), while *Clostridium sensu stricto* 1 (r = 0.687),

Dubosiella (r = 0.571), *Faecalibaculum* (r = 0.515), *Peptostreptococcaceae*

unclassified (r = 0.525), and *Romboutsia* (r = 0.651) displayed negative correlations with isobutyrate (*p* < 0.001).
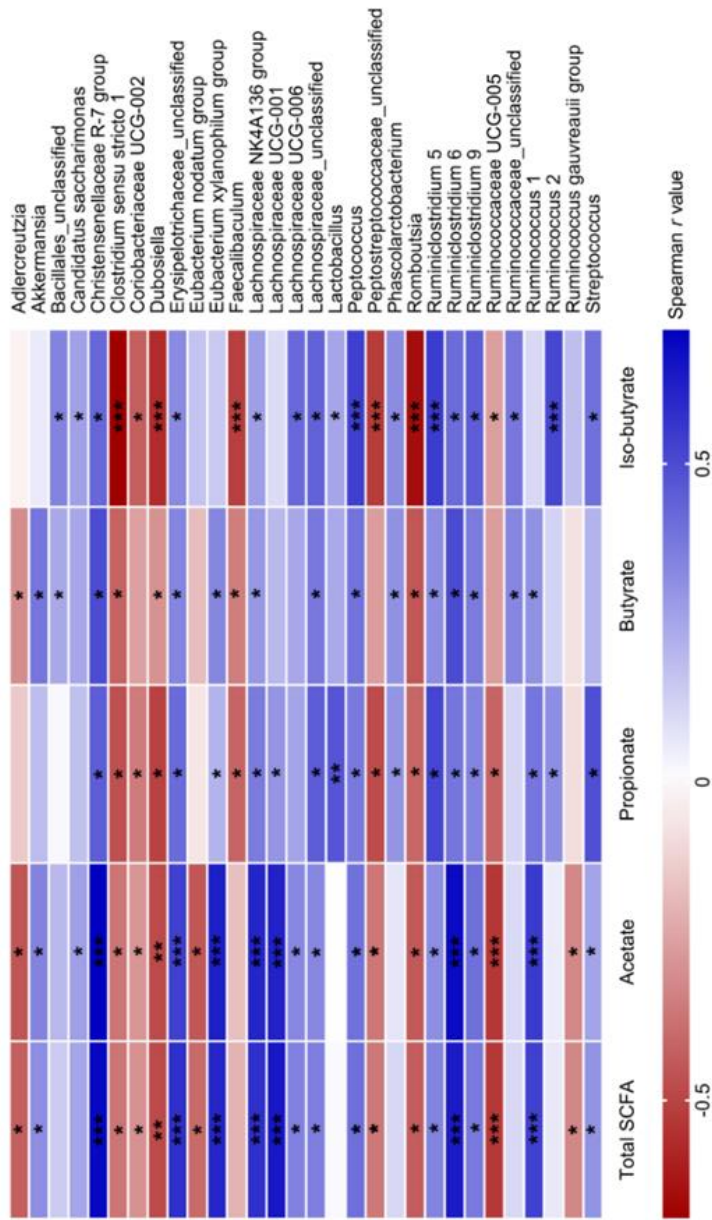
Figure 19 Heatmap of Spearman's correlation between cecal microbiota at the genus level and short-chain fatty acids (SCFAs), including total SCFAs, acetate, propionate, butyrate, and isobutyrate in the cecum of experimental rats.

The color scale indicates the Spearman's correlation coefficients, with blue denoting a positive association, red denoting a negative association, and white denoting no association. FDR-adjusted p-values were obtained using the Benjamini-Hochberg multiple test correction. Significance was indicated by *, **, and *** for $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

4.7 Discussion

This study investigates the effects of DFP on gut health in rats fed a normal or high-fat, high-fructose (HFHFr) diet. Previous research has shown that the HFHFr diet negatively impacts gut function, leading to increased intestinal barrier dysfunction, imbalances in gut bacteria, and changes in the gut microbiota composition. These effects ultimately contribute to systemic inflammation and metabolic disturbance (Rachael G. Horne et al., 2020; Rizzello et al., 2019). The study showed that rats consuming the HFHFr diet exhibited increased fluid and calorie intake, while their food consumption decreased by 33.0% compared to those on a normal diet during the 12-week feeding period. However, there were no significant differences in body weight gain between the HFHFr and normal diet groups. These effects may be attributed to the palatability of fructose, which enhances taste and promotes increased fluid and calorie consumption (Cavalcanti, Roseira, Leandro, & Arruda, 2022; Mamikutty et al., 2014). The decreased food intake may be a compensatory mechanism to regulate energy balance homeostasis and prevent excessive weight gain, given the higher energy density of the HFHFr diet (5 kcal/g) (Cavalcanti et al., 2022). In terms of intestinal morphology, the findings of this study demonstrate that chronic consumption of a Western-style diet leads to an increase in the length of the rat small intestine, which aligns with previous research (Segú et al., 2022). This observation suggests the existence of a dietary adaptation that aims to enhance the availability of calories from food intake. Similar phenomena have been observed in

other research studies, which have consistently established a connection between the consumption of dietary macronutrients and morphological changes in the intestine (Teshfam et al.,2010; Segú et al., 2022).

Intriguingly, the addition of DFP effectively counteracted the increase in body weight associated with both the normal diet and the HFHFr diet. Furthermore, DFP supplementation led to a reduction in fluid and calorie intake while simultaneously increasing the mass of cecal content in the group that received the HFHFr diet. These positive effects can be attributed to the significant amount of dietary fiber present in DFP, constituting 65% of its composition, along with its unique hydration properties (Chumroenvidhayakul et al., 2022). By increasing the viscosity of the stomach digesta and promoting moisture retention in the cecal region. This is achieved by slowing down the gastric emptying rate and enhancing feelings of satiety, effectively reducing hunger (Chumroenvidhayakul et al., 2022; Tan et al., 2017). As a result, the rats experienced a reduction in body weight gain and fluid intake.

A well-balanced fecal profile, characterized by reduced pH, and increased moisture, is closely associated with optimal gut health and function (Shang et al., 2017; Spiller, 2001). Our findings revealed that the inclusion of DFP in both diets led to higher moisture content and lower pH levels in the feces. These outcomes can be attributed to the unique physicochemical properties of DFP, such as its capacity to retain water and its swelling ability, which contribute significantly promote SCFA production and improve of fecal water content (Chumroenvidhayakul et al., 2022;

Shang et al., 2017; Spiller, 2001). Additionally, when DFP was combined with the HFHFr diet, there was a marked increase in the excretion of fecal fat. The dietary fibers present in DFP have the ability to bind with bile acids and form physical viscous gels, thereby interfering with fat digestion and absorption. Our unpublished data suggests that DFP has the potential to bind both primary and secondary bile acids, effectively delaying the digestion and absorption of dietary fats. As a result, favorable fecal characteristics are observed, and excessive uptake of dietary fats is prevented (Usuda, Okamoto, & Wada, 2021).

Previous studies have documented that the consumption of a high-fat, high-fructose diet or a Western-style diet can disturb the intestinal flora, stimulate the production of pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS) and compromise the integrity of the intestinal barrier (Lambertz, Weiskirchen, Landert, & Weiskirchen, 2017; Usuda et al., 2021). These disruptions contribute to the development of endotoxemia, chronic low-grade systemic inflammation, and metabolic disturbance (Lambertz et al., 2017; Rohr, Narasimhulu, Rudeski-Rohr, & Parthasarathy, 2020). The binding of LPS to a pathogen recognition receptor, Toll-like receptor 4 (TLR4), on entherocytes triggers the production of pro-inflammatory such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)– 6, and IL-1 via nuclear factor-κB (NF-κB) pathway, subsequently promotes cell apoptosis, which contribute to increases in intestinal permeability

(Lambertz et al., 2017). Metabolic endotoxemia is often observed in patients with inflammatory bowel disease (IBD) and other intestinal disorders like necrotizing enterocolitis (Rohr et al., 2020). While measuring serum LPS levels is challenging due to fluctuations and technical limitations, serum lipopolysaccharide-binding protein (LBP) is a reliable long-term marker for assessing exposure to metabolic endotoxemia (Fuke et al., 2023). In this study, we found that HFHFr diet increased serum LBP concentration and upregulated mRNA expression of TLR4 expression in colonic tissue compared to the normal diet group, indicating an exposure of bacterial endotoxemia. However, DFP supplementation attenuated this effect by restoring LBP levels and TLR4 gene expression to those observed in the normal group. This suggests that DFP might have the potential to reduce bacterial LPS translocation by increasing the expression of tight junction proteins, thereby potentially lowering LBP concentration and inhibiting the inflammatory cascade. Interestingly, previous studies have demonstrated that incorporating plant-derived natural compounds, such as dietary fibers and phytochemicals, into high-fat or high-fructose diets can effectively reduce the expression of TLR4 gene and inhibit the inflammatory signaling pathway mediated by NF-κB (Li et al., 2019). Furthermore, these compounds play a key role in protecting the integrity of the intestinal epithelial barrier and promoting the restoration of a disrupted gut microbiota, characterized by an increase in the levels of cellulolytic and anti-inflammatory bacteria (Sun et al., 2021; Yin et al., 2019). These positive effects have significant implications in reducing inflammation, both locally within the

gut and systemically throughout the body. Additionally, Dietary fibers and active compounds also showed the positive impact on the growth of beneficial bacteria and SCFA production in the gut (Chen et al., 2022; T. Jiang et al., 2016; Li et al., 2019). This leads to lower endotoxemia, improved intestinal permeability, and reduced metabolic disturbances.

Epithelial tight junction (TJ) proteins, such as ZO-1, Occludin, and Claudin-1, are crucial for maintaining the integrity and preventing the passage of harmful substances like LPS from the gut into the bloodstream (Usuda et al., 2021). The study revealed significant alterations in the gene expression of TJ proteins in the colonic mucosa of rats fed a HFHFr diet. These changes in TJ protein expression were directly associated with inflammation and damage to colon cells (Agus et al., 2016). Disruption of TJ proteins compromise the integrity of the intestinal barrier, resulting in hyperpermeability, and low-grade systemic inflammation (Lambertz et al., 2017; Rohr et al., 2020). However, the detrimental effects of the HFHFr diet on TJ protein gene expression were mitigated by supplementing with DFP, as evidenced by a substantial increase in the expression of ZO-1, Occludin, and Claudin-1 genes. This upregulation of TJ proteins helps restore the integrity of the intestinal barrier, reducing permeability and alleviating inflammation in the small intestine. Moreover, DFP supplementation enhances the production of SCFAs, the important fuel for intestinal epithelial cells, in the large intestine, which can promote the growth and health of intestinal cells by supporting their proliferation, differentiation, and strengthening the mucosal barrier.

Consequently, these effects contribute to an increased expression of TJ proteins, further supporting the overall health and integrity of the intestines epithelial (Ji et al., 2022; Li et al., 2019; Liu et al., 2021). Therefore, DFP has the potential to modulate TJ protein expression, safeguarding the integrity of the intestinal barrier and reducing systemic inflammation.

Scientific evidence from human and animal studies strongly supports the connection between diet and the composition of the gut microbiome (Usuda et al., 2021). Consuming a Western diet is known to bring about changes in the diversity and makeup of the gut microbiome, resulting in an increased presence of potential pathogenic bacteria that produce endotoxins causing intestinal inflammation and barrier dysfunction (Agus et al., 2016; Rachael G. Horne et al., 2020). The current study yielded different results regarding the impact of a HFHFr diet on the richness and diversity of the bacterial community, as depicted in Figure 14. In contrast to previous studies where rats fed high-fat diets (with 60% kcal/g from fat) or high-fructose diets (with 65% kcal/g from fructose) exhibited distinct differences in microbiota composition and diversity after 12-18 weeks (Ji et al., 2022; Yu et al., 2021), clear effects were not observed. This discrepancy may be attributed to the lower caloric intake from fat and fructose in this study, which amounted to 48% and 36% kcal/g, respectively. It is possible that these reduced levels were not sufficient to induce significant alterations in the bacterial community. Additionally, the supplementation of DFP did not lead to changes in microbial diversity, aligning with previous research

findings (Hua et al., 2021; Kim et al., 2020). This could be attributed to the predominant presence of insoluble fibers, such as cellulose, hemicellulose, and lignin, in DFP (Chumroenvidhayakul et al., 2022). These fibers have limited fermentability compared to soluble fibers, which may have hindered their ability to modulate microbial diversity effectively (Chen et al., 2019). Nevertheless, both the HFHFr diet and DFP supplementation exerted influences on the proportions of cecal microflora at the phylum and genus levels. The gut microbiota primarily consists of *Firmicutes* and *Bacteroidetes*, which make up more than 90% of the microbial community (Mazhar, Zhu, & Qin, 2023). Other less dominant phyla, including *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia,* are also present (Mazhar, Zhu, & Qin, 2023). Dietary fiber, a complex polysaccharide, is metabolized by the microbiota in the cecum and colon to produce SCFAs such as acetate, propionate, and butyrate (Abreu y Abreu et al., 2021; Mazhar et al., 2023). Studies have shown that regular consumption of dietary fiber can influence specific SCFA-producing families within the *Firmicutes* phylum (*Ruminococcaceae*, *Lachnospiraceae*, *Erysipelotrichaceae*, and *Clostridiaceae*), leading to an increased *Firmicutes*-to-*Bacteroidetes* (F/B) ratio (Abreu y Abreu et al., 2021). This increase in the F/B ratio was also observed with the consumption of DFP along with a normal diet, indicating a higher abundance of beneficial bacteria within the *Firmicutes* phylum (Hua et al., 2021; Mazhar et al., 2023). Furthermore, the study revealed a decrease in the abundance of *Actinobacteria* but an increase in the *Lentisphaerae* phylum in rats fed

a HFHFr diet compared to those on a normal diet. This shift in microbial composition is associated with gut dysbiosis and inflammation (Binda et al., 2018; Sánchez-Alcoholado et al., 2020). Conversely, DFP consumption attenuated the overabundance of *Lentisphaerae* induced by the HFHFr diet, suggesting a potential protective effect against colorectal cancer (Sánchez-Alcoholado et al., 2020).

At the genus level, rats fed the HFHFr diet showed increased abundance of harmful bacteria, including *Romboutsia*, *Dubosiella*, *Clostridium sensu stricto* 1, and *Peptostreptococcaceae*. These bacteria are known to disrupt intestinal integrity and contribute to inflammation through their metabolites' interaction with the immune system (Hu et al., 2021; Jena et al., 2020; Zhuge et al., 2022). However, the supplementation of DFP resulted in contrasting effects, particularly in inhibiting the growth and colonization of *Romboutsia*. This finding supports the idea that DFP's potential to reduce HFHFr-induced gene expression associated with tight junction proteins supports the maintenance of intestinal barrier function. DFP supplementation in rats, regardless of their diet type, increased beneficial bacterial families *Lachnospiraceae*, *Christensenellaceae*, *Ruminococcaceae*, and *Erysipelotrichaceae* known for their positive effects on gut barrier integrity (Abreu y Abreu et al., 2021; Jena et al., 2020; A. Li et al., 2021). *Lachnospiraceae* showed a positive correlation with SCFA concentrations (Abreu y Abreu et al., 2021), while the *Christensenellaceae* R-7 group (Waters & Ley, 2019) and *Erysipelotrichaceae* exhibited associations with SCFA production and reduced gut inflammation (Abreu y Abreu et al., 2021).

Ruminococcaceae, a cellulose-degrading bacterium, correlated with cecal SCFA content and lower plasma LBP levels (Fuke et al., 2023; Portincasa et al., 2022). The components of DFP also have an impact on other potential probiotic bacteria. For instance, DFP's phenolic compound has the ability to increase *Lachnospiraceae* NK4A136, which has been correlated with anti-inflammatory effects in gut inflammation rodents (Vacca et al., 2020; Wang et al., 2020). Additionally, the hemicellulose present in DFP can promote the growth of the butyrate-producing bacterium *Eubacterium xylanophilum* (Duncan et al., 2016). *Coriobacteriaceae*_UCG-002 and *Lachnospiraceae*_UCG-001 were associated with SCFA production and intestinal homeostasis (Mazhar et al., 2023; Waters & Ley, 2019).

In addition to stimulating the growth of SCFA-producing bacteria, the incorporation of DFP into both diets resulted in a significant elevation in the concentrations of all SCFAs. This finding is consistent with the observed increase in the abundance of SCFA-producing bacteria within families such as *Lachnospiraceae*, *Christensenellaceae*, *Ruminococcaceae*, and *Erysipelotrichaceae* in our study. A previous study examining the effects of prickly pear peels, which are closely related to dragon fruit peel, suggested that the substantial presence of polyphenols and fiber in DFP is responsible for this effect (Chumroenvidhayakul et al., 2022; Tejada-Ortigoza et al., 2019). These components serve as abundant sources of fermentable substrates and prebiotics for the gut microbiota, ultimately promoting increased production of SCFAs (Bernardi et al., 2020; Usuda et al., 2021). SCFAs, including acetate, propionate, and butyrate, are

beneficial metabolites renowned for their protective effects against diseases. Previous studies have demonstrated the metabolic benefits of acetate and propionate, such as the regulation of postprandial satiety hormones, specifically Glucagon-like peptide 1 and Peptide tyrosine tyrosine, along with improved insulin sensitivity and enhanced glucose tolerance in rodents (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016; Mazhar, Zhu, & Qin, 2023). While butyrate serves as a vital energy source for colonocytes and contributes to the production of mucin, which protects epithelial cells, it also enhances the expression of tight-junction proteins (Liu et al., 2021; Usuda et al., 2021). Collectively, these mechanisms synergistically improve metabolic disturbance, reduce intestinal permeability, and effectively preventing the entry of harmful pathogen byproducts from the intestinal lumen into the bloodstream. These findings provide evidence for the potential of DFP as a prebiotic ingredient, contributing to the establishment of a healthy gut microbiota and prevention of gut barrier inflammation.

4.8 Conclusion

DFP supplementation had several positive effects, including reducing body weight gain, improving fecal properties, and increasing fecal lipid excretion. It also effectively regulated the intestinal barrier function by reducing serum LBP levels, and suppressing TLR4 gene expression. These changes were associated with the upregulation of barrier-associated TJ proteins. DFP supplementation also promoted the growth of beneficial bacteria associated with SCFA production. These findings highlight the

potential of DFP, derived from fruit waste, in promoting gut health by modulating the microbiota, supporting colonic barrier function, and improving overall gastrointestinal well-being.

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CHAPTER V

CONCLUSION

Dragon fruit peel powder (DFP) is a natural ingredient derived from the by-product of dragon fruit consumption. It is characterized by its high content of dietary fiber, specifically cellulose and pectin, as well as bioactive phytochemicals such as phenolic compounds and betacyanins. DFP exhibits remarkable water holding capacity and swelling capacity, making it an effective viscosity modifier. When added to various cooking flours such as potato, glutinous rice, rice, and wheat flour, the incorporation of DFP resulted in a significant change in gelatinization enthalpy and peak viscosity, positively influencing the amorphous part and interfering with the crystalline region. Furthermore, the inclusion of DFP in these flours reduces starch digestibility, including rapidly digestible starch and slowly digestible starch, while increasing the proportion of undigested starch. Moreover, the bioactive compounds present in DFP are highly bioaccessible and retain their functionality even after undergoing simulated gastrointestinal digestion.

Expanding on these findings, DFP was introduced into cookies, which are known for their high levels of refined carbohydrates and heat-induced toxicants. Incorporating DFP into cookies at varying concentrations (1%, 2%, and 5% *w/w* of raw cookie dough) led to a distinct improvement in bioactive compound levels and antioxidant activity, while significantly reducing the presence of dietary oxidized

compounds such as malondialdehyde, methylglyoxal, and dietary advanced glycation end products. From a nutritional perspective, DFP-enriched cookies displayed a lower hydrolysis index and predicted glycemic index, indicating a slower digestion rate and a higher proportion of undigested starch. Importantly, sensory evaluation scores indicated that even with up to a 2% incorporation level, DFP-treated cookies were highly accepted, achieving an overall acceptability score greater than 7.

Owing to its rich content of dietary fiber and bioactive compounds, DFP holds promise as an ingredient for modulating gut health in rats exposed to a Western diet. Supplementing a high-fat, high-fructose diet (HFHFr) with DFP resulted in noteworthy effects, including a significant attenuation of body weight gain and an increase in fecal lipid excretion in rats. Furthermore, rats fed with the DFP-treated diet showed considerable improvements in cecum digesta weight, total short-chain fatty acids, and the abundance of SCFA-producing bacteria. In terms of gut barrier function, DFP supplementation up-regulated the expression of genes associated with tight junction-related proteins in the colonic tissue. Additionally, it down-regulated the expression of Toll-like receptor 4 and reduced lipopolysaccharide leakage, indicating a reduction in inflammation and an enhancement in gut permeability induced by HFHFr diet.

Collectively, DFP exhibits the potential to serve as a functional ingredient that enhances nutritional properties and promotes gut health. By utilizing DFP, valuable resources can be created from food waste, offering sustainable solutions to reduce the environmental burden while simultaneously increasing overall value.

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