Bioactivity of prebiotics from commonly consumed vegetables and root crops under simulated gastrointestinal conditions

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野菜や根菜のプレバイオティクス効果について

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CHAPTER 1

Literature Review

Polyphenols and its fermentation characteristics during colonic fermentation by gut microbiota

1.1.General introduction

Food is any nutritious and edible substance eaten by people or animals to maintain life and growth. Moisture, lipids, protein, fiber, carbohydrate, minerals and vitamins are essential nutrients in Food. Polyphenols are a food component and categorized as micronutrients. They are secondary metabolites mostly found in fruits and vegetables including seeds and cereals with high variability in structure and formation [1,2]. They are synthesized by plants as a defensive mechanism against radiation and microbial infection [3,4], and depending upon the chemical structure, polyphenols can either absorb in the small intestine or reaches the colon for microbial attack. There are more than 8,000 polyphenols identified so far, and out of which, more than 4,000 are flavonoids that can be grouped into four categories, namely, flavonoids, phenolic acids, polyphenolic amides, and other polyphenols [5-7]. The growing interest in polyphenols has led researchers in the recent past to discover its potential health attributes and thus, studies associated with polyphenols have become an increasingly important area of human nutrition research. Polyphenols are poorly absorbed in the upper gastrointestinal tract. On the other hand, the non-absorbable polyphenols (more than 90%) exited the upper gastrointestinal tract into the large intestine and become a substrate for microbial fermentation [8,9]. Owing to the limited absorption of polyphenols leads to the assumption that its health attributes would be involved in a certain role in the gut, and this raised questions which enable researchers to elucidate on the effects associated with the microbial community, and later found that the derived phytochemicals displayed positive attributes associated with health [10,11]. This was highly supported by researchers regarding its biological role in counteracting degenerative diseases such as cancers, cardiovascular diseases, and neurodegenerative diseases [12,13], which leads to intensive investigation regarding other biological effects of polyphenols. For example, studies have reported that polyphenols are strong

antioxidants, which means that they (polyphenols) can counteract oxidative stress caused by reactive oxygen species (ROS) – a potential health risk associated with various diseases and disorders such as cardiovascular disease and cancer [14,15]. Recently, studies reported the modulatory effects of polyphenols in the microbiota with its prebiotic potential in enriching the beneficial bacteria [16-18]. Besides, cecal putrefactive products (ammonia, indole, and skatole) and intestinal immunity-related substances (Mucin and Immunoglobulin A (IgA)) were reportedly been positively affected by the polyphenols [19,20].

1.2. Polyphenol and Health

Epidemiological studies and a lot of research studies both *in vitro* and *in vivo* ascribed to polyphenols as an antioxidant, anti-inflammatory, an anticarcinogenic, antiviral and antiallergic agent which improves human health [,21-26]. Studies have also indicated that a lower risk of type 2 diabetes was attributed to polyphenols, by lowering of the blood-glucose spike, higher glucose tolerance, and increased insulin sensitivity by preventing the breakdown of starch to simple sugars [27,28]. Similarly, a study was conducted and reported that a high intake of polyphenol-rich food lowered the risk of type 2 diabetes by more than 50% [20]. Further, it was reported that anthocyanins could be the most potent antidiabetic polyphenol amongst the many, which can be found in food sources such as berries, currants, grapes, and purple sweet potatoes [30,31]. Because polyphenols can exert beneficial health effects to the host, it was believed that this is attributed to the polyphenol's antioxidant potential, which alleviates such diseases as chronic inflammation and risk factors associated with heart diseases such as lowering of blood pressure and low-density lipoprotein (LDL)-bad cholesterol level [32-36]. Besides, many studies have linked high

polyphenol-rich food intake in reducing the risk of cancer, particularly attributed to antioxidant and anti-inflammatory effects [37-40]. A meta-analysis study highlighted that a high intake of flavonols and flavones reduced the risk of breast cancer in women [41]. Additionally, other studies have suggested that polyphenols may potentially block the growth and development of cancerous cells which may link to lower the risk of breast and prostate cancers, but more studies are warranted to further elucidate before strong evidenced-based conclusions can be made [37,40,43]. Polyphenols, upon reaching the large intestine, modulates the microbial composition and depending upon the amount and type may exert specific response with ameliorative effects [14]. Consequently, it promotes or proliferates the population of beneficial bacteria while reducing the pathogenic ones [44,45]. For instance, green tea polyphenols increase the population of *Bifidobacterium* while reducing the harmful bacteria such as *C. difficile*, *E.coli*, and *Salmonella* [18,46,47]. Similarly, fermentation of polyphenols stimulated the proliferation of *Bifidobacterium* while decreasing the ratio of Firmicutes to Bacteroidetes, relative to controls. Besides, polyphenols also stimulate the production of short chain fatty acid (SCFA) by the bacteria [48].

1.3. Colonic microbiota and health

The human large intestine especially the distal region is the largest reservoir and diversity of microflora of about 10^{11}–10^{12} bacteria per gram of colonic content, and this accounts for approximately 60% of fecal mass [49,50]. Colonization of bacteria started at birth and this is influenced by the delivery mode, infant diet, and medication effects [51]. It was reported that early colonizers of the large intestine were *Enterobacteria* and *Bifidobacterium* and it may vary depending on the incidence of infection and breast-to-formula-fed infants [52]. Other studies have

reported that colonic flora of vaginally born infants was dominated by *Lactobacillus*, *Prevotella*, *Atopobium* or *Sneathia sp*. with higher levels of *Clostridium sp*, while cesarean section infants have increased the levels of skin-associated bacteria such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium sp*. [53-55]. This put early colonizers or pioneer bacteria at an advantageous position in the colon, and subsequently modulate the gene expression which will create a conducive environment for them and thus, inhibit the introduced bacteria [56]. While the diversity and variability associated with the microbial composition is linked with the host's health, this is critically equilibrated and disturbances (dysbiosis) may consequently lead to chronic conditions such as inflammatory bowel diseases, colorectal cancer (CRC), obesity, diabetes, cardiovascular diseases, and neurological diseases [57-59]. Besides, influence on the microbial composition may be due to inactivity, and most importantly diet, and by modulating the microbiota may subsequently trigger the susceptibility to diseases [60]. Therefore, diet is the most common modulator of colonic flora. Studies are suggesting that a diet high in fiber is the model diet for healthy colonic flora because of its health attributes. For example, inulin selectively increases the population of *Bifidobacterium* while reducing the pathogenic ones [61,62]. Similarly, fermentation of complex carbohydrates increases the population of *Prevotella* which is associated with higher levels of fecal SCFA production [63]. Moreover, it was reported that soluble fiber through microbial fermentation could be more effectively improve the colonic barrier function by upregulating gene expressions of the gut barrier [64].

1.4. Polyphenols and colonic microbial interaction and health

As have been mentioned earlier, most polyphenols (more than 95%) ended up in the large intestine intact in the form of glycosides and become a substrate for colonic flora [65,66]. Apart from the 'prebiotic-like' and other health-associated effects of polyphenols, colonic modulation of microbiota and its associated health implications is being intensively investigated recently in an attempt to elucidate on other specific gut microbial species that may provide health benefits to the host. The effect of polyphenol depends very much on the type and concentrations [67-69]. For example, tea polyphenols inhibited pathogenic bacterial growth such as *Clostridium perfringens* with limited effect on *Bifidobacterium* and *Lactobacillus* [18]. Studies were recently reported that polyphenols can modulate the microbial composition with beneficial health attributes. For example, wild blueberry proanthocyanidins distinctively shaped the gut microbiota profile and influence the glucose homeostasis and intestinal phenotypes in high-fat high-sucrose fed mice [70]. Similarly, blueberry supplementation influences gut microbiota, inflammation, and insulin resistance in high-fat-diet-fed rats [71]. In another study, chitin–glucan and pomegranate polyphenols improve the endothelial dysfunction by microbial modulation [72]. Fotschki *et al*. [73] reported that anthocyanins in strawberry polyphenolic extract exert a positive response in the rat cecal environment. Likewise, Peng *et al*. [74] also reported the long-term intake of anthocyanins from *Lycium ruthenicum* Murray on the organism's health and gut microbiota and could be potentially developed into functional food ingredients. Notably, these studies and many other polyphenol-related studies have demonstrated that polyphenol-microbial interaction can potentially contribute to the host's health.

1.4.1. Polyphenol and SCFA production

SCFA (primarily acetate, propionate, and butyrate) are produced as a result of non-digestible carbohydrates fermentation, with beneficial health effects [75]. Acetate, for example, can

potentially delay the proliferation of cancerous cells and prevent oxidative damage of the distal colonic cells [76,77]. Propionate, on the other hand, is metabolized in the liver and subsequently used for gluconeogenesis [78], while butyrate is an important fuel for colonocytes [79]. The mechanism by which polyphenol increases the production of SCFA is not fully understood. But it could be associated with anaerobic bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Ruminococcus* [80-82]. Besides, it could be due to the inhibitory effect of polyphenols towards α-amylase and α-glucosidase in saliva and small intestine, thus, provide colonic flora with residual carbohydrates for SCFA production [83]. Additionally, it was reported that while polyphenols influence the microbial composition variations, polyphenol conversion by the microbiota affects other pathways including pathways associated with SCFA productions [84]. Larrosa *et al*. [85] reported that grapes polyphenol increases the population of *Bifidobacterium sp.* and *Lactobacillus*. Whereas, high acetate level may be correlated with the metabolic activities of *Bifidobacterium* and *Lactobacillus* [86,87]. In another study, fecal microbial metabolism of polyphenols specifically increased the population of *Bifidobacterium* and simultaneously increase the metabolites, 3-hydroxyphenylacetic acid, and 3-hydroxyphenylpropionic acid [51]. Besides, the correlation of butyrate production with bacterial metabolites was also reported [88]. Conversely, other authors reported that a high level of polyphenol in the diet may reduce the microbial composition and SCFA level [89-91]. Additionally, Wallace *et al*. [92] and Etxeberria *et al*. [93] reported that boysenberry and trans-resveratrol did not cause any significant changes in SCFA production respectively, particularly due to the different types of polyphenols, experimental model, and complexity of the microbiota.

1.4.2. Polyphenol and cecal putrefactive products

Putrefactive products (phenol, ammonia, *p*-cresol, indole, 4-ethylphenol, and skatole) are consequences of indigestible protein fermentation by proteolytic bacteria (mainly *Bacteroides* and *Propionibacterium*) [94]. For example, *p*-cresol is an aromatic compound produced by gut microbiota during the fermentation of *L*-tyrosine [95,96]. Likewise, tryptophan-rich food such as soy protein is likely to increase the level of indole by the intestinal bacteria [94]. Production of putrefactive products in access are considered putative and could pose some serious health issues associated with colon cancer [96]. Any studies have indicated the ammonia is detrimental to the host, as Davila *et al*. [97] highlighted that an increase in ammonia concentration can affect the energy metabolism of colonic epithelial cells. Studies have also reported that a higher concentration of *p*-cresol is associated with chronic disease development and liver failure [98,99], while phenol and indole are putrefactive products that are directly associated with the epithelium by stimulating the strength of carcinogenic factors [100,101]. The putrefactive products are diet-related, and although they are detrimental and considered putative carcinogenic, they can be suppressed by the choice of diet. For example, a high intake of fruits and vegetables is inversely associated with health risks [58,102,103]. Likewise, including polyphenols in the diet could be a potential remedy for the high production of putrefactive products. Studies have shown that polyphenols can reduce or suppress the production of putrefactive products. For example, Yamakoshi *et al*. [104] showed that grape proanthocyanidin-rich extract significantly increased the fecal number of *Bifidobacterium*, while reducing the putrefactive bacteria. Similarly, Goto *et al*. [19] reported that tea polyphenols positively affect the growth of *Bifidobacterium sp*. while reducing the putrefactive bacteria like *Enterobacteriaceae sp*. and *Clostridium sp.* and consequently reduce the levels of sulfides and ammonia. In another study, Jurgoński *et al*. [105] showed that blackcurrant pomace supplementation reduced the putrefactive metabolites, similar to

a report by Zduńczyk *et al*. [91] on the ammonia-nitrogen level. Nagata *et al*. [106] showed that polyphenol-containing adzuki bean extract reduced the ammonia concentration in the rat cecum, and Bilić-Šobot *et al*. [107] reported that hydrolyzable tannins reduced the intestinal skatole production via lower synthesis of androstenone due to tannins. Thus, based on studies regarding the positive attributes associated with polyphenols, it could be potentially developed into functional food ingredients for human consumption aiming to alleviate the detrimental effects associated with putrefactive products.

1.4.3. Polyphenol and intestinal immunity-related substances - Mucin and IgA

Immunity can be defined as the organism's ability to resist a particular infection or toxin by the action of specific antibodies or sensitized white blood cells. Intestinal immunity is associated with the epithelial response to any invasion by upregulating the defense mechanisms or signals for immediate protection. The intestinal mucosal lining is prone to constant challenges by the antigens, digestion products, and drugs, and thus, the microbial-epithelial crosstalk is such an important component central to the immune system [108-110]. In most cases, diet is the main factor influencing the immune system. For example, a diet high in resistant starch and dietary fiber enhances the mucin and IgA secretions via large bowel fermentation [111-113]. Besides, phytochemicals or polyphenols are another group of active compounds associated with the immunomodulatory activity which enhances the secretions of mucin and IgA [20,106,114].

1.4.3.1. Mucin

The gel-like substance of mucus covering the epithelial layer can be regarded as the first line of defense against pathogenic bacterial growth [115,116]. It was reported that the major component of the mucus is mucin produced by the goblet cells in a single layer in the upper gastrointestinal

tract/small intestine and a double layer in the colon [117]. Studies have shown that mucin secretion by the goblet cells was stimulated by SCFA production [118,119], and was reported that genes of mucin are up-regulated by the derived substances from bacteria such as lipopolysaccharides [120]. Besides, mucin was up-regulated by the prebiotic treatment [121], influenced by the modulation of microbiota, which is associated with the type of diet [111-114]. A lot of studies have been conducted to verify that polyphenols are very influential in mucin secretions. For example, Nagata *et al*. [106] and Taira *et al*. [20] reported that adzuki bean extract and dietary polyphenols increased the cecal mucin concentration respectively. In another study, ellagic acid and proanthocyanidin enhanced the secretion of mucin [122]. Besides, dietary polyphenols can enhance the viscoelastic modulus of the mucus layer via mucin cross-link, thus, mucus layer stabilization [123,124]. Further, it was reported that polyphenols are immune regulators [125], but the mechanism by which polyphenol influences the secretion of mucin is not fully understood. There are reports which could indicate the initiatial steps towards its secretion and up-regulation. For example, polyphenols might influence the enzymatic activity via microbial modulation and subsequently reduce the mucin-degrading enzymes and consequently increase the secretion of mucin [126,127].

1.4.3.2. IgA

Immunoglobulin A (IgA) is an important immunoglobulin in the body and responsible for mucosal homeostasis playing a vital role against antigens [128,129]. Per day, 66 mg/kg is produced in adults, which is the highest amongst immunoglobulin [130]. IgA is secreted by the plasma cells or mucosal-associated lymphoid tissues as a homogeneous population of cells and released as dimer joined by J-chain in external secretions as the first line of defense preventing accessibility of antigens to the submucosa and circulation [131-133]. Yazdani *et al*. [134] reported that deficiency in IgA can lead to the production of anti-IgA antibodies which can weaken the mucosal barrier

functions and subsequently expose the body to external pathogens. It was reported that recurrent sino-pulmonary infection is commonly associated with IgA deficiency [135]. Other studies also reported that IgA deficiency is associated with gastrointestinal infections, allergic, and autoimmune diseases [136,137]. As have been discussed earlier [61-64], diet is the main influencer of colonic microbiota, and it was shown that microbial composition influences the up-regulation of IgA responses and consequently selects for a microbiota composition in a reciprocal positive feedback loop for the host's mucosal homeostasis [138]. Recently, polyphenols have become a focus of investigation regarding ther modulatory effects and fermentation characteristics. Many of these studies reported that polyphenols can up-regulate the level of IgA. For example, Taira *et al*. [20] reported that Aronia, Haskap, and Bilberry markedly elevated the amount of fecal IgA as an intestinal barrier function and ameliorated the disturbance in gut microbiota caused by a high-fat diet in rats. Okazaki *et al*. [139] reported that consumption of Curcumin elevates fecal IgA, an index of intestinal immune function in rats fed a high-fat diet. Likewise, grape pomace supplementation increased the level of IgA in pigs [140]. In another study, Peng *et al*. [78] reported that cecal IgA content of mice was increased after anthocyanin intervention and a polyphenol-rich cocoa diet improved the barrier integrity and prevents intestinal inflammation in diabetic rats [141].

1.5. Aims of the study

As have been discussed previously in this chapter, polyphenols are associated with lowering the risk of chronic disease development due to their antioxidant potential. Besides, a higher percentage of polyphenols escaped the upper gastrointestinal tract into the colon and become a substrate for the colonic microbiota. Consequently, the microbial fermentation of polyphenols has been linked

to positive health attributes to the host with prebiotic potential. In light of this, studies associated with the health attributes of polyphenols have been conducted using a variety of polyphenol sources ranging from low to very high polyphenol content with concentrations vary depending upon the polyphenol content, with reports indicating the therapeutic potentials of some polyphenols. Given the strong evidence from scientific studies regarding the association of dietary polyphenol and health, still there could be other sources of polyphenols yet to be elucidated on factors associated with health attributes. Hence, the study aims to examine in the next three chapters the biological activity of polyphenols from commonly consumed root crop, purple sweet potato with particular attention to the fermentation characteristics to elucidate its potential as a healthy substrate for colonic microbiota, and the results obtained could contribute to a better understanding of the potential health benefits of colored foods.

CHAPTER 2

In vitro colonic fermentation characteristics of purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) polyphenols

2.1. Introduction

2.1.1. Distribution of purple sweet potato

Purple sweet potato is exceptionally one of the most well-known crops grown worldwide as a rich source of carbohydrates containing essential components such as dietary fiber, minerals (Ca, Mg, K and Zinc), vitamins (B1, B2, C and E), and phytochemicals [142-144]. At least, its domestication can be traced back to 5000 years ago [145,146]. Purple sweet potato has been grown globally with the highest of approximately over 95% are grown in the developing countries and more than 80% are grown in Asia with 15% in Africa, and the rest of the world is about 5%. The plant's adaptability to fluctuating environmental conditions has led some countries to count on the sweet potato as a backup crop. Some countries have linked sweet potato growing with domesticated animals. For example, in Vietnam, sweet potato production has been linked to pig production and in China, sweet potato production is associated with animal feed [147]. In Brazil, purple sweet potato is ranked fourth as a most consumed vegetable and source of carbohydrates [148]. In Japan, purple sweet potato was introduced to Okinawa in the early 1600s by the Portuguese and became a staple food since then when rice harvests were poor [149-152]. Today, purple sweet potato has become popular because of its health attributes associated with the high polyphenolic contents. In Asian countries, different varieties of purple sweet potatoes are grown with variations in phenolic contents and recommended for the diet of other countries [153]. In Japan, purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) was bred by selecting tubers with a large anthocyanin content [154].

2.1.2. Anthocyanin contained in purple sweet potato

Anthocyanins are associated with blue, red, or purple pigments found in plants, especially flowers, fruits, and tubers with color reflection is pH-dependent (red in acidic condition and blue in alkaline condition) [155]. Physically, anthocyanins are disease stressors packed with a defensive mechanism against radiation and microbial infection [3,4,155]. Purple sweet potatoes contain substantial amounts of anthocyanins compare to other anthocyanin-containing fruits and vegetables such as grapes, plums, sweet cherries, raspberries, and eggplant [156]. Anthocyanin is the glycosylated form of anthocyanidin (aglycon). It was reported that cyanidin, delphinidin, pelargonidin, peonidin, malvidin, and petunidin are commonly distributed anthocyanidins in the plants with a percentage distribution of 50%, 12%, 12%, 12%, 7%, and 7%, respectively [157]. In figure 2.1, it shows the basic anthocyanin structure with an anthocyanin structure isolated from purple sweet potato. It was reported that purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) contains a total of eight major anthocyanins in mono- or di-acylated forms [158].

2.1.3. Bioavailability of polyphenol/anthocyanin

In nutritional science, bioavailability can be defined as the proportion of the administered substance capable of being absorbed and available for use or storage [159]. Thus, the bioactivity of dietary polyphenols depends on its bioavailability properties [160]. Thus, polyphenols with low bioavailability reduce their potential or efficacy to scavenge free radicals, while high bioavailability polyphenols are efficient in their scavenging ability to reduce the risk of diseases. There are a host of factors associated with polyphenol's bioavailability. For example, environmental factors such as sun exposure, rainfall, a different type of culture, and fruit yields [15,161], food processing factors such as thermal treatment [162,163], storage factors such as cold storage, warm storage, or storage for long periods [164-166], and food related-factors such as the food matrix and chemical structure [167,168]. Studies have indicated that amongst the

anthocyanins, the bioavailability of cyanidin-3-glucoside and malvidin-3-glucoside are the most reported. Besides, it was reported that malvidin 3-glucoside was absorbed after ingestion of red wine and red grape juice [169,170]. Other studies reported that the bioavailability of anthocyanin is less than 1% and more recently, it was reported that it may have been underestimated [171,172].

2.1.4. Physiological functionality of polyphenols/anthocyanin

It was suggested that the health and therapeutic effects of polyphenols are mainly antioxidative attributes and in the recent past, studies have reported that anthocyanins are antioxidative and free radical scavengers [173,174], preventing carcinogenesis [175] and platelet aggregation [176] and improving visual function [177]. An epidemiological study highlighted that high intakes of anthocyanin-rich fruits and vegetables are related to health benefits [178]. Previous studies on polyphenol/anthocyanin have shown the positive health attributes associated with anthocyanin. For example, the highest antioxidant activity was reported for malvidin-3-glucoside followed by catechin, malvidin, and resveratrol [179]. This was similar to a study reported by Bub *et al*. [170] where malvidin-3-glucoside was higher in red grape juice than red wines. It was also reported that delphinidin, an anthocyanin pigment is known for combating melanoma cells [180]. Besides, pelargonidin-3-glucoside, cyanidin-3-glucoside, and delphinidin-3-glucoside isolated from *Phaseolus vulgaris L*. (black bean) seed coat have strong antioxidant activity [181]. Further, studies on an anthocyanin-rich extract from berries (wild blueberry, bilberry, cranberry, elderberry, and strawberry) were reported with high antioxidant potential and were able to suppress the hydrogen peroxide and TNF-α-induced vascular endothelial growth factor (VEGF) expression in HaCaT cells (human keratinocytes) [182]. A similar study was also reported that bilberry anthocyanidins (delphinidin, cyanidin, and malvidin) and anthocyanin-rich purple corn extract inhibited the VEGF-induced tube formation in a co-culture of human umbilical vein endothelial

cells and fibroblasts and attenuates the endothelial expression of VEGF and hypoxia-inducible factor (HIF)-1α respectively [183,184]. Previously, physiological and functionality studies of polyphenols have been confined to grapes and berries [185-189]. Later, studies on the physiological functions of sweet potato polyphenols have been conducted [190,191] and noted for its stability [192,193] and because of that, more studies have since been conducted to verify the health potential of sweet potato polyphenols. For example, purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) have been reported with high antioxidant activity *in vivo* as well as in vitro [194]. Han *et al*. [195] also reported that dark purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) can potentially modulate the antioxidant enzymes and oxidative status in the serum and liver and may counter any possible oxidative damage.

2.1.5. Aim of the study

We have so far seen that polyphenols are very low with regards to their absorption potential in the small intestine, and since polyphenol's absorption in the small intestine is very low, high amounts are entering the colon and become a potential substrate for the microbial community. Previously, studies on purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) have been conducted to examine its antioxidative potentials [194-196] but with very little information on its fermentation characteristics. Keppler and Humpf [197] reported that polyphenols entering the large intestine are deglycosylated by the gut microbiota. Purple sweet potato polyphenols from different varieties have been studied previously for their effects concerning absorption, antioxidative, anti-inflammatory, anti-tumor, and hypoglycemic effect [194,195,198]. Although these studies focused on health implications associated with polyphenols, however, there are remarkably very few studies addressing the effects on colonic microbiota. Therefore, this study aims to assess the hypothesis that the effects of purple sweet potato polyphenols (*Ipomoea batatas* cv. Ayamurasaki**)**

during colonic fermentation improve the intestinal environment with beneficial health attributes. Thus, in this study, purple sweet potato polyphenols will be combined with cellulose (partly fermentable dietary fiber) or inulin (fermentable dietary fiber) in a mixed culture of swine fecal bacteria to assess with particular attention on the fermentation characteristics to elucidate its potential as a healthy substrate for colonic microbiota.

2.2. Materials and Methods

This study used the purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) as the main material of focus, and all the chemicals used in this study were of analytical grade.

2.2.1. Preparation and determination of sweet potato polyphenols

Preparations of purple sweet potato polyphenols [PSPP] extract were done by weighing 50 g of the potato powder into a 3-L beaker and subjected to 70% acetone and the solution was sonicated for 20 minutes. After sonication, the solution was allowed to stand at room temperature for 10 minutes giving time for sediments to settle at the bottom. After that, the suspensions were filtered and centrifuged at 14,600 × g and 4 ◦C for 30 minutes. The extraction process (with 70%) was done three times so that, as much as possible, all the colored pigments were extracted. The combined acetone fraction was removed by using the rotary evaporator at 35 ◦C. The pigment extract was reconstituted with distilled water and applied directly to the pretreated Diaion HP-20 resin (Nippon Rensui Co., Tokyo, Japan) column. There were three types of solutions added consecutively to the elution column, namely, water, 20% ethanol, and 80% acetone (Fig. 2.2). The eluate fractions from water, 20% ethanol, and 80% acetone were determined for their polyphenol content with 1.54%, 5.06%, and 93.4% as polyphenol contents respectively. The water and ethanol fractions were not used for the study as it contained impurities like free sugars, proteins, salts, and lower molecular weight polyphenol fractions. The pooled eluate from 80% acetone was evaporated using a rotary evaporator to remove the acetone at 35 ◦C, and the concentrate was reconstituted in distilled water and vacuum filtered to remove any possible bacterial contamination by using 0.22 μm sterile disposable filter (Nalgene rapid-flow disposable filter units with PES membrane, Thermo Fisher Scientific, Tokyo, Japan) and sample were stored for *in vitro* studies. The total

polyphenol content of purple sweet potato extract was determined according to the procedures by the Folin-Ciocalteu method [199] with brief modification. In this study, 0.5 mL of the diluted sample was mixed with 5 mL of $NaCO_3$ and then shaken vigorously. After that, the samples were allowed to stand at room temperature for 5 minutes. After 5 minutes, 0.5 mL of Folin-Ciocalteu reagent was added to the mixture and vigorously shaken. After 30 min, the absorbance was measured at 750 nm using Shimadzu 1600-UV spectrophotometer. The total phenolic content of the extract was approximately 79% as gallic acid equivalents.

2.2.2. Feces and *In Vitro* Fermentation

The *in vitro* fermentation study was conducted under an anaerobic condition with a mixture of fresh pig feces collected from Tokachi Hills farm in Obihiro. We used swine feces because of the similarities in the digestive systems and the intestinal ecology between pigs and humans. The pig's feces were collected directly from the anus of three pigs and inserted into an anaerobic double zipper plastic bag containing AnaeroPack-Anaero (Mitsubishi Gas Chemical, Tokyo, Japan) instantly without any exposure to air. The pig's feces were collected on-site on the day of the experiment. The fecal slurry (10× dilution) was prepared by homogenizing equal amounts from the three pigs in a stomacher (Exnizer 400, Organo Co., Tokyo, Japan) and filtered through a stomacher bag (Eiken Chemical Co., Ltd., Tokyo, Japan) filled with 0.85% NaCl solution and used as a microbial source. The pH-controlled jar fermenters (220 mL working volume, Able & Biott, Tokyo, Japan) were inoculated with the fecal slurry to give a final concentration of 2.0% (v/v). In this study, four pH-controlled jar fermenters were used and before sample treatment, a pre-incubation period of 12 hours was scheduled to stabilize the growth of microbes in the fermenters. After pre-incubation period of 12 hours, one of the following samples was added to each fermenter, 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU, Fuji FF), and 3% inulin

+ 0.16% PSP (INUP). This study uses the polyphenol content equivalent per jar based on a study reported by Nagata *et al*. [106]. The final concentration of 0.8% (w/v) of the autoclaved basal nutrient broth (Difco, Sparks, MD, USA) was added to each fermenter. The fermentation design was anaerobically maintained under CO2 gas at 37 ◦C for 48 h at a lower pH limit of 5.50. At 0-, 6-, 12-, 24- and 48-hour time points, pH was recorded and aliquots were collected in 2-mL tubes and stored at −80 ◦C for the analysis (Fig. 2.3). This experimental design was reviewed and approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine (no. 18–32).

2.2.3. Microbiological analysis

2.2.3.1. Viable plate count method

Bacterial populations were analyzed using selective media by the viable plate count method. This was done by using samples from fermenters (500 µL into 4.5 mL of sterilized saline solution (0.85% NaCl)) and were serially diluted (10^1 to 10^7). The diluted samples (100 µL) were cultured to enumerate the specific bacterial species with selective media and incubated at 37 ◦C (TVN680DA Advantec® incubator, Toyo Seisakusho Kaisha Ltd., Tokyo, Japan) by using an AnaeroPack-Anaero (Mitsubishi Gas Chemical) in sealed anaerobic containers. In this study, Coliform bacteria and *Cl. Perfringens* from the fermenters were inoculated for 24 hours on EMB agar (Eiken Chemical Co., Ltd.) and OPSP agar (Oxoid, Hampshire, UK) respectively. The Anaerobes, *Lactobacillus*, and Lactic Acid Bacteria (LAB) were incubated for 48 hours on BL (Eiken Chemical Co., Ltd.), Rogosa (Oxoid), and MRS (Oxoid, Hampshire, UK) agar respectively. The *Bifidobacterium* was incubated for 72 hours on TOS-propionate agar (Yokult pharmaceutical Industry, Tokyo, Japan).

2.2.3.2. DNA Extraction and 16S Ribosomal RNA (16S rRNA) Gene Sequences

The samples used for bacterial DNA extraction were 48-hour samples. The samples (non-diluted) were thawed at room temperature and centrifuged (16,000×g, 4°C, 5 min) before DNA extraction using a modified phenol-free repeated bead beating plus column (RBB + C) method described by Yu & Morrison [200]. Following the removal of the supernatant, 300 µL of lysis buffer (500 mM NaCl, 50 mM Tris-HCl, 50 mM EDTA, 4% sodium dodecyl sulfate; pH 8.0) was pipetted into 2-ml Eppendorf® tubes containing samples with thoroughly mixing using pipette aspiration. After well-mixed, the contents were completely pipetted into the screw-capped tube (Watson® BioLab, Fukaekasei Co., Ltd., Kobe, Japan) containing 0.4 g of beads (a mixture of 0.3 g of 0.1 mm + 0.1 g of 0.5 mm). The screw-capped tubes were subjected to the cell destroyer (4,260 rpm, 15 min, 3 cycles; PS1000, Prosense Inc., Tokyo, Japan) and after that, the tubes were incubated at 70°C for 15 min in the water bath. After incubation, the tubes were centrifuged (16,000×g, 4°C, 5 min) and the supernatants were pipetted into new sterile 1.5 mL Eppendorf® tubes. The screw-capped tubes with beads were once again washed with another 300 µL of lysis buffer and the procedures were repeated once more. After cell lysis, 260 µL ammonium acetate (10 mM) was added into the supernatant and well-mixed by aspiration. After 5 minutes on ice, the samples were centrifuged (16,000×g, 4°C, 10 min) and the supernatants were pipetted into 2-ml Eppendorf® tubes with added equal volumes of isopropanol and well-mixed again and after, the samples were kept for 30 minutes on ice. After 30 minutes, the samples were centrifuged (16,000×g, 4°C, 10 min) and the supernatant was carefully removed and the DNA pellets were re-suspended in 70 % (v/v) ethanol and once more centrifuged (16,000×g, 4°C, 10 min). The supernatant was completely removed and allowed to completely dry for approximately 40 min. After drying, the DNA pellet was

dissolved with 200 µL Tris-EDTA (TE) buffer (1 M Tris, 0.5 M EDTA; pH 8.0) and stored at -30°C until further purification procedures.

In the genomic DNA purification, the extracted DNA was purified (QIAamp DNA Stool Mini Kit, QIAGEN, Valencia, CA, United States) by subjecting 2 µL of DNAse-free RNAse into each sample with aspiration. After, the samples were incubated for 15 minutes at 37°C then 15 µL of proteinase K was added, mixed well by aspiration and 200 µL of AL buffer was added with aspiration and incubated for 10 minutes at 70°C. After, 200 µL of 99.5% (v/v) ethanol was added with aspiration, and transferred to a collection tube column and centrifuged (16,000×g, 20°C, 1 min). 500 µL of AW1 buffer and 500 µL AW2 buffer were added to the column one after the other and centrifuged (16,000×g, 20°C, 1 min) respectively. Once more with a new collection tube attached, the samples were centrifuged (16,000×g, 20°C, 1 min) to dry. Finally, 50 µL of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0) was added into the column with 1.5 mL Eppendorf® tube as a collecting tube and centrifuged (16,000×g, 20°C, 1 min). The collected DNA samples were measured for their quality and concentration (NanoDrop 2000c spectrophotometer, Thermo Fisher Scientific, Tokyo, Japan) with adjustments (5 ng/µL with Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0)). The samples were stored at -30°C for further analysis.

After DNA purification, polymerase chain reaction (PCR) and gel electrophoresis were conducted. The PCR reaction mix of 24 µL (Table 2.1) was combined (without DNA sample) and into each PCR tube pipetted 1 µL of template DNA or sample DNA and subjected to the thermal cycler (2720, Applied Biosystems, CA, United States) with the conditions in Table 2.2. After PCR, the 25-ml gel was prepared by weighing 0.25 g of agarose and added 2.5 ml of 10X TAE buffer (10%) and volume-up with distilled water (22.5 ml). The gel was solidified after 20 minutes with a DNA ladder. After the gel was solidified, the DNA ladder was removed (creating wells), transferred,

and submerged into the electrophoresis tank (Mupid®- 2 Plus, Mupid Co., Ltd., Tokyo, Japan) with 1X TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0). DNA ladder (DM2100, Excel Band TM, 100 bp DNA ladder, SMOBIO Technology Inc., Hsinchu City, Taiwan) was pipetted into the first well followed by blank and samples (after mixing 4 µL sample with 1 µL loading buffer), and the electrophoresis was started (100 V; 30 to 40 min/25 mL gel). After, the gel was photographed for band visualization (Fig. 2.4). In the first stage of PCR, V3-V4 variable regions of the 16S rRNA gene were amplified using the following bacterial overhang adapters and universal forward primer (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3') and the reverse primer (5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA TTA CHV GGG TAT CTA ATC C-3'). The reaction mixes and conditions are in tables 2.3 and 2.4 respectively. In the second stage of PCR, Illumina sequencing adapters and dual index barcodes were added to the amplicons using Nextera® XT Index Kit (Illumina Inc., San Diego, CA, USA). After quantification of PCR products using QuantusTM fluorometer (Quantifluor® dsDNA System, Promega, Madison, WI, USA), the successful PCR products were pooled in one tube with equal volumes and subjected to paired-end sequencing by Illumina MiSeq System (Illumina Inc.). The analysis of retrieved raw 16S rRNA gene sequences was conducted according to Warren *et al*. [201] and the generated biome table was normalized using an equal subsampling size of 6727 sequences. The calculation of distances between bacterial communities in different samples was done by the weighted UniFrac distance metric and the preparation of principal coordinate analysis (PCoA) plot was conducted in Quantitative Insight Into Microbial Ecology (QIIME) software (version 1.9.1) [202], and calypso version 8.84 [203] was used to visualize the α-diversity (observed species and Shannon index) and hierarchical clustering plots at the phylum level.

2.2.4. Short-Chain Fatty Acid (SCFA) Analysis

Aliquots collected from the fermenters at 0, 6, 12, 24, and 48 hours were centrifuged (9,200 × g and 4 ◦C for 15 min) and 450 µL of sample supernatant was pipetted into 2 mL Eppendorf® tubes with 1 mL of 0.5 N $HClO_4$ (60% v/v) was added into each tube. After centrifuging, the supernatant was filtered into new 1.5 mL Eppendorf® tubes using a 1 mL syringe and a micron membrane filter (0.45 µm; DISMIC-03CP, Advantec, Toyo Roshi Kaisha, Tokyo, Japan), and filtrates were subjected to HPLC in a Shimadzu LC-10AD (Kyoto, Japan) equipped with an ST3-R post-column. The analytical conditions were as follows: Column, RSpak KC-811 (8.0 mm × 300 mm, Shodex, Tokyo, Japan), eluent and flow rate, 2 mM perchloric acid at 1.0 mL/min, column temperature, 47 ◦C, reaction reagent and flow rate, ST3-R (10× diluted, Shodex) at 0.5 mL/min, UV detector wavelength, 430 nm.

2.2.5. Measurement of Putrefactive Products

The concentration of ammonia-nitrogen was measured using a commercially available kit (Wako Pure Chemical Industry, Ltd., Tokyo, Japan). Samples from fermenters were mixed well on a vortex and centrifuged (3,000 rpm, 4°C, 15 min). The dilution of samples depends upon the ammonia-nitrogen content, normally 100 or 200 folds with phosphate buffer (0.1M; pH 5.5) was used. Standards (0, 100, 200, 300, 400 µg/dL) were prepared by using a standard solution and diluent provided by the kit. In this study, 50 µL of samples or standards were mixed with a deproteinizing solution on a vortex and centrifuged (3,000 rpm, 4°C, 5 min). After that 200 µL of the deproteinized samples or standards were pipetted into new 1.5 mL Eppendorf® tubes and 200 µL of color reagent A was added and mixed well on a vortex. After, 100 µL of color reagent B and 200 µL color reagent C were added with mixing by a vortex. Finally, the samples were incubated

at 37°C in a water bath for 20 minutes. After incubation, the absorbance was measured at 630 nm using a UV-vis spectrophotometer (UV-1600, Shimadzu) and the ammonia concentration in the fermenters was calculated as follows; Ammonia-nitrogen concentration (mg/mL) = (Sample absorbance – y-intercept) / Gradient of line * dilution *1/1000. The concentration of *p*-cresol at 48 h was measured according to Ikeda *et al*. [204]. The samples from fermenters were mixed well on a vortex and centrifuged (3,000 rpm, 4°C, 15 min). In a tube of a 0.1 mL sample, was added acetonitrile, anhydrous sodium sulfate, and acetonitrile-saturated hexane. The mixture was then shaken vigorously using a vortex for 2 minutes and centrifuged (1500 × g, 5 min). Acetonitrile-saturated hexane was added to the middle of the acetonitrile phase in a new tube, and the mixture was vigorously shaken for 1 minute and centrifuged (1500 × g, 5 min) again. The lower acetonitrile phase was used for the analysis after filtration with a syringe and a micron membrane filter (0.45 μm; DISMIC-03CP, Advantec, Toyo Roshi Kaisha, Tokyo, Japan). A CTO-10A Shimadzu column (150 × 4.6 mm, Shimadzu Co, Tokyo, Japan) was used in a column oven at 40 ◦C. The mobile phase, consisting of acetonitrile/water (30:70, v/v), was flowed at a constant flow rate of 1.0 mL/min and the UV detector wavelength was set at 280 nm.

2.2.6. Statistical Analysis

All data are presented as a mean and standard error (SE). The *in vitro* fermentation was conducted in a block design with replicates of five. Two-way ANOVA was performed to assess the effect of fiber (cellulose and inulin), PSP, and their interaction. Differences of $p < 0.05$ was taken to be statistically significant. If the variance was observed in the main effect of interaction, Tukey's test was used for this comparison ($p < 0.05$). Analyses were performed using PASW Statistics 17.0 software (SPSS Institute, Armonk, NY, USA).

2.3. Results and Discussion

2.3.1. Microbiota composition in the fermenter media during the fermentation period

2.3.1.1. Bacterial enumeration by plate count

As have been mentioned in chapter one, polyphenols are antioxidative, anti-inflammatory, anticarcinogenic, antiviral, and antiallergic agent which improves human health. In this study, purple sweet potato polyphenols (PSP) (*Ipomoea batatas* cv. Ayamurasaki) were investigated for their fermentation characteristics during *in vitro* colonic fermentation. Reports indicated that polyphenols can modulate the microbial composition [18,71,74]. In this study, the microbial count for *Bifidobacterium* and Coliform bacteria were not affected by PSP (Fig. 2.5). However, PSP affects ($p < 0.05$) the population of Anaerobic bacteria at 24 h time point. Anaerobic bacteria cannot replicate or thrive in the presence of oxygen and die within minutes in less than 0.5% oxygen and most of the anaerobic bacteria are commonly found in the gastrointestinal tract. Examples include *Clostridium perfringens*, *Clostridium difficile*, *Bacteroides*, *Bifidobacterium*, and *Lactobacillus*. In this study, the anaerobes were increased in the PSP supplemental groups (CELP and INUP). It was reported that polyphenols do not affect the commensal bacterial growth but reduce the pathogenic bacterial growth [18]. That could be the case with the current study because the commensal bacteria, *Lactobacillus* was unaffected or increased by PSP. *Lactobacillus* count was also affected by PSP ($p < 0.05$) and the combination (Fiber/PSP) effect ($p < 0.05$), particularly at 24 h and 48 h time points. It was increased in the supplemental groups (CELP and INUP). Other studies also reported similar findings. For example, Dolara *et al*. [205] reported that red-wine polyphenols increased the population of *Lactobacillus spp.* [57]. Similarly, grape polyphenols also increased the population of *Lactobacillus* [85]. *Lactobacillus* is a commensal

bacterium and associated with high acetate levels which can delay the proliferation of cancerous cells and prevent oxidative damage of the distal colonic cells [76,77]. It is worth noting that *Lactobacillus* was also affected due to the interaction of fiber and PSP. Tuohy *et al*. [206] reported that consuming dietary fiber and polyphenol-rich chocolate will simultaneously up-regulate the commensal bacteria. This finding was in line with the current study which, at the end of 48 h, *Lactobacillus* was up-regulated in the supplemental groups (CELP and INUP). Lactic Acid Bacteria (LAB) in this study were affected by the interaction of fiber and PSP at 48 h time point. LAB are probiotics such as *Lactobacillus* and are generally considered beneficial for health. It is interesting to note that while LAB was reduced in the CELP group compared with the CEL group, they were increased in the INUP group compared with the INU group. Tabasco *et al*. [207] reported that Flavan-3-ols are inhibitory of lactic acid bacteria and this depends on the concentration and composition. In this study, it could be associated with the effect of PSP with the fermentability of dietary fiber associated with (CEL-less fermentable and INU-fermentable). Consequently, INUP favored LAB growth while CELP hindered the growth of LAB. At 24 h time point, *Cl. Perfringes* was affected ($p < 0.05$) by the interaction of PSP and fiber. It was reported that *Cl. Perfringes* is a pathogen responsible for many gastrointestinal illnesses [208]. Lopez-Legarrea *et al*. [209] reported that intake of polyphenol-rich fruits and vegetables increases the counts of beneficial bacteria and decreasing the population of *CL.perfringens*. In this study, CELP reduces significantly ($p < 0.05$) the counts for *CL.perfringens* compared with the CEL group without any effect on INUP. It seems PSP was more influential towards CEL (less fermentable fiber) than INU (fermentable fiber).

2.3.1.2. 16S Ribosomal RNA (16S rRNA) gene sequences

In this study, the microbial composition from 48-h samples were analyzed using the 16S rRNA amplicon. The α-diversity (Shannon index (Fig. 2.6a) and observed species (Fig. 2.6b)) were not affected by PSP as indicated by the Two-way ANOVA. The differences in α-diversity is associated with the effect of dietary fiber (DF) (CEL and INU), that is, low and high fermentability, respectively. To evaluate the similarities and differences between groups, the principal coordinate analysis (PCoA) (β-diversity) was performed to portray the relationship between microbiota structures amongst the groups (Fig. 2.6c). The β-diversity of species showed three distinct clusters indicating the different responses of gut microbiota to the substrate. As have been shown in Fig. 2.6c, INU and INUP groups clustered together. Interestingly, the CELP group was distinctively separated along the PC2 axis indicating a different microbial response from that of the CEL group. Accordingly, the clustered bar-chart at the phylum level (Figure 2.7a and b) shows that the INU and INUP groups were clustered together while the CEL and CELP groups formed two distinctively separate clusters. The Linear Discriminant Analysis (LDA) effect size (LEfSe) plot (Fig. 2.7c) also highlighted the individual response to the diet by certain bacterial groups. Interestingly, the redundancy analysis (RDA) identified that when PSPP was added to the diet, there was a significant ($p < 0.05$) difference between the cecal microbiota of PSPP-fed rats and non-PSPP-fed rats (Fig. 2.7c). The present demonstrated that microbial response to substrate depends on its physicochemical properties. For example, CEL and INU are less fermentable and fermentable DF respectively, and this will influence the microbial composition as seen in this study. In particular, CELP shifted the microbial composition due to the effect of PSP. It was reported that grape polyphenols demonstrated a distinct alteration in microbial diversity [210]. This is in line with this study where the CELP group was separated along the PC2 axis, which in fact, was affected by PSP supplementation. Consistently, Espley *et al*. [211] highlighted that

supplementation with apple anthocyanin affects species richness and diversity. Besides, it was highlighted in this study that PSP improved the fermentability condition for cellulose. This phenomenon could be associated with the upregulation of certain bacterial enzymes by PSP, as a result of the combined effect.

The microbial composition in this study was dominated by phyla Firmicutes and Bacteroidetes followed by Actinobacteria and proteobacteria (Fig. 2.7b). It is interesting to note that the findings of this study also indicated that when CEL was combined with PSP (CELP), it increased the relative abundance of Actinobacteria but reduced when combined with INU (INUP). This shows that bacterial proliferation can be differentially enhanced or thwarted by PSP depending on the fermentability of dietary fiber. At the genus level, the relative abundances of *Bacteroides*, *Bifidobacterium*, and *Sharpea* were not affected by PSP (Table 2.5). However, the relative abundance of *Prevotella*, *Lactobacillus*, *Coprococcus*, *Bulleidia*, and *Acidaminococcus* were significantly affected ($p < 0.05$) by PSP. Besides, *Clostridium*, *Bulleidia*, and *Acidaminococcus* were significantly affected ($p < 0.05$) by the interaction effect of fiber and PSP. Studies have reported that *Prevotella* is associated with a diet rich in fiber or non-digestible carbohydrates [212,213]. This might be the reason for the high abundance in the cellulose groups (CEL and CELP). Similarly, Kovatcheva-Datchary *et al*. [214] highlighted that the proliferation of *Prevotella* is diet-specific. In this study, the relative abundance of *Prevotella* in the CEL and CELP groups were almost the same, while in the inulin groups (INU and INUP), INUP increased the relative abundance to almost two times compared with the INU group. As have been mentioning earlier, PSP can potentially modulate the microbial composition depending on the fermentability of DF. The relative abundance of *Lactobacillus* was significantly affected ($p < 0.05$) by PSP. This result was different from the viable count result mentioning earlier above. *Lactobacillus* is a probiotic

with attributes associated with the host's health. The relative abundance of *Lactobacillus* was increased in both the supplemental groups (CELP and INUP). Other studies reported that polyphenols can increase the abundance of beneficial bacteria such as *Lactobacillus* [18,85]. Likewise, Zhang *et al*. [215] reported that anthocyanin increased the abundance of *Lactobacillus* by the metabolism of polyphenols and their corresponding monomers. This is consistent with the present study in which the increase in the relative abundance of *Lactobacillus* was attributed to PSP. Like *Lactobacillus*, the relative abundance of *Coprococcus* was affected ($p < 0.05$) by PSP, particularly when the PSP was supplemented with INU. *Coprococcus* is a bacterial genus responsible for the fermentation of dietary fiber and other complex carbohydrates to butyrate, the metabolite associated with the inhibition of colonic inflammation and carcinogenesis [216]. Further, *Clostridium* was significantly affected ($p < 0.001$) by the interaction of fiber and PSP, particularly, the abundance was suppressed in the CELP group, which is a positive response because *Clostridium* is a pathogen associated with many gastrointestinal illnesses [208]. Additionally, it was reported that *Acidaminococcus* is associated with type 2 diabetes [217]. Interestingly, the relative abundance of *Acidaminococcus* in the supplemental groups (CELP and INUP) was suppressed, particularly in the CELP group which was significantly different ($p < 0.05$) compared with the CEL group, which also explains that PSP can differentially influence certain bacterial enzymes depending upon the fermentability of dietary fiber. In this case, *Acidaminococcus* was suppressed when PSP was combined with CEL (less fermentable), which is a positive attribute. *Bulleidia* was significantly increased ($p < 0.05$) by both the PSP and the interaction effect in both the supplemental groups (CELP and INUP). *Bulleidia* is a beneficial bacterium associated with SCFA production [218] and PSP increased the abundance to a very significant level. PSP was responsible for the proliferation and suppression of beneficial and

potentially pathogenic bacterial species respectively (Fig. 2.8). For instance, the increase ($p < 0.05$) in *Collinsella stercoris*, *Bulleidia p1630c5*, *Bifidobacterium sp*., and *Lactobacillus sp*., and the decrease ($p < 0.05$) in *Acidaminococcus sp*. in this study were attributed to both PSP and the interaction effect. Molan *et al*. [219] reported that the blackcurrant extract increases the counts of beneficial bacterial species while decreasing the counts of potentially harmful species. Besides, β-glucosidase and β-glucuronidase increases and decrease respectively. This could be at play in the current study. That is, the enzymes responsible for beneficial bacterial growth and pathogenic bacterial growth were up-regulated and suppressed by anthocyanin contained in PSP respectively.

2.3.2. Short chain fatty acid production

SCFA (primarily acetate, propionate, and butyrate) production from non-digestible carbohydrates fermentation are essential with attributes associated with health [75]. Acetate prevents oxidative damage of the distal colonic cells while propionate is metabolized in the liver and subsequently used for gluconeogenesis and butyrate is an important fuel for colonocytes [76-79]. In this study, PSP did not either have any positive or negative effects on SCFA production, although the INU and INUP groups were significantly higher than the CEL and CELP groups, the attribution due to the differences in the fermentability of cellulose and inulin (Table 2.6).

2.3.3. pH in the fermenter

Colonic pH can be a biomarker associated with health as well. For example, fermentable fibers are fermented to SCFA and consequently reduce the colonic pH and suppressed the proliferation of pathogenic bacterial growth [220]. In this study, PSP was also responsible for the reduction ($p < 0.05$) of colonic pH from 12 to 48 h time points (Fig. 2.9). One reason could be associated with sugar moiety present in PSP which was utilized by the microbiota and subsequently acidify the

colonic environment. The other possibility could be the microbial conversion of dietary fiber and PSP might influence the production of organic acid, and consequently reduces the level of pH. Other studies have reported similar findings. For example, Zhu *et al*. [84] reported that microbial conversion of polyphenols affects other colonic pathways and processes, such as the production of formic or lactic acid. In this study, one of the main reasons for the acidic colonic environment is the high fermentability of dietary fiber, particularly inulin, that consequently increased the production of SCFA and subsequently acidify the colonic environment.

2.3.4. Putrefactive products in the fermenter

Fermentation of indigestible protein leads to the production of putrefactive products (phenol, ammonia, *p*-cresol, indole, 4-ethylphenol, and skatole), which is associated with some serious health issues affecting the host [99-104]. In this study, PSP neither has any positive or negative effects on ammonia production, although the INU and INUP groups were significantly lower than the CEL and CELP groups (Fig. 2.10a). Conversely, the *p*-cresol level was suppressed ($p < 0.05$) by PSP (Fig.2.10b). This could be associated with PSP structure that might suppress the production of *p*-cresol, or, the enzyme activation responsible for *p*-cresol production was inhibited by PSP. As have been mentioned in the introductory section *p*-cresol is an aromatic compound produced by gut microbiota during fermentation of *L*-tyrosine and is associated with chronic diseases and liver failure. Other studies also reported on the positive attributes of polyphenols in reducing the putrefactive products [104-107], which is in line with the present study.

2.3.5. Conclusions to Chapter 2

The purple sweet potato polyphenols displayed the modulatory effect on the colonic microbiota in this study. It differentially proliferated and inhibited the beneficial and pathogenic bacterial growth

respectively, depending on its association with the fermentable and non-fermentable dietary fiber. Accordingly, purple sweet potato polyphenols could be a material conducive to improving the conditions for the fermentation of non-fermentable dietary fiber. Besides, purple sweet potato polyphenols drastically reduce the putrefactive products, particularly *p*-cresol to a significant level. Therefore, this study demonstrated that purple sweet potato polyphenols might be a potential material for functional food ingredients that will confer health benefits to the host. Although this study elucidated the positive impact PSP has on the colonic microbiota, conclusions based on this study require further study, particularly a suitable *in vivo* animal model is warranted.

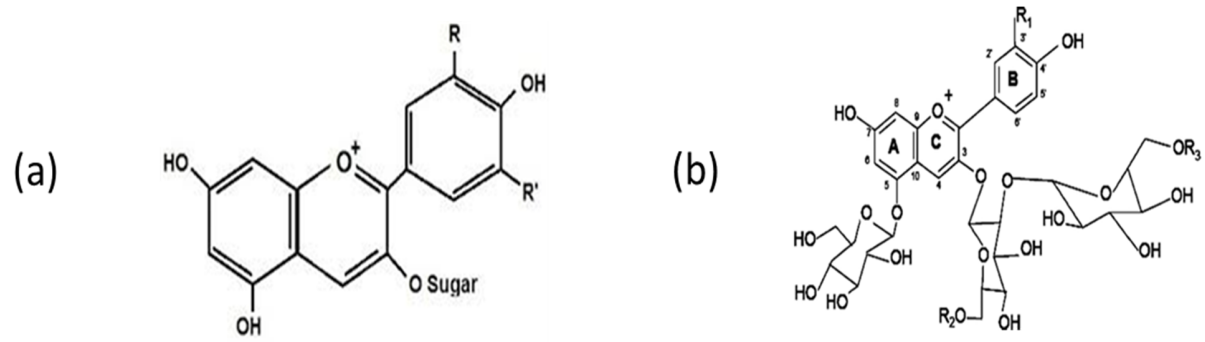


Fig. 2.1. *(a) basic chemical structure of anthocyanins and (b) structure of anthocyanins isolated from purple-fleshed sweet potatoes: cyanidin, R_1 = OH; pelargonidin, R_1 = H; peonidin, R_1 = OCH_3; R_2, R_3 = H, caffeic acid, ferulic acid, p-hydroxybenzoic acid, and p-coumaric acid [166].*

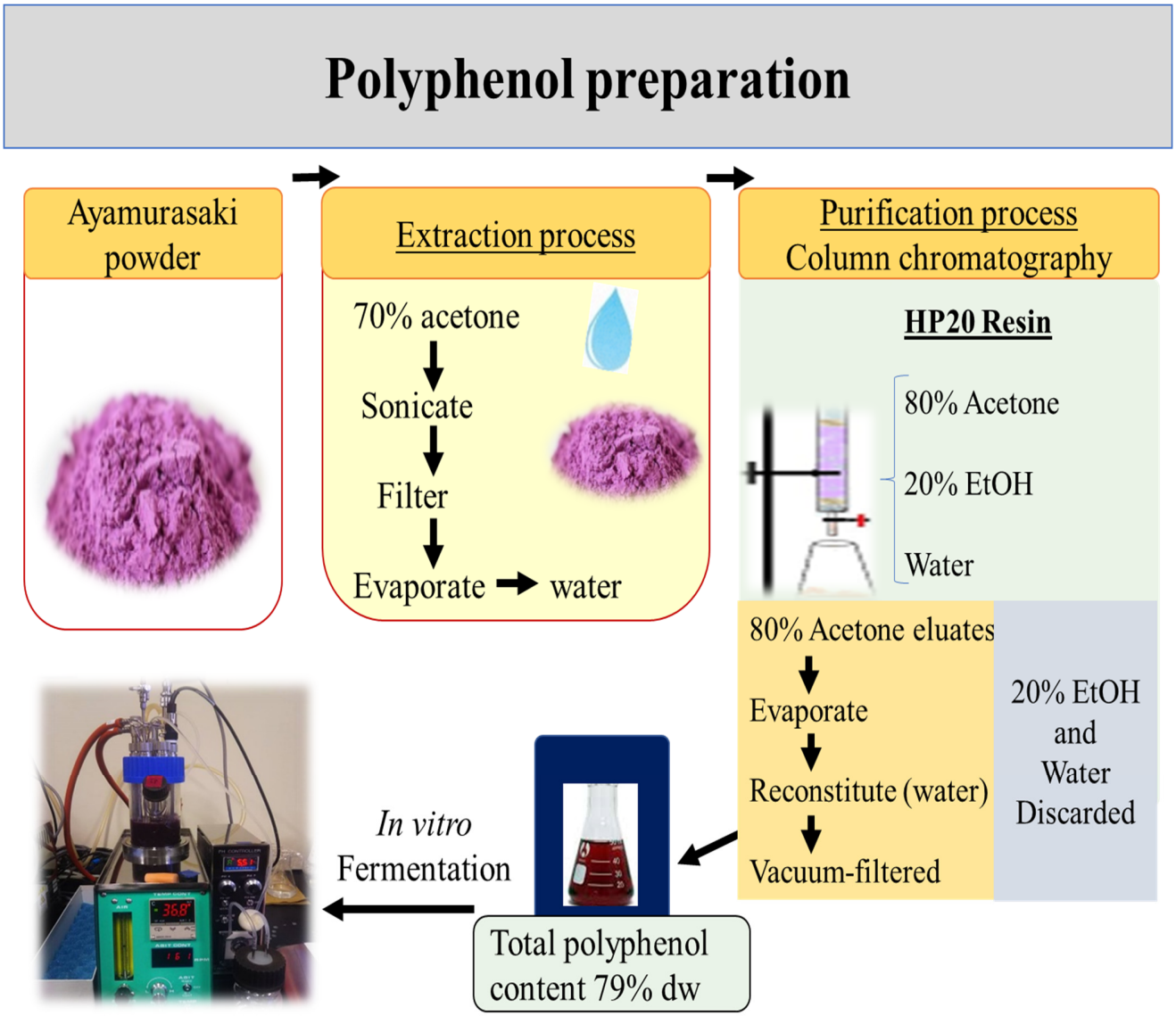


Fig. 2.2. *Procedure for polyphenol extraction*

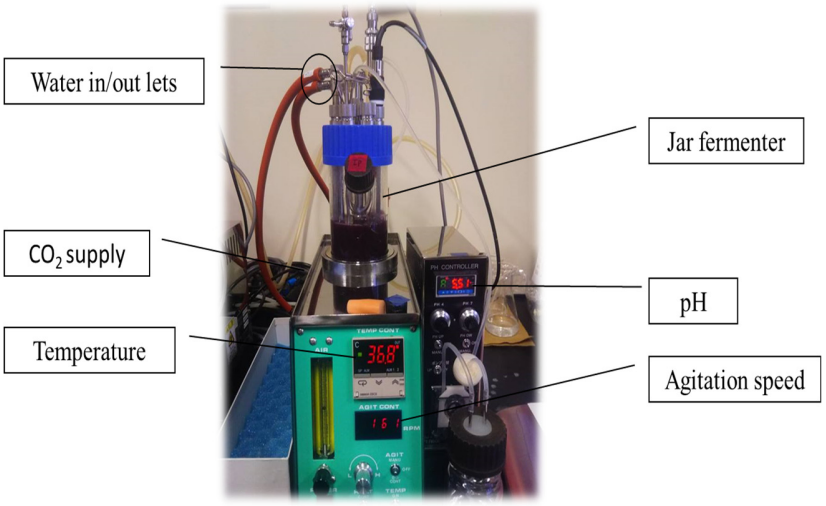


Fig. 2.3. *In vitro fermentation apparatus*

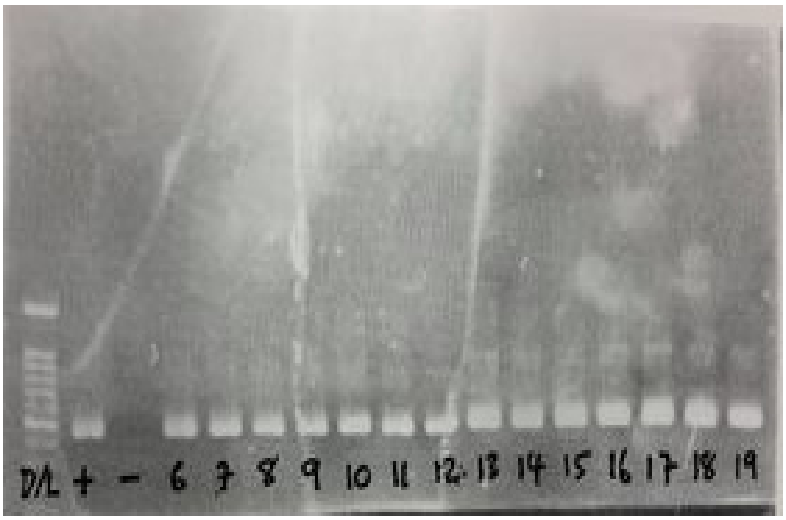


Fig. 2.4. *Gel electrophoresis bands*

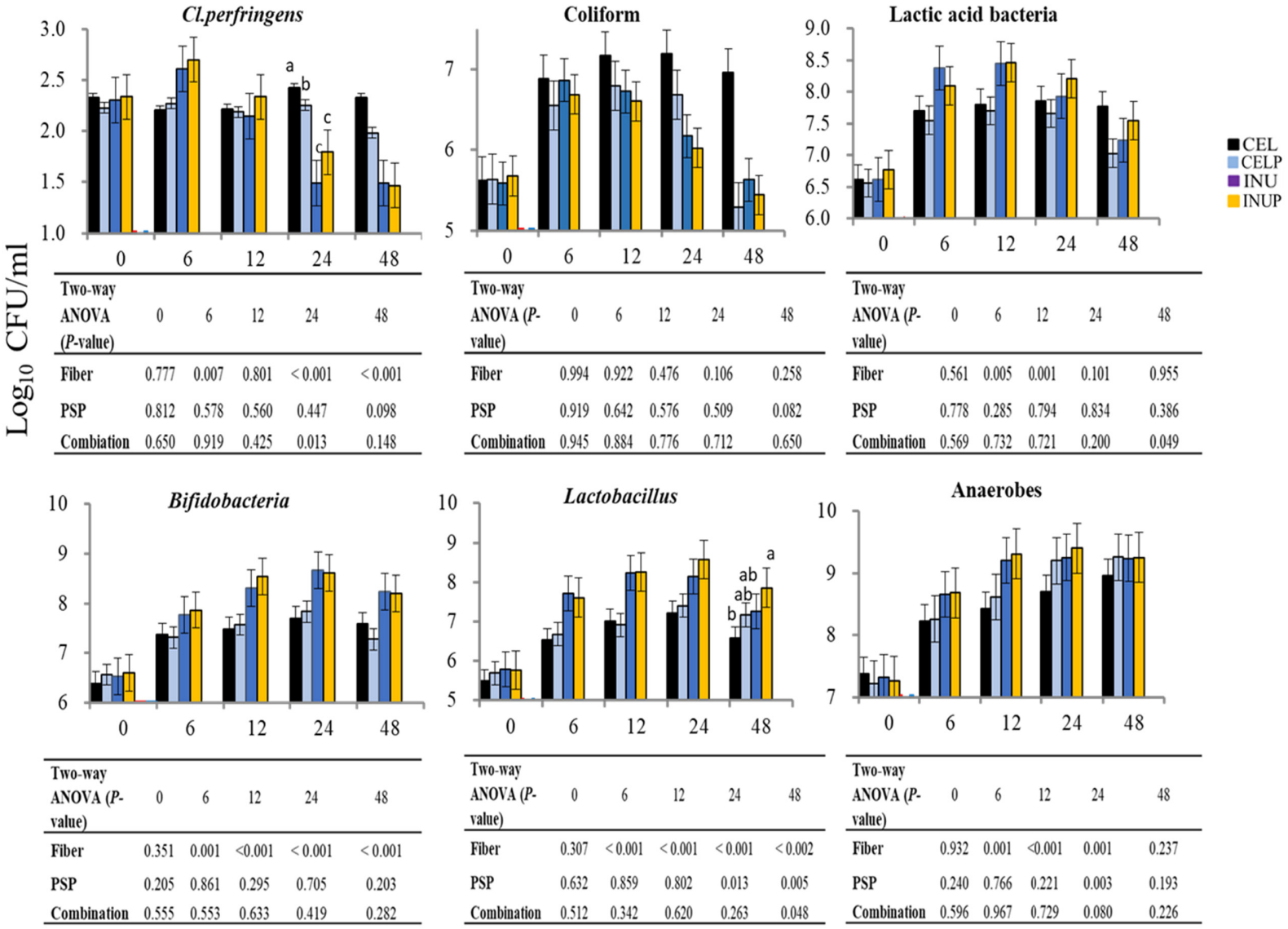
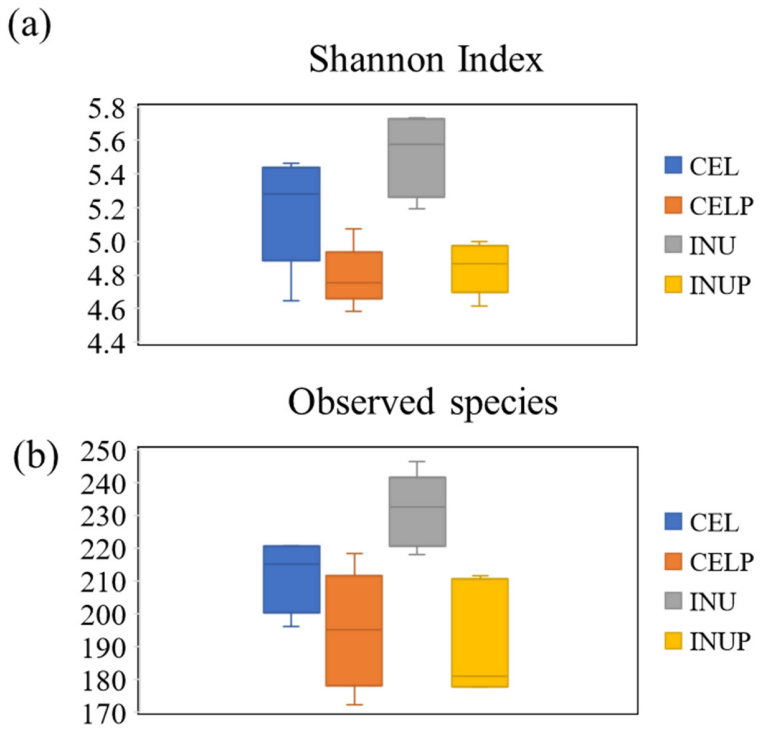


Fig. 2.5. *Changes in bacterial populations during in vitro colonic fermentation of 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU, Fuji FF), and 3% inulin + 0.16% PSP (INUP). Shannon diversity index (a) and Observed species (b) were compared by using the non-parametric Kruskal-Wallis rank sum test.*



Two-way ANOVA (*p*-values)	α-diversity	
	Shannon index	Observed species
Fiber	0.001	0.001
PSP	0.091	0.221
Interaction	0.217	0.096

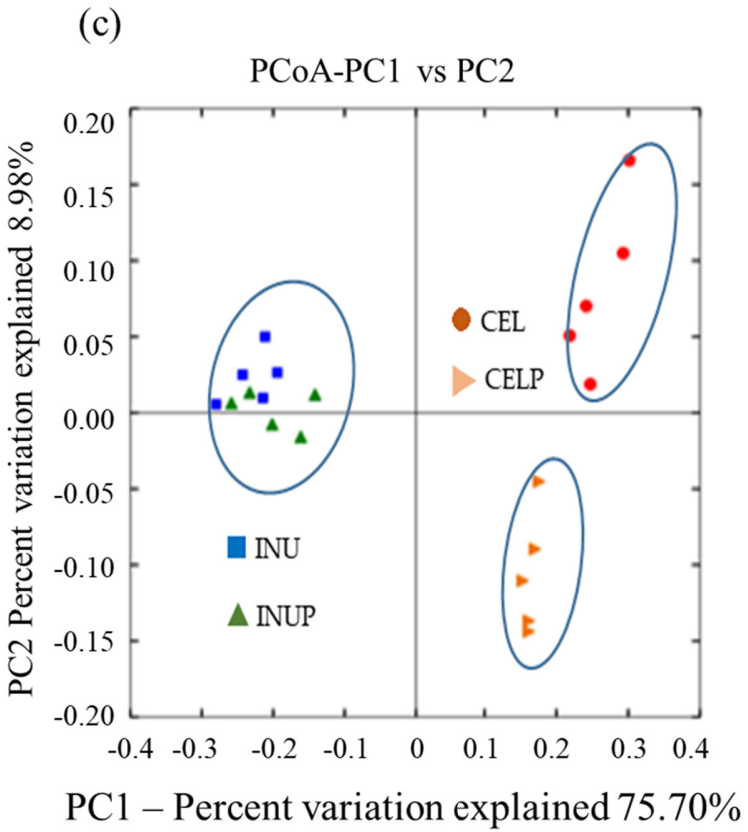


Fig. 2.6. *α-diversity (Shannon diversity index (a) and observed species (b)), β-diversity (c) comparisons of microbiota during in vitro colonic fermentation of 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU, Fuji FF), and 3% inulin + 0.16% PSP (INUP). Shannon diversity index (a) and Observed species (b) were compared by using the non-parametric Kruskal-Wallis rank sum test.*

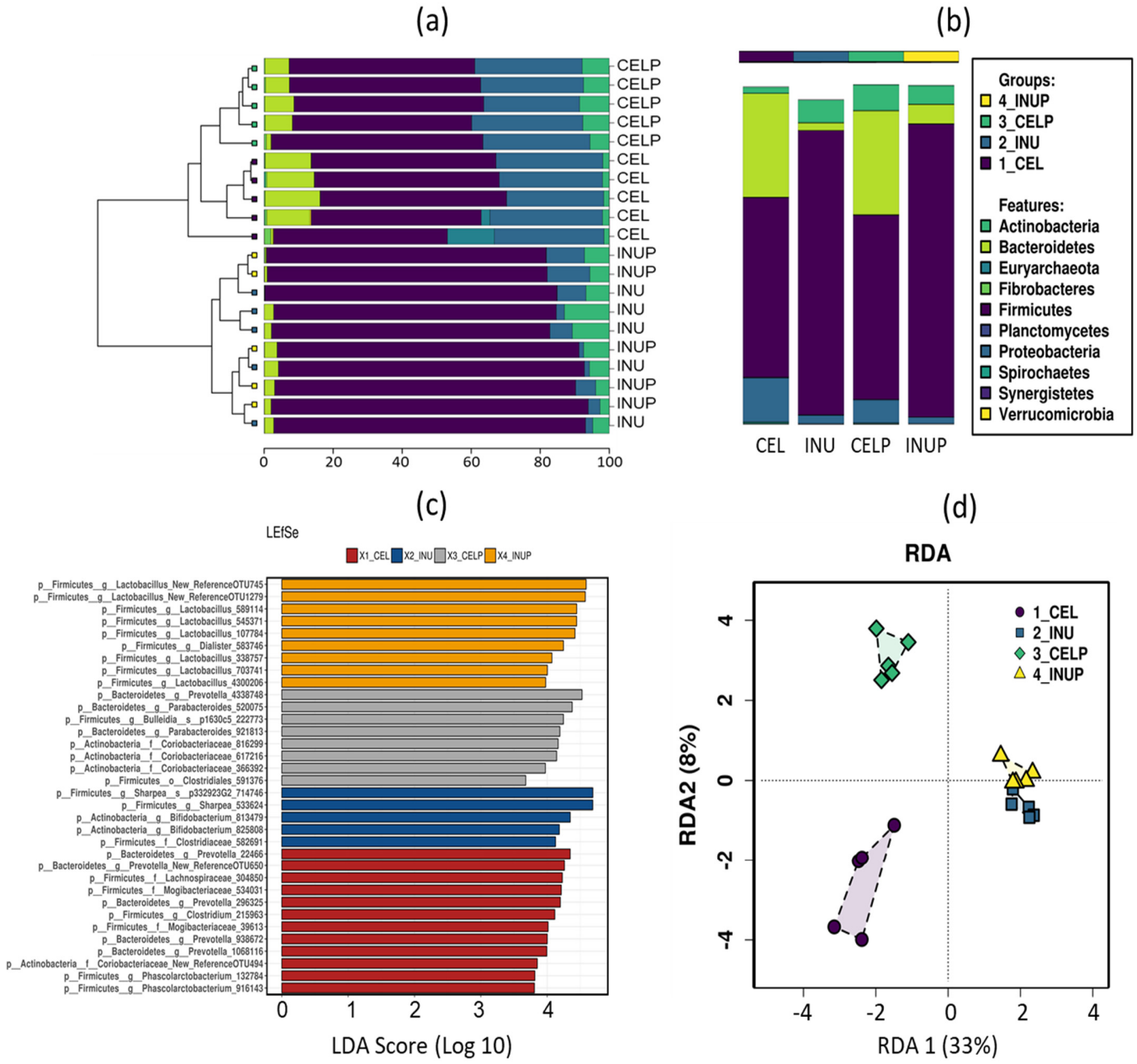
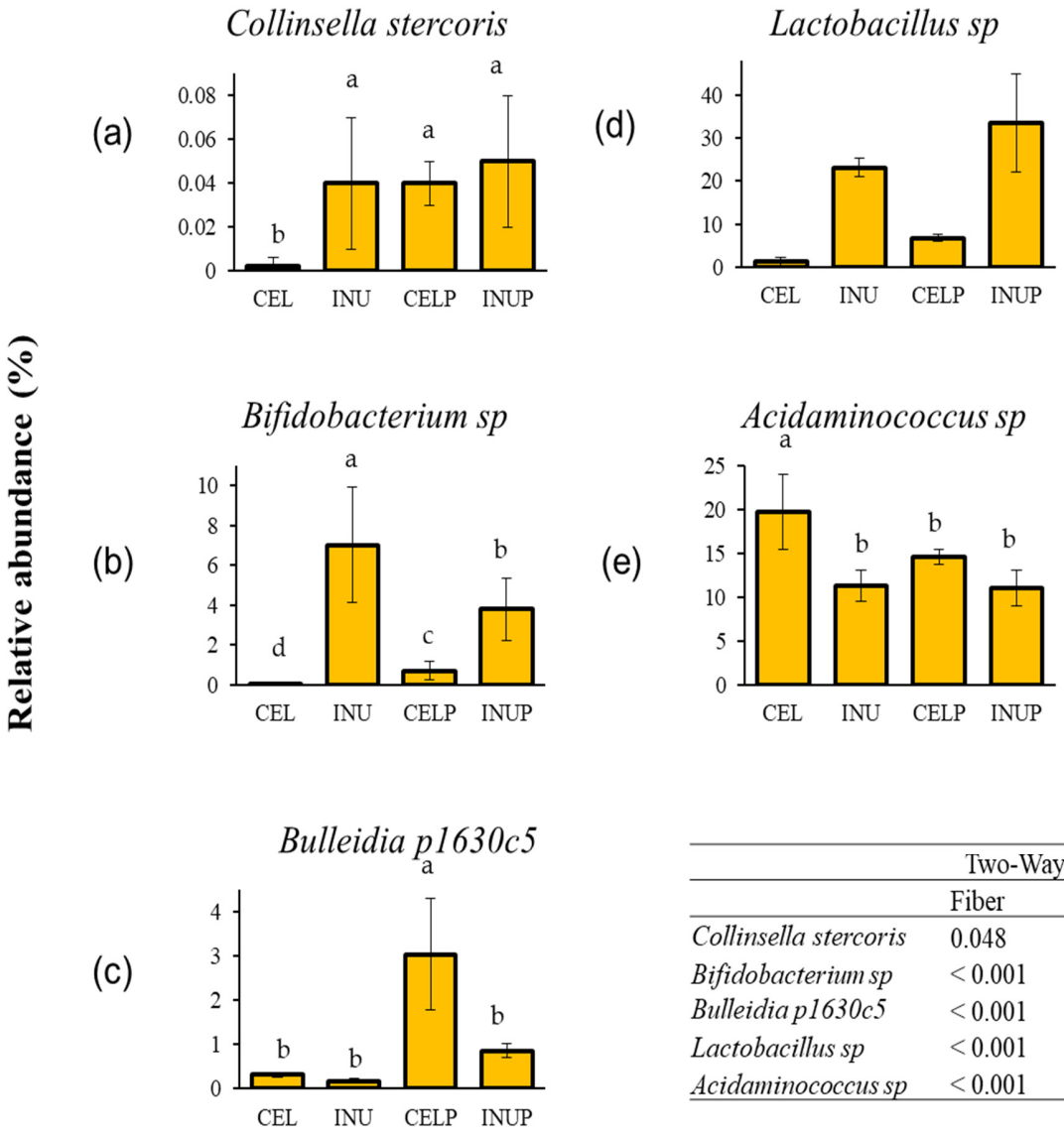
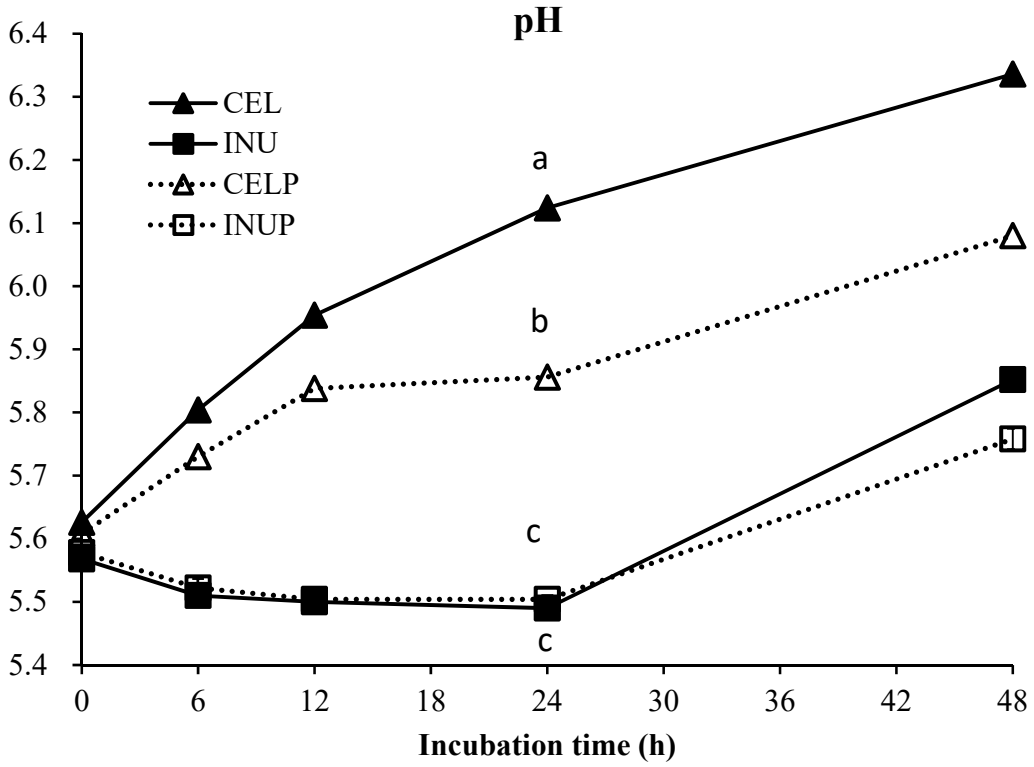


Fig. 2.7. *Clustered bar chart at the phylum level (a), predominant bacterial phyla (b), the linear discriminant analysis (LDA) effect size (LEfSe) plot (c), and redundancy analysis (RDA) (d) during in vitro colonic fermentation of 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU, Fuji FF), and 3% inulin + 0.16% PSP (INUP).*



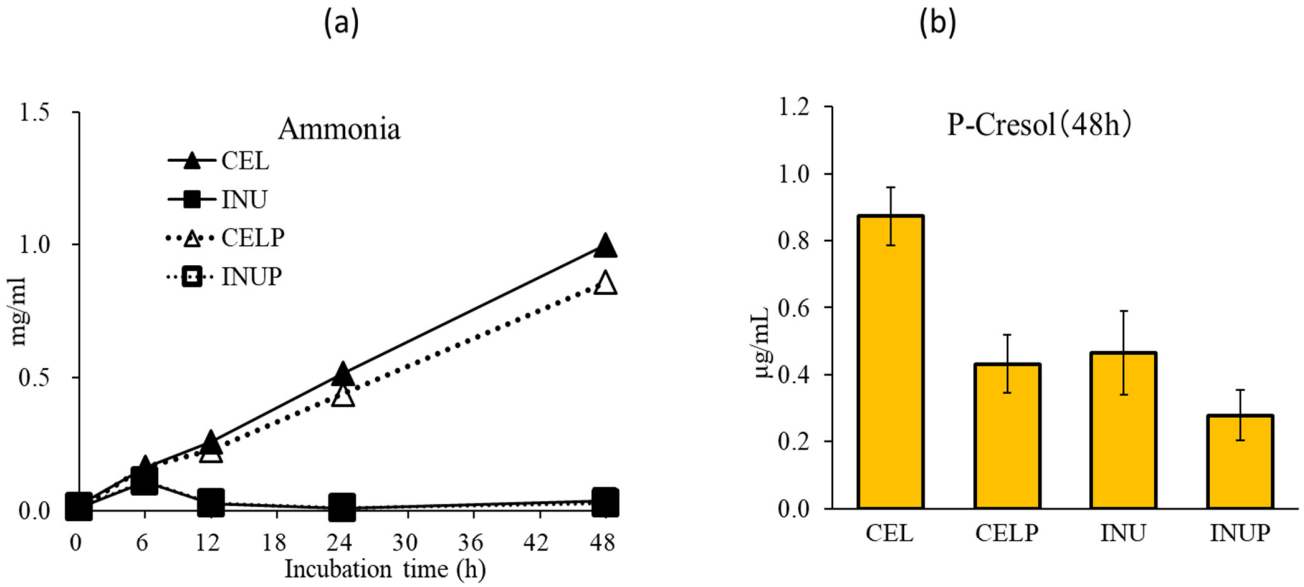
	Two-Way ANOVA (*p* - value)		
	Fiber	PSP	Combination
Collinsella stercoris	0.048	0.260	0.048
Bifidobacterium sp	< 0.001	0.108	0.018
Bulleidia p1630c5	< 0.001	< 0.001	0.010
Lactobacillus sp	< 0.001	0.013	0.460
Acidaminococcus sp	< 0.001	0.034	0.044

Fig. 2.8. *Relative abundances at species level during in vitro colonic fermentation of 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU, Fuji FF), and 3% inulin + 0.16% PSP (INUP). Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CEL and INU), PSP and their combination. P < 0.05 was considered to be statistically significant. If the variance was observed in the main effect of the interaction, Tukey's test was used for this comparison (p < 0.05).*



	Two-Way ANOVA (p-value)				
	0 h	6 h	12 h	24 h	48 h
Fiber	0.079	< 0.001	< 0.001	< 0.001	< 0.001
PSP	0.867	0.390	0.050	< 0.001	< 0.001
Combination	0.559	0.238	0.090	< 0.001	0.063

Fig. 2.9. *pH in the fermenters during in vitro colonic fermentation of 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU, Fuji FF), and 3% inulin + 0.16% PSP (INUP). Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CEL and INU), PSP and their combination. P < 0.05 was considered to be statistically significant. If the variance was observed in the main effect of the interaction, Tukey's test was used for this comparison (p < 0.05). Mean values designated by different letters (a-c) amongst the groups are significantly different (p < 0.05).*



Two-way ANOVA (*p*-value)					
	0 h	6 h	12 h	24 h	48 h
Fiber	0.221	< 0.001	< 0.001	< 0.001	< 0.001
PSP	0.256	0.936	0.376	0.330	0.548
Combination	0.100	0.979	0.315	0.306	0.591

Two-way ANOVA (*P-value*)	
Fiber	< 0.003
PSP	< 0.001
Combination	< 0.137

Fig. 2.10. *Putrefactive products (ammonia and p-cresol) in the fermenters during in vitro colonic fermentation of 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU, Fuji FF), and 3% inulin + 0.16% PSP (INUP). Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CEL and INU), PSP and their combination. P < 0.05 was considered to be statistically significant.*

Table 2.1. Polymerase chain reaction (PCR) mix for quality assurance PCR.

Component	Volume per sample (μL)
Distilled water	13.875
5X buffer	5.0
25mM $MgCl_2$	1.75
dNTP	0.75
Fw primer (10 mM)	1.25
Rv primer (10 mM)	1.25
Polymerase	0.125
Template DNA/Sample DNA	1.0
Total volume	25

Table 2.2. PCR conditions for total bacteria.

Step 1		Step 2			Step 3	
Initial denaturation	Denaturation	Annealing	Extension	Final extension		Store
94˚C 2 min	94˚C 15 sec	55˚C 15 sec	72˚C 30 sec	72˚C 7 min	94˚C 2 min	4˚C ∞
1 cycle		35 cycles			1 cycle	

Table 2.3. First stage polymerase chain reaction (PCR) mix.

Component	Volume per sample (μL)
Genomic DNA (5 ng/μL in 10 mM Tris pH 8.5)	2.5
PCR Fw primer (1 μM)	5
PCR Rv primer (1 μM)	5
2x KAPA HiFi HotStart ReadyMix	12.5
Total volume	25

Table 2.4. First stage PCR conditions.

Step 1		Step 2			Step 3
Initial denaturation	Denaturation	Annealing	Extension	Final extension	Store
95˚C	95˚C	55˚C	72˚C	72˚C	4˚C
3 min	30 sec	30 sec	30 sec	5 min	∞
1 cycle		25 cycles			1 cycle

Table 2.5. Changes in the relative abundances of genus during *in vitro* colonic fermentation.

	Groups				Two-Way ANOVA (*p*-value)		
	CEL	INU	CELP	INUP	Fiber	PSP	Combination
Genus (Relative abundance, %)							
Bacteroides	1.74 ± 0.30	20.2 ± 1.6	3.79 ± 1.20	16.9 ± 6.5	< 0.001	0.409	0.302
Prevotella	20.0 ± 2.4	3.64 ± 2.90	20.0 ± 2.4	6.39 ± 4.70	< 0.001	0.008	0.367
Bifidobacterium	0.01 ± 0.01	7.03 ± 2.90	0.71 ± 0.50	3.78 ± 1.60	0.002	0.072	0.071
Clostridium	2.18 ± 1.20 [a]	0.29 ± 0.10 [b]	1.61 ± 0.70 [a]	0.33 ± 0.20 [b]	< 0.001	0.108	0.018
Lactobacillus	1.27 ± 0.90	23.1 ± 2.1	6.76 ± 0.80	33.5 ± 11.0	< 0.001	0.039	0.334
Sharpea	2.01 ± 1.50	0.02 ± 0.01	0.62 ± 0.60	0.02 ± 0.03	< 0.001	0.354	0.356
Coprococcus	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.03	0.021	0.035	0.219
Bulleidia	0.82 ± 0.50 [b]	0.33 ± 0.10 [b]	3.42 ± 1.30 [a]	1.06 ± 0.10 [a]	< 0.001	< 0.001	0.010
Acidaminococcus	19.8 ± 4.3 [a]	11.3 ± 1.8 [b]	14.6 ± 0.9 [b]	11.1 ± 2.1 [b]	< 0.001	0.034	0.044

Values are reported as mean and standard error (n = 5). Two-way ANOVA was performed to assess the effect of fiber (cellulose and inulin), PSP, and their interaction. Differences of p < 0.05 was taken to be statistically significant. If the variance was observed in the main effect of the interaction, Tukey's test was used for this comparison. Mean values designated by different letters (a and b) amongst the groups are significantly different (p < 0.05).

Table 2.6. Changes in short chain fatty acid (SCFA) during *in vitro* fecal fermentation.

	Incubation time (h)	CEL	INU	CELP	INUP	Two-Way ANOVA (*p*-value)		
		μmol mL^{-1}				Fiber	PSP	Combination
Acetate	0	1.92 ± 2.00	2.00 ± 2.00	1.94 ± 0.22	1.94 ± 0.19	0.840	0.935	0.865
	6	4.54 ± 0.70	9.00 ± 1.50	5.68 ± 0.60	9.36 ± 1.50	0.003	0.529	0.744
	12	8.30 ± 0.70	34.2 ± 10.9	10.7 ± 0.4	31.6 ± 10.5	0.007	0.992	0.748
	24	12.0 ± 1.6	126 ± 20	16.6 ± 1.0	107 ± 25	< 0.001	0.672	0.482
	48	32.8 ± 9.6	200 ± 18	32.9 ± 4.4	182 ± 27	< 0.001	0.591	0.588
Propionate	0	0.57 ± 0.06	0.63 ± 0.07	0.54 ± 0.11	0.68 ± 0.11	0.255	0.935	0.664
	6	1.97 ± 0.92	2.55 ± 1.86	1.90 ± 1.01	2.60 ± 1.77	0.660	0.992	0.967
	12	3.97 ± 0.76	21.2 ± 12.5	5.10 ± 0.30	20.0 ± 13.3	0.097	0.995	0.898
	24	5.56 ± 0.85	83.8 ± 26.2	6.50 ± 0.60	65.5 ± 29.1	0.003	0.663	0.629
	48	13.5 ± 4.1	178 ± 12	12.6 ± 1.4	150 ± 23	< 0.001	0.290	0.319
n-Butyrate	0	0.11 ± 0.05	0.08 ± 0.03	0.11 ± 0.05	0.07 ± 0.04	0.396	0.902	0.975
	6	0.57 ± 0.13	1.06 ± 0.19	0.70 ± 0.13	1.00 ± 0.20	0.017	0.846	0.789
	12	1.68 ± 0.27	2.25 ± 0.22	2.00 ± 0.18	1.88 ± 0.09	0.276	0.903	0.111
	24	3.07 ± 0.30	3.81 ± 0.34	3.29 ± 0.11	3.43 ± 0.28	0.127	0.754	0.288
	48	5.33 ± 0.92	10.2 ± 1.3	5.16 ± 0.33	9.55 ± 2.39	0.005	0.770	0.861
Total SCFA	0	2.60 ± 0.22	2.71 ± 0.30	2.58 ± 0.36	2.70 ± 0.31	0.722	0.960	0.994
	6	7.10 ± 1.70	12.6 ± 3.2	8.30 ± 1.70	13.0 ± 3.2	0.062	0.760	0.887
	12	14.0 ± 1.7	57.6 ± 22.8	17.8 ± 0.8	53.5 ± 24.1	0.030	0.992	0.812
	24	20.7 ± 2.5	213 ± 46	26.4 ± 1.4	176 ± 53	< 0.001	0.661	0.549
	48	51.6 ± 14.6	388 ± 31	50.6 ± 6.1	341 ± 52	< 0.001	0.447	0.465

Values of short chain fatty acids (SCFA) for each sample treatments during in vitro colonic fermentation of 3% cellulose (CEL), 3% cellulose + 0.16% PSP (CELP), 3% inulin (INU) and 3% inulin + 0.16% PSP (INUP). Values are reported as mean and standard error (n = 5). Two-way ANOVA was performed to assess the effect of fiber (cellulose and inulin), PSP, and their interaction.

CHAPTER 3

Colonic fermentation characteristics of purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) polyphenols *in vivo*

3.1. Introduction

As has been reported in chapter 2, the purple sweet potato polyphenols (PSP) have been implicated to modulate the microbial composition, particularly its association with the less-fermentable dietary fiber (CEL). Notably, the effect of PSP according to the principal coordinate analysis (PCoA) plot upon treated with cellulose (CELP), shifted away from the cellulose (CEL) group indicating different microbial composition. Although the effect was not so pronounced between inulin and PSP, it was more implicated on CEL, which can be seen as a material that could conducive for the fermentation of less-fermentable dietary fiber. Besides, in chapter 2, PSP was more effective in reducing the putrefactive products, which is sometimes associated with the inhibition of putrefactive bacteria by the polyphenols. Although the results were convincing regarding the colonic modulation of microflora, nevertheless, it is warranted that an animal model should be used to further elucidate the fermentation characteristics of PSP. Apart from the anthocyanin structures and physiological functions of PSP discussed previously in the introductory section and the modulatory effect of PSP *in vitro* presented in chapter 2, could the *in vivo* result display a similar effect regarding the modulatory trend in the microbiota?

3.1.1. Physiological functionality of polyphenols from purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki)

Physiological functionality of polyphenols with brief information on purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) physiological functionality was discussed previously in chapter 1 and chapter 2. Purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) is a polyphenol/anthocyanin-rich cultivar. This cultivar was developed in 1995 particularly for industrial natural food colorants. Later it was discovered that it was packed with phytochemicals (anthocyanin) such as mono- or

di-acylated forms of peonidin and cyanidin, ferulic, and/or hydroxybenzoic acids, and was reported with strong antioxidant potential [194,221-223]. Studies have reported that polyphenols isolated from purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) were antimutagenic, angiotensin I-converting enzyme-inhibitory, α-glucosidase-inhibitory, and suppression of atherosclerotic lesions [224,225]. Stahl *et al*. [226] reported that lower carbohydrate concentration rather than polyphenols was responsible for the health attributes. Later Han *et al*. [195] ascribed to polyphenols isolated from purple sweet potato being a key player in the antioxidative status of rats. Still, information regarding the non-absorbable polyphenols *in vivo* from purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) is less well appreciated. Thus, in chapter 2, an *in vitro* approach was devised to elucidate the effects of polyphenol extract isolated from purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) during colonic fermentation using pig's feces with results indicating microbial modulation. This is in line with a study reported by Zhang *et al*. [215] which uses the same approach with the human colonic microbiota. While these studies may be important regarding the health attributes associated with sweet potato polyphenols/anthocyanin, it warrants further animal studies.

3.1.2. Aims of the study

Daily intake and opportunities regarding a polyphenol/anthocyanin-rich meal are by far quite high in some countries, but due to its limited knowledge regarding the physiological functionality, certainty regarding the non-absorbable fraction of polyphenol plays in the large intestine, particularly regarding the purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) is still unclear. Thus, this study aims to assess the effects of purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) on microbiota and biomarkers of colonic fermentation in rats (*in vivo*). Hence, two types of dietary fiber, namely cellulose (CEL) (partly fermentable dietary fiber) and inulin (INU

(Fuji FF)) (readily fermentable dietary fiber) (Fuji Nihon Seito Co. (Tokyo, Japan)) was designed as they are commonly used fibers for colonic fermentation studies to assess the fermentation characteristics of polyphenols to elucidate its health attributes and potential substrate for colonic microbiota.

3.2. Materials and Methods

3.2.1. Preparation of polyphenols from purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki)

The purple sweet potato tubers (*Ipomoea batatas* cv. Ayamurasaki) were bought from Kyushu, Japan in 2019. The tubers were thoroughly washed with water and dried using a paper towel. After drying with the paper towel, the tubers were peeled, cut into tiny slices, and freeze-dried. The dried potato slices were grounded to power. The extraction of purple sweet potato polyphenols (PSPP) was done by weighing 50g of potato powder and subjecting to 70% acetone and sonicate for 20 minutes. Chosen of 70% acetone was based on the previous study by Han *et al*. [227]. In a process of sonication, almost all colored pigments were extracted. The suspensions were filtered and centrifuged (14,600 × g, 4˚C, 30 min). After centrifuging, the acetone fraction was evaporated using a rotary evaporator at 35˚C, and the pigment extract was reconstituted with distilled water and ready for column chromatography. The recovered amount of PSP after extraction was approximately 2.40% (w/w). During column chromatography, the pigment extracts were applied to the pretreated Diaion HP-20 resin (Nippon Rensui Co., Tokyo, Japan) column so that the selected eluate fraction was collected. There were three types of solutions consecutively added to the elution column, namely, water, 20% ethanol, and 80% acetone. Based on the previous chapter 2, the pooled eluate fraction from 80% acetone was used for the study because of high polyphenol content. The acetone fraction was entirely evaporated using a rotary evaporator at 35˚C, and the concentrate was reconstituted with distilled water. The reconstituted samples were freeze-dried and samples were ready for *in vivo* study (Fig. 3.1). The total polyphenol content of the purple sweet potato extract was determined according to the Folin Ciocalteu method [199] with approximately 0.77mg/g as gallic acid equivalents in the dried purple sweet potato extract.

3.2.2. Animals and diets

The male Fischer rats (7-week-old) weighing 125–155 g were purchased from Charles River Laboratories (Yokohama, Japan). Upon arrival, all the rats were housed individually in transparent plastic cages with the following conditions; 23 ± 1 °C, 60 ± 5% relative humidity, and 12-h light/dark cycle (Fig 3.2). Animal model using rats have been preferred for study due to their anatomical, physiological, and genetic similarity to humans. Besides, the small size, ease of maintenance, and short life cycle made it an easy choice to use. The Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine approved the design of the animal study (approval number: 18-86). The rats were housed and treated according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council. Guide for the care and use of laboratory animals, 1996) [228]. In diet preparation, before treatment, the freeze-dried polyphenol extract reported above (Section 3.2.1) were well-mixed with the cellulose (CELP and CEHP) or inulin (INLP and INHP) using an electric food stand mixer (KPL9000S, Kenmix, Toda, Japan). The initial diet treatment commenced after a 7-day acclimation period, in which rats were allowed to stabilize in a new environment with free access to water and food. After a period of acclimation, the rats were randomly assigned to six groups (n=6 per group) and fed one of the experimental diets based on a modified AIN-93G diet (Oriental Yeast Co., Ltd., Tokyo, Japan): 5% cellulose (CEL), 5% CEL + 0.2% purple sweet potato polyphenol extract (CELP), 5% CEL + 1% purple sweet potato polyphenol extract (CEHP), 5% inulin (INU), 5% INU + 0.2% purple sweet potato polyphenol extract (INLP), and 5% INU + 1% purple sweet potato polyphenol extract (INHP) (Table 3.1). Inulin (Fuji FF) was gifted from Fuji Nihon Seito Co. (Tokyo, Japan). Polyphenol concentration of 1% was chosen based on a study by Han *et al.* [195]. During the start of diet treatment, body weights were determined without any significant difference amongst the

groups. The rats had access to feed and water *ad libitum* for 4 weeks. During the course of the study, body weights and feed intake were determined weekly and daily, respectively. At the end of the experimental period on the 27th day, before the animal sacrifice, blood samples (1 mL) were collected from the fasted rats (12 hours) from abdominal vena cava under anesthesia induced by Nembutal (sodium pentobarbital, 40 mg/kg body weight; Abbott Laboratories, Chicago, IL, USA), and the serum was separated by centrifugation (1500 × g for 20 min, Iwaki Glass Co., Ltd., Tokyo, Japan) and stored at -80°C for biochemical analysis. At the end of the experimental period, the tissues were excised and weighed, and the caecal content was diluted in sterile distilled water (1 g of caecal content/5 mL of deionized water) and the solution pH was measured. The remaining caecal content suspensions were stored at −80 °C until further analyses.

3.2.3. Serum lipid analysis

The blood collected on the 27th day (Section 3.2.1) was allowed to stand at room temperature for 2 hours. After 2 hours, the serum was then prepared by centrifugation (1500 × g for 20 min, Iwaki Glass Co., Ltd., Tokyo, Japan) and the serum was stored at -80°C for further biochemical analysis. The determination of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were done enzymatically using TBA-120FR/JA (Canon Medical Systems Co., Ltd., Tochigi, Japan). To determine the serum non-HDL-C concentration, it was calculated as follows: [non-HDL-C] = [total cholesterol] - [HDL-C].

3.2.4. Cecal bacterial DNA extraction and 16S ribosomal RNA (16S rRNA) gene sequences

Previously in chapter 2 (Section 2.2.3.2), the samples were obtained from the *in vitro* 48-h samples. In this chapter, *in vivo* samples were used, thus bacterial DNA was extracted from the caecal content without dilution. In this study, the procedures were employed according to chapter 2

(Section 2.2.3.2). That is, the extraction of bacterial DNA from the cecal digesta, DNA sample purification, polymerase chain reaction (PCR), Gel electrophoresis, first and second stage of PCR. The retrieved raw 16S rRNA gene sequences and the generated biome table were normalized using an equal subsampling size of 6824 sequences. Distances between bacterial communities in different samples, principal coordinate analysis (PCoA) plot, Calypso version 8.84 for bacterial visualization procedures were also employed from chapter 2 (Section 2.2.3.2).

3.2.5. Cecal organic acid analysis

The caecal organic acid contents in the diluted caecal digests were vortexed and centrifuged (16,000 × *g*, 4°C, 15 min), and the methods for organic acid analysis were employed according to the methods presented in chapter 2 (Section 2.2.5) by using the high-performance liquid chromatography (Shimadzu LC-10AD, Kyoto, Japan) for the analysis with the following analytical conditions: Column, RSpak KC-811 (8.0 mm × 300 mm, Shodex, Tokyo, Japan), eluent and flow rate, 2 mM perchloric acid at 1.0 mL/min, column temperature, 47 ◦C, reaction reagent and flow rate, ST3-R (10× diluted, Shodex) at 0.5 mL/min, UV detector wavelength, 430 nm.

3.2.6. Cecal ammonia-nitrogen analysis

The samples were vortexed and centrifuged (3,000 rpm, 4°C, 15 min) followed by the cecal ammonia-nitrogen concentration determination according to the procedures presented in chapter 2 (Section 2.2.6) using a commercially available kit (Wako Pure Chemical Industry, Ltd., Tokyo, Japan).

3.2.7. Cecal Immunoglobulin A (IgA) analysis

IgA in the samples was analyzed by an enzyme-linked immunosorbent assay (ELISA) kit (Montgomery, TX, United States). To determine the IgA level in the cecal sample, 0.05M coating

buffer was prepared (carbonate-bicarbonate buffer, pH 9.6) in 1 µL:1 µL:100 µL ratio of sample, antibody, and buffer, respectively which was then pipetted into the wells (100 µL/well) and allowed to stand for 1 hour at room temperature. After 1 hour, the well plate contents were discarded and wash with wash buffer (0.05 M Tirs, 0.14 M NaCl, 0.05% Tween20, pH 8.0) in the microplate washer (Thermo Fischer Scientific Oy, Vantaa, Finland) five times. After, the plate was removed and dried by tapping on a paper towel followed by the addition of 200 µL of block solution (50 mM Tris, 0.14 M NaCl, 1% bovine serum albumin powder, pH 8.0) and kept at room temperature for 30 minutes and subsequently stored in the refrigerator at 4°C overnight. On the next day, the block solution was discarded and wells were washed as before with microplate washer. The centrifuged (3,000 rpm, 4°C, 15 min) samples and standards were prepared and 100 µL each from sample and standard was pipetted into wells in duplicates and allowed to stand for 1 hour at room temperature. After 1 hour, the plate was washed once more and 100 µL biotinylated detection antibody (HRP) was added into each well and allowed to stand for 1 hour at room temperature. After, the contents were discarded once more and washed as before with the plate washer. After 100 µL of tetramethylbenzidine (TMB) was added into each well and subsequently allowed to stand in the dark for 20 minutes. After 20 minutes the stop solution (0.18 M H2SO4) was added and the samples in the well plate were measured in the well plate reader (MultiskanTM FC, Thermo Fisher Scientific).

3.2.8. Cecal mucin analysis

The caecal mucin level was determined according to the method described by Bovee-Oudenhoven *et al.* [229]. The samples were mixed properly by vortex and incubated for 10 minutes at 95°C and again for 90 minutes at 37°C consecutively. After, the samples were vortex and centrifuged (20,000×g, 4°C, 15 min). The supernatant (100 µL) was pipetted into a 1.5 mL Eppendorf® tube

followed by 0.4 M acetate buffer (pH 4.75) and well mixed. After the 10.4 µL of amyloglucosidase (300 U/mL) was added and the samples were incubated once more for 20 minutes at 50°C. After incubation, 310 µL of cold ethanol (99%) was added, vortex, and stored overnight at -30°C. On the next day, the samples were vortexed and centrifuged (20,000×g, 4°C, 10 min) and supernatants were carefully removed and 1.5 mL of PBS buffer (1.9 mM NaH2PO4, 8.1 mM Na2HPO4, 0.154 M NaCl, 0.05% NaN3, pH 7.2) was added to the precipitate to solubilized. Following this, the standard was prepared and 300 µL each from standard and samples were pipetted into tubes followed by 360 µL of 0.6 M cyanoacetamide diluted in NaOH (cyanoacetamide:NaOH = 1:5 v/v) was added and mixed well on vortex and incubated for 30 minutes at 100°C. After cooling in water, 3 mL of 0.6 M borate buffer (pH 8.0) was added and vortex and measured using a fluorescence spectrophotometer (FP-6200, Jasco International Co., Ltd., Tokyo, Japan) at 336 nm and a measurement wavelength of 383 nm. Therefore, the mucin content (µmol/mL) = (Sample absorbance – y-intercept) Gradient of line * (dilution factor*1000)/221.

3.2.9. Cecal indole analysis

Cecal indole level was determined according to Ikeda *et al*. [204]. The procedures for cecal indole determination were done according to the procedures reported previously in chapter 2 (Section 2.2.6).

3.2.10. Statistical Analysis

All data are presented as a mean and standard error (SE) (n=6). Two-way ANOVA was performed to assess the effect of fiber (cellulose and inulin), PSPP, and their combination and differences of $p < 0.05$ was taken to be statistically significant. If the variance was observed in the main effect of

combination, Tukey's test was used for this comparison ($p < 0.05$). The analyses were performed using the SPSS software (version 17; IBM Corporation, Armonk, NY, USA).

3.3. Results and Discussion

3.3.1. Feed intake, body weight, and tissue weight

Amongst the groups, feed intake, body weight, perirenal fat weight, and epididymal fat weight were reduced ($p < 0.05$) by the IN treatment while the cecal wall and cecal contents were increased by IN; however, these parameters were not affected by the PSPP treatment (Table 3.2). The liver weight differed neither between the fiber- nor between the PSPP-treated groups (Table 1). And, there were no significant differences in the combined effect of fiber and PSPP on those parameters as well.

3.3.2. Serum lipid and ammonia levels

The effect of fiber, particularly IN treatment reduces ($p < 0.001$) the serum TC, non-HDL, and TG level. When PSPP was added to the diet, the level of TG dose-dependently increases to a significant ($p < 0.05$) level in the PSPP-treated groups. Besides, the serum HDL-C level was significantly ($p = 0.025$) increased by the PSPP treatment (Table 3.3) while the effect of PSPP on serum non-HDL-C level was not different amongst the groups. On the other hand, and interestingly, the serum ammonia level was significantly ($p < 0.05$) reduced dose-dependently by the PSPP treatment (Table 3.3).

Inulin is a well-known fermentable dietary fiber and thus, reduces the cholesterol level. Kilua *et al*. [230] also reported that kidney fiber reduces cholesterol level. Interestingly, in this study, the level of serum TG was increased by PSPP supplementation. High TG level increases the incidence associated with stroke, heart attack, and acute inflammation of the pancreas (pancreatitis). It was reported that serum TG was originated from the dietary fat and through the action of lipases, it can hydrolyze to fatty acids and monoglycerides [231]. Many studies have reported that polyphenols

positively decrease serum lipid levels [232,233]. However, some studies reported the opposite results showing that polyphenols increased or did not link with the change in the serum lipid levels [232,234]. Possibly, it could be associated with the high level of purified polyphenol in the diet as Nakagawa *et al*. [235] stated that pure anthocyanins were shown to elevate the plasma levels of homocysteine in rats. The total polyphenol content of PSPP was 77.0% (w/w) as gallic acid equivalents in the dried purple sweet potato extract. Similarly, the high level of HDL-C was attributed to PSPP, and a lower level of HDL-C is associated with metabolic syndrome (MetS) [236] and regarded as good cholesterol because of its cardiovascular protective factor. Other studies also reported that a high level of HDL-C is associated with polyphenol intake [237,238] and this is in line with this study as well. In this study, the level of serum ammonia was reduced by PSPP. A high serum ammonia concentration is associated with a high incidence of cerebral edema and herniation [239]. Interestingly, the level of serum ammonia was reduced by PSPP. Likewise, a similar result was also reported by Julia *et al*. [240] and Goto *et al*. [19].

3.3.3. 16S Ribosomal RNA (16S rRNA) gene sequences

The α- diversity (Shannon index and species richness) and β-diversity were not affected by PSPP, although the differences were associated with the differences in the fermentability of cellulose (CE) and inulin (IN) (Fig. 3.3), which was indicated by two distinctively microbiota compositions between CE- and IN-fed rats in Figure 3.3c. Similar to the previous *in vitro* study in chapter 2, the effect of PSPP on IN-fed rats in this *in vivo* study was not seen. Besides, the clear distinction in the microbial composition when PSPP was supplemented with CE (as was the case in chapter 2) was not seen in this *in vivo* study. However, the effect of PSPP in exerting an individual response to the diet by the microbiota can be seen in this study as indicated by the Linear Discriminant Analysis (LDA) effect size (LEfSe) plot and the bar chat (Fig. 3.4). Further, it is interesting to note

the significant effect ($p = 0.001$) of PSPP at a higher concentration, according to the Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA) (Fig. 3.5). There are many *in vivo* studies previously been conducted on the fermentation characteristics of polyphenols. For example, it was reported that grape polyphenol extract increased the microbial α-diversity [241], and polyphenol-rich Canarium album extract altered the gut microbiota [242]. Previously, studies regarding the purple sweet potato polyphenols (PSPP) (*Ipomoea batatas* cv. Ayamurasaki) have been reported [158,195,224,226], but knowledge regarding the non-absorbable polyphenols in the large intestine, particularly PSPP is less well appreciated, therefore, this *in vivo* study was conducted to elucidate the fermentation characteristics of PSPP. This study indicated that the α-diversity and β-diversity were not changed by the PSPP. Seemingly, though not significant, the β-diversity was impacted by PSPP depending on the fermentability of dietary fiber. Besides, the individual microbial response and the differences in the microbial composition between PSPP-fed rats and non-PSPP-fed rats attributed to PSPP was also seen in the polyphenol-rich Canarium album extract, in which the microbial composition was shifted away from the model control, indicating altered gut microbiota [242]. According to the bubble plot (Fig. 3.6), the predominant bacterial phyla distributed equally amongst the groups was Firmicutes, and at this level, the effect of PSPP was not seen. The dominant of Firmicutes in this *in vivo* study was similar to the previous *in vitro* study in chapter 2. The bacterial genera used for the results were based on the relative abundance expression of above 2% in CEL-fed rats and the taxa that change significantly according to the diet treatments. The bacterial genera in this study were affected ($p < 0.05$) by the dietary fiber. For example, IN-fed rats reduced ($p < 0.05$) the relative abundances of *Oscillospira* and *Bacteroides* compared with the CE-fed rats. Interestingly, supplementation of the diet with PSPP significantly reduced ($p < 0.05$) the relative abundances of *Oscillospira* and *Bacteroides*

without any effect due to combination. On the other hand, while IN-fed rats increased the relative abundance of *Dorea*, PSPP supplemented diets significantly ($p < 0.05$) increased the relative abundance of *Dorea* without any combination effect. The relative abundances of *Ruminococcus*, *Parabacteroides, and Coprococcus* were not affected by PSPP nor the combination of fiber and PSPP (Table 3.4). While *Bacteroides* a predominant bacterial genus in the colon, *Oscillospira* on the other hand is associated with high-energy diet [243,244]. It was reported that polyphenols isolated from purple sweet potato inhibited the growth of *Bacteroides in vitro* [215]. Likewise, *Oscillospira* was also suppressed by polyphenols [245]. These results are in line with the present *in vivo* study and might indicate that PSPP is a potential material for the reduction of pathogenic bacterial growth. Besides, the quorum sensing mechanisms of bacteria is reportedly been disrupted by polyphenols [246,247]. In this study, the modulatory effects could be associated with the chemical structure of polyphenols because Sun *et al*. [248] reported that acylated peonidin exhibits a higher inhibition sensitivity on harmful bacteria. Further, *Dorea* is a genus related to the digestion of a high-fiber diet [249] and is associated with the host health. It was reported that polyphenols increase the abundance of *Dorea* [250], which is in line with the present study. Other studies reported that peonidin enhanced the growth of beneficial bacteria while the highly acylated anthocyanin prevent pathogenic bacterial growth [248,251,252]. Thus, PSPP and its metabolite may be a potential material conducive to increase certain beneficial intestinal microbiota as reported by Hidalgo *et al.* [253] that anthocyanin and their metabolites can exert a positive effect on the colonic microbiota.

3.3.4. Cecal biomarkers

3.3.4.1. Cecal organic acid production

All the cecal organic acids were impacted by the fiber (CE and IN) ($p < 0.05$). However, the cecal level of acetate was affected by the combination of fiber and PSPP ($p = 0.003$), particularly the CELP group was significantly ($p < 0.05$) higher than the other groups (Table 3.5). Besides, the cecal succinate production was affected by PSPP ($p < 0.05$), particularly the supplemental groups with a lower concentration of PSPP (CELP and INLP). Likewise, the cecal level of iso-butyrate affected ($p < 0.001$) by both the PSPP and its combination with fiber. It was reported that organic acids (mainly acetate, propionate, and butyrate) were associated with the host's health [254]. In this study, the cecal levels of acetate, propionate, and butyrate were not affected by PSPP alone. This was similar to the previous *in vitro* result in chapter 2 and was also similar to a study reported recently [250]. Interestingly, the level of acetate alone was affected by the combined effect of fiber and PSPP, particularly with the lower concentration of PSPP and the fermentability of dietary fiber. It was reported that acetate was produced by many groups of bacteria [255], and this result might suggest that specific acetic acid-producing bacteria might be suppressed by PSPP because α- and β-galactosidases and β-glucosidase in the intestinal digests were suppressed by polyphenols [91]. Similarly, succinate was increased particularly with lower PSPP concentration. Further, the formation of branched-chain fatty acid (BCFA) was the result of valine, leucine, and iso-leucine fermentation. In a pre-diabetic patient which was reported previously in a clinical study, high levels of BCFA were observed [256], and interestingly, cecal iso-butyrate production was suppressed in a dose-dependent manner in the CELP and CEHP groups and agrees with other studies [257,258]. Generally, PSPP was more effective when it combines with less fermentable fiber as was seen in the previous chapter 2 of the study.

3.3.4.2. Cecal pH

The cecal pH was significantly ($p < 0.05$) affected by PSPP in this study (Fig. 3.7). Acidifying the cecal environment provides a conducive environment for beneficial bacterial growth while suppressing the growth of pathogenic bacterial [220]. It was decreased dose-decently in the cellulose groups, while no significant effect could be seen in the inulin groups, which could be associated with the high organic acid production as a result of degradation of the de-glycosylated PSPP/anthocyanins and consequently reduce the cecal environment

3.3.4.3. Cecal putrefactive products-Ammonia and Indole

Higher levels of putrefactive products are an indication of protein fermentation and it is of great concern when produced at a toxic level. For example, tryptophan is catabolized to indole and subsequently facilitate the toxic material across the gut barrier and consequently destroys the gut epithelial permeability [259,260]. In this study, the effect of fiber and PSPP, and their combined effect could not be seen on the production of cecal ammonia. Likewise, PSPP did not affect the cecal production of indole although it was reduced at a higher level of PSPP concentration (CEHP and INHP) (Fig. 3.7).

3.3.4.4. Intestinal immunity-related substances - Mucin and IgA

Interestingly, in this study, the mucin level was increased ($p < 0.05$) by PSPP and its combination with dietary fiber (Fig. 3.7), particularly when it combines with CE at a lower concentration (CELP), which was significantly higher ($p < 0.05$) than the CE group. Besides, there were no significant changes when PSPP was combined with IN group. IgA on the other hand, was unaffected by PSPP, although the inulin groups significantly increased ($p < 0.05$) the IgA production compared with the cellulose groups. The biomarkers (mucin and IgA) are an intestinal

immunity-related substance which are important for the host's health. Mucin, for example, is a first line defense against pathogen while IgA inhibits the bacterial attachment to the epithelium [139,261].

3.3.5. Conclusions to Chapter 3

The modulatory effect of PSPP on the cecal microbiota in this study was positively affecting the biomarkers of colonic fermentation properties. Besides, PSPP was more effective at a lower concentration with less fermentable dietary fiber, which could indicate a material conducive for the fermentation of less fermentable dietary fiber. This was similar to the previous *in vitro* result in chapter 2. Therefore, in this chapter, the result indicated that the inclusion of PSPP in the diet could be a material positively affecting the colonic microbiota and biomarkers of colonic fermentation that will simultaneously confer beneficial health to the host.

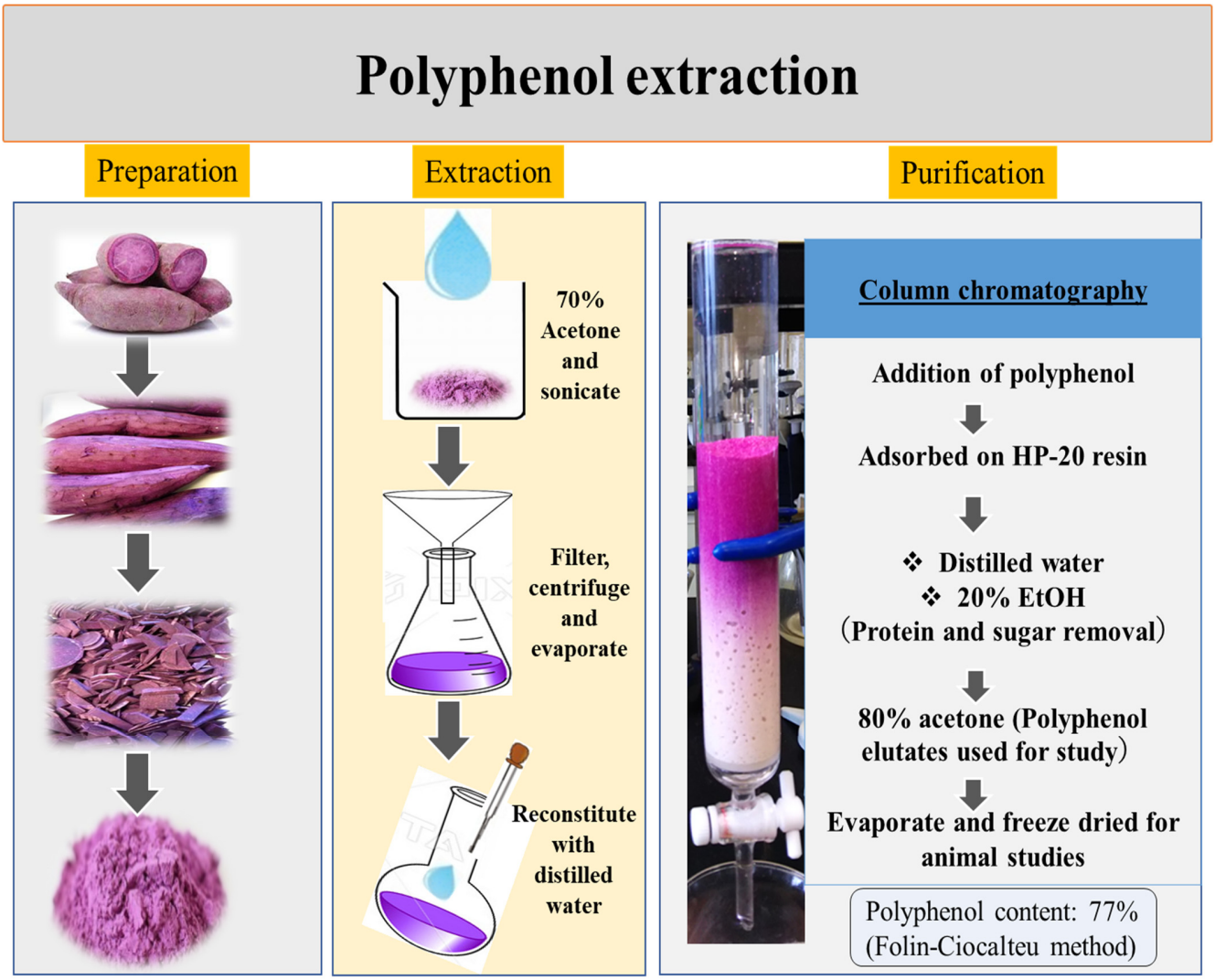


Fig. 3.1. *Procedure for polyphenol extraction*

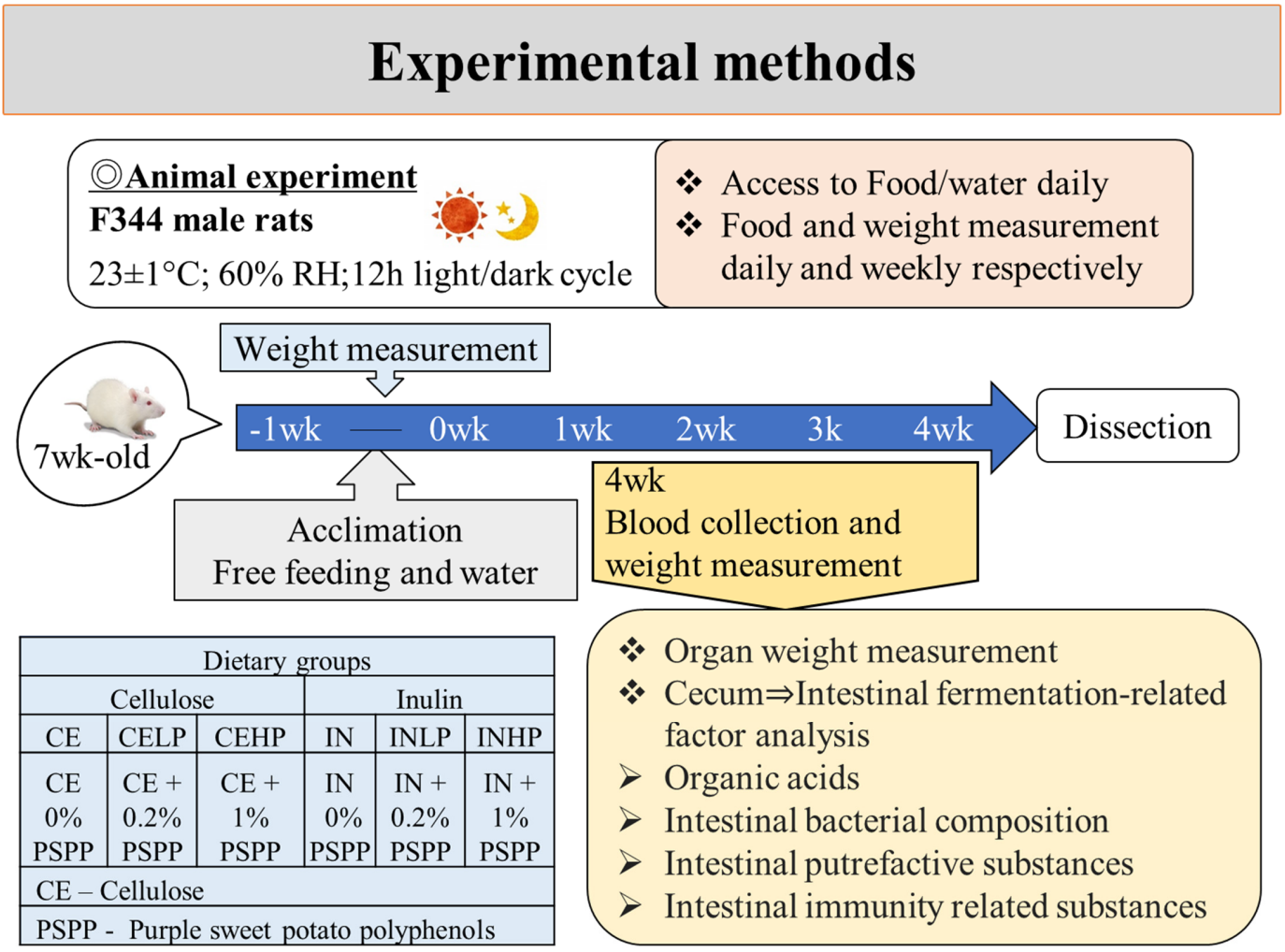
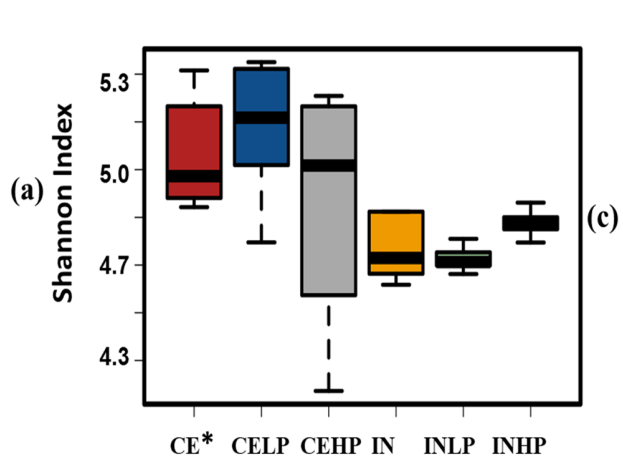
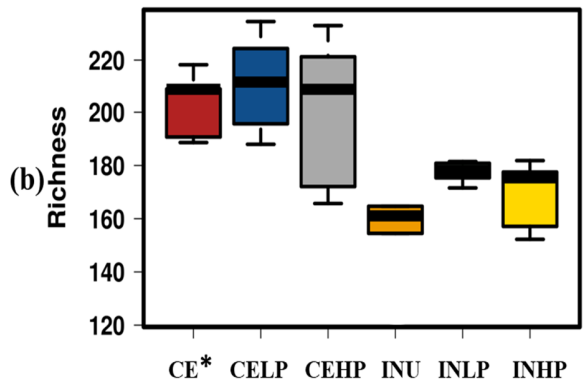
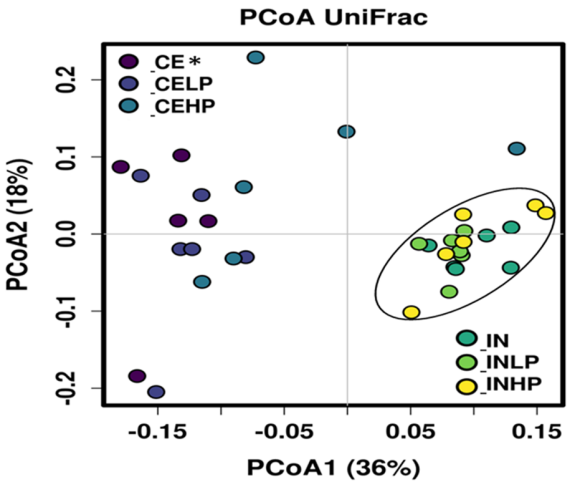


Fig. 3.2. *Schematic representation of the animal experimental design.*







Two-way ANOVA (*p*-value)	*α*-diversity		*β*-diversity
	Shannon Index	Richness	
Fiber	0.745	< 0.001	< 0.001
PSPP	0.070	0.159	0.352
Combination	0.682	0.401	0.453

Fig. 3.3. *α-Diversity (Shannon index (a) and species richness (b)), and β-diversity (c) comparisons of microbiota during colonic fermentation in rats for 4 wks. Shannon index (a) and species richness (b) were compared using the non-parametric Kruskal–Wallis rank-sum test. CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple sweet potato (PSPP); CEHP, 5% cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; INHP, 5% inulin + 1% PSPP. Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CE and IN), PSPP, and their combination. P < 0.05 was considered to be statistically significant. Data were expressed as mean and SE (n=6, but *n=5).*

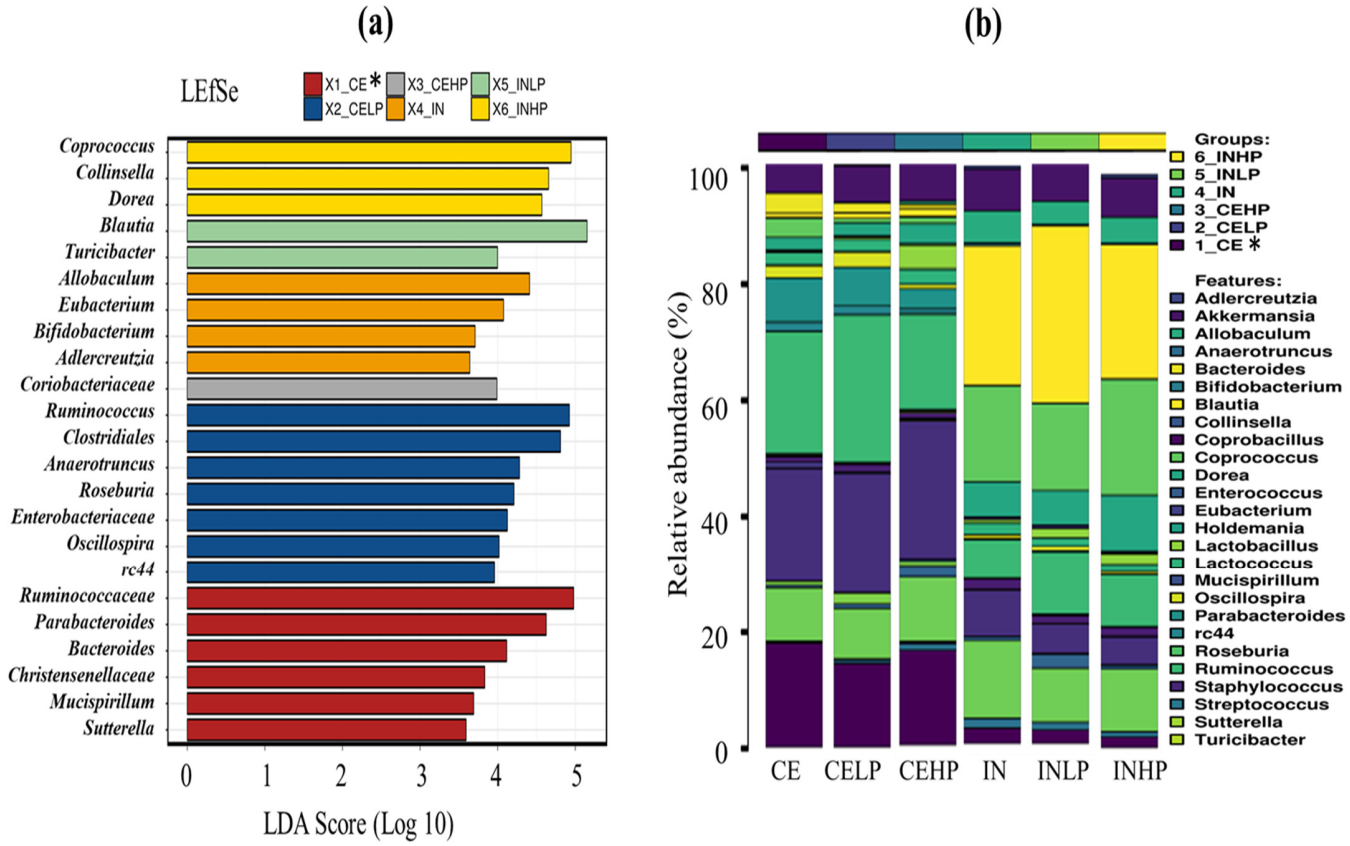
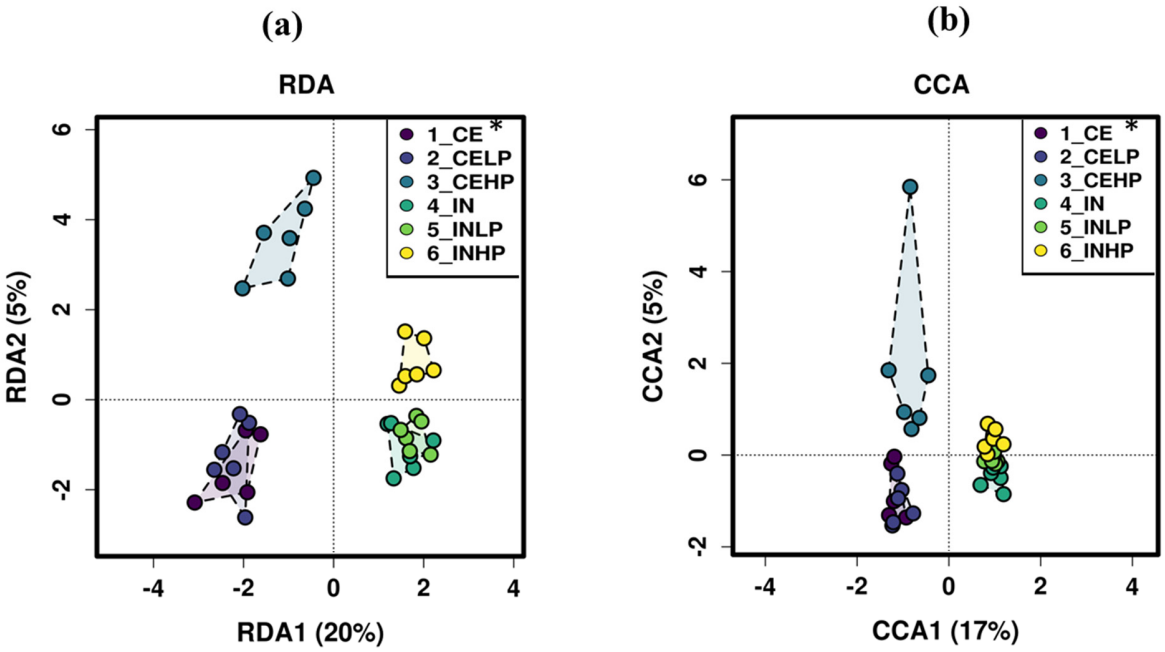


Fig. 3.4. *Linear Discriminant Analysis (LDA) effect size (LEfSe) plot (a) and bar chart (b) compa risons of microbiota during colonic fermentation in rats for 4 wks. CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple sweet potato (PSPP); CEHP, 5% cellul ose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; INHP, 5% inulin + 1% PSPP. St atistical significance amongst the groups was determined by two-way ANOVA analysis to assess t he effect of fiber (CE and IN), PSPP, and their combination. P < 0.05 was considered to be statis tically significant. Data were expressed as mean and SE (n=6, but *n=5).*



RDA significance	
Variance	124.1
F-value	2.76
P-value	0.001

CCA significance	
ChiSquare	0.98
F-value	2.5
P-value	0.001

Fig. 3.5. *Redundancy Analysis (RDA) (a) and Canonical Correspondence Analysis (CCA) (b) co mparisons of microbiota during colonic fermentation in rats for 4 wks. CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple sweet potato (PSPP); CEHP, 5% ce llulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; INHP, 5% inulin + 1% PSPP. Data were expressed as mean and SE (n=6, but *n=5).*

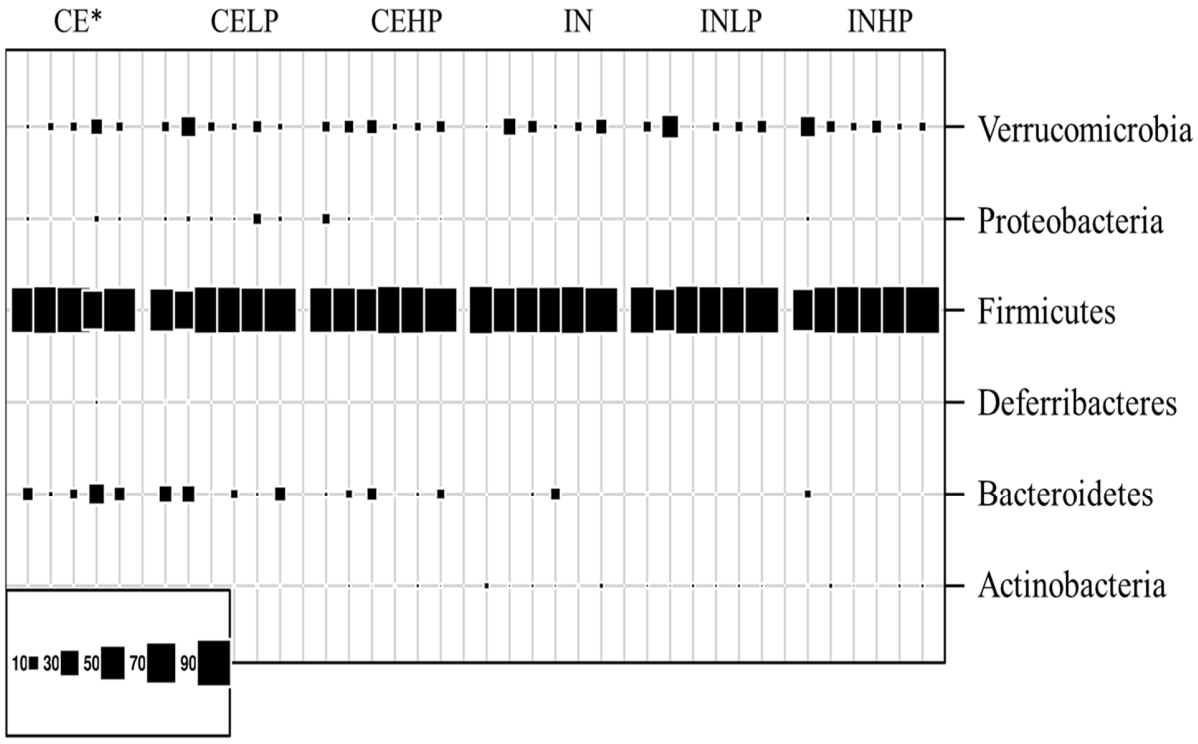
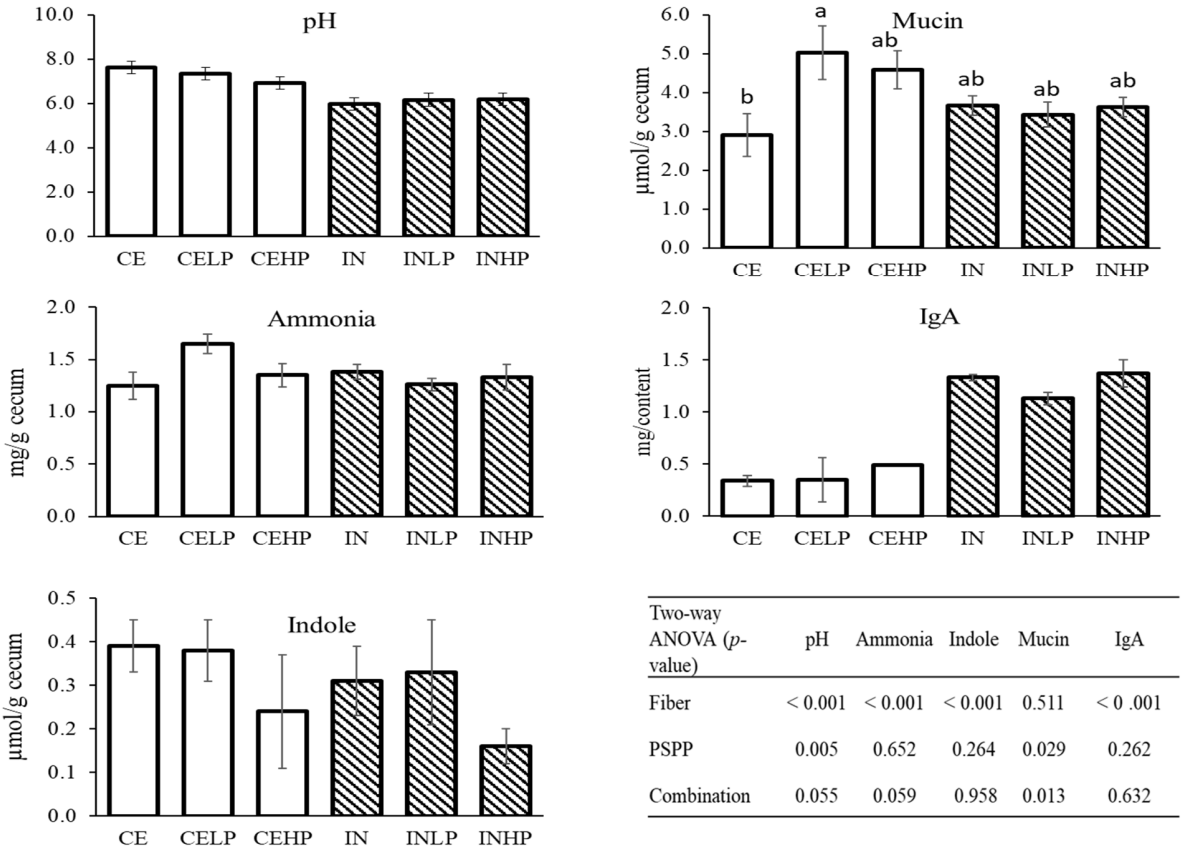


Fig. 3.6. *Bubble plot of the predominant bacterial phyla during colonic fermentation in rats for 4 wks. CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple swe et potato (PSPP); CEHP, 5% cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSP P; INHP, 5% inulin + 1% PSPP. Data were expressed as mean and SE (n=6, but *n=5).*



Two-way ANOVA (*p*-value)	pH	Ammonia	Indole	Mucin	IgA
Fiber	< 0.001	< 0.001	< 0.001	0.511	< 0 .001
PSPP	0.005	0.652	0.264	0.029	0.262
Combination	0.055	0.059	0.958	0.013	0.632

Fig. 3.7. *Cecal pH, ammonia, indole, mucin and IgA during colonic fermentation in rats for 4 wk s. CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple sweet p otato (PSPP); CEHP, 5% cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; I NHP, 5% inulin + 1% PSPP. Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CE and IN), PSPP, and their combination. P < 0.05 was considered to be statistically significant. If the variance was observed in the main effect of the interaction, Turkey's test was used for this comparison. Mean values designated by differe nt letters (a and b) are significantly different (p < 0.05). Data were expressed as mean and SE (n =6).*

Table 3.1. Compositions of experimental diet.

Ingredients (g/kg diet)	Dietary group					
	CE	CELP	CEHP	IN	INLP	INHP
Casein	200	200	200	200	200	200
L-Cystine	3	3	3	3	3	3
Sucrose	100	100	100	100	100	100
Soybean oil	70	70	70	70	70	70
t-Butylhydroquinone	0.014	0.014	0.014	0.014	0.014	0.014
Mineral Mix (AIN-93G-MX)	35	35	35	35	35	35
Vitamin Mix (AIN-93VX)	10	10	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Cellulose	50	50	50	-	-	-
Inulin（Fuji FF）	-	-	-	50	50	50
α-Cornstarch	529.486	527.486	519.486	529.486	527.486	519.486
PSPP	0	2	10	0	2	10
Sum (g)	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

PSPP, polyphenol extract isolated from purple sweet potato (Ipomoea batatas cv. Ayamurasaki). CE, 5% cellulose; CELP, 5% Cellulose + 0.2% PSPP; CEHP, 5% Cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; INHP, 5% inulin + 1% PSPP.

Table 3.2. Feed intake, final body weight, body weight gain, tissue weight, and cecal content weight of rats fed with the CE, CELP, CEHP, IN, INLP and INHP diets for 4 wks.

Groups	Feed intake (g/4 wk)	Final body weight (g/4 wk)	Body weight gain (g/4 wk)	Liver (g)	Perirenal fat tissue (g)	Epididymal fat tissue (g)	Cecal wall (g)	Cecal content (g)
CE	357 ± 9	253 ± 3	69.4 ± 2.8	9.64 ± 0.36	4.67 ± 0.31	5.01 ± 0.28	0.65 ± 0.05	1.98 ± 0.24
CELP	359 ± 7	258 ± 4	73.9 ± 2.4	9.73 ± 0.21	4.89 ± 0.49	4.76 ± 0.10	0.65 ± 0.04	1.76 ± 0.10
CEHP	359 ± 8	255 ± 5	70.5 ± 2.8	9.74 ± 0.55	4.81 ± 0.17	5.18 ± 0.26	0.71 ± 0.08	2.31 ± 0.25
IN	310 ± 8	238 ± 4	54.0 ± 2.5	9.34 ± 0.27	2.85 ± 0.27	3.26 ± 0.17	1.39 ± 0.06	3.98 ± 0.28
INLP	317 ± 7	239 ± 3	54.4 ± 1.9	9.61 ± 0.36	3.48 ± 1.49	3.84 ± 0.29	1.15 ± 0.07	3.47 ± 0.26
INHP	318 ± 7	239 ± 3	54.4 ± 2.4	9.22 ± 0.26	3.66 ± 0.22	3.89 ± 0.18	1.14 ± 0.07	3.36 ± 0.33
	---------- Two-way ANOVA (*p*-value) ----------							
Fiber	< 0.001	< 0.001	< 0.001	0.256	< 0.001	< 0.001	0.016	< 0.001
PSPP	0.790	0.659	0.786	0.803	0.219	0.088	0.507	0.366
Combination	0.904	0.924	0.820	0.802	0.513	0.315	0.586	0.179

Values are expressed as mean ± SE (n=6). CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract is olated from purple sweet potato (PSPP); CEHP, 5% cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0. 2% PSPP; INHP, 5% inulin + 1% PSPP. Statistical significance amongst the groups was determined by two-wa y ANOVA analysis to assess the effect of fiber (CE and IN), PSPP, and their combination. P < 0.05 was conside red to be statistically significant.

Table 3.3. Serum total cholesterol (TC), high-density-lipoprotein-cholesterol (HDL-C), non-HDL-C, triglyceride (TG), and ammonia (NH_3) in rats fed with the CE, CELP, CEHP, IN, INLP, and INHP diets for 4 wks.

Groups	TC (mmol/L)	HDL-C (mmol/L)	Non-HDL-C (mmol/L)	TG (mmol/L)	NH_3 (μmol/L)
CE	1.92 ± 0.06	0.488 ± 0.010	1.43 ± 0.05	3.13 ± 0.33	168 ± 6
CELP	1.93 ± 0.05	0.490 ± 0.010	1.45 ± 0.04	3.81 ± 0.31	157 ± 6
CEHP	2.02 ± 0.06	0.501 ± 0.008	1.52 ± 0.05	4.00 ± 0.32	145 ± 5
IN	1.72 ± 0.04	0.479 ± 0.016	1.25 ± 0.03	1.67 ± 0.18	164 ± 5
INLP	1.74 ± 0.07	0.481 ± 0.020	1.26 ± 0.05	2.13 ± 0.34	149 ± 5
INHP	1.85 ± 0.04	0.515 ± 0.008	1.33 ± 0.04	2.25 ± 0.20	143 ± 7
	---------- Two-way ANOVA (*p*-value) ----------				
Fiber	< 0.001	0.455	< 0.001	< 0.001	0.336
PSPP	0.057	0.025	0.214	0.011	0.001
Combination	0.795	0.296	0.994	0.591	0.871

Values are expressed as mean ± SE (n=6). CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple sweet potato (PSPP); CEHP, 5% cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; INHP, 5% inulin + 1% PSPP. Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CE and IN), PSPP, and their combination. $P < 0.05$ was considered to be statistically significant.

Table 3.4. Relative abundance of bacterial genus during caecal fermentation in rats fed with CE, CELP, CEHP, IN, INLP, and INHP diets for 4 wks.

	Genus (Relative abundance, %)					
Groups	*Ruminococcus*	*Oscillospira*	*Parabacteroides*	*Dorea*	*Coprococcus*	*Bacteroides*
CE	18.5 ± 2.7	2.25 ± 0.45	7.59 ± 2.71	2.47 ± 0.84	4.21 ± 1.66	2.41 ± 0.54
CELP	22.6 ± 3.9	2.35 ± 0.29	6.51 ± 2.28	1.79 ± 0.44	0.77 ± 0.24	1.44 ± 0.46
CEHP	14.9 ± 5.3	0.88 ± 0.31	3.00 ± 1.02	3.96 ± 1.41	1.39 ± 0.67	1.00 ± 0.37
IN	6.73 ± 0.69	0.80 ± 0.24	0.94 ± 0.86	5.74 ± 0.80	13.6 ± 3.6	0.78 ± 0.54
INLP	10.1 ± 0.4	0.74 ± 0.18	0.07 ± 0.03	6.57 ± 1.13	13.9 ± 1.8	0.080 ± 0.040
INHP	9.08 ± 2.60	0.43 ± 0.07	0.67 ± 0.61	9.12 ± 0.94	17.5 ± 3.5	0.180 ± 0.140
	---------- Two-way ANOVA (*p*-value) ----------					
Fiber	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
PSPP	0.338	0.043	0.273	0.029	0.654	0.010
Combination	0.510	0.470	0.267	0.612	0.375	0.429

Values are expressed as mean ± SE (n=6). CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple sweet potato (PSPP); CEHP, 5% cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; INHP, 5% inulin + 1% PSPP. Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CE and IN), PSPP, and their combination. P < 0.05 was considered to be statistically significant.

Table 3.5. Cecal organic concentrations in rats fed with CE, CELP, CEHP, IN, INLP, and INHP diets for 4 wks.

	Organic acids (μmol/g cecum)								
Groups	Total	Acetate	Propionate	*n*-Butyrate	Succinate	Formate	Iso-butyrate	Iso-valerate	Valerate
CE	171 ± 5	102 ± 5b	15.4 ± 1.1	7.69± 0.80	0.992 ± 0.637	1.79 ± 0.96	31.8 ± 0.9	2.88 ± 0.70	8.04 ± 0.73
CELP	195 ± 9	128 ± 7[a]	15.8 ± 1.8	7.96 ± 0.72	6.61 ± 2.59	3.05 ± 1.16	23.3 ± 1.66	3.35 ± 0.59	6.48 ± 1.10
CEHP*	172 ± 11	116 ± 10[b]	18.3 ± 1.1	6.90 ± 0.46	2.16 ± 0.41	1.14 ± 0.36	16.9 ± 2.0	3.20 ± 0.41	7.25 ± 0.49
IN	233 ± 11	97.3 ± 6.2[b]	11.9 ± 3.4	41.2 ± 3.9	76.4 ± 16.1	0.059 ± 0.050	1.36 ± 0.47	1.53 ± 0.46	3.43 ± 1.09
INLP	238 ± 12	79.2 ± 3.04[b]	8.58 ± 1.71	35.9 ± 4.5	110 ± 3	< 0.001	0.780 ± 0.216	1.14 ± 0.18	2.15 ± 0.93
INHP	236 ± 14	84.6 ± 6.5[b]	9.05 ± 1.34	49.9 ± 8.5	86.8 ± 6.2	< 0.001	1.48 ± 0.43	1.54 ± 0.32	2.41 ± 0.63
	---------- Two-way ANOVA (*p*-value) ----------								
Fiber	0.208	< 0.001	0.001	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001
PSPP	0.641	0.640	0.651	0.857	0.040	0.830	< 0.001	0.800	0.121
Combination	0.275	0.003	0.163	0.806	0.161	0.752	< 0.001	0.469	0.986

*Values are expressed as mean ± SE (n=6), but *n=5. CE, 5% cellulose; CELP, 5% cellulose + 0.2% polyphenol extract isolated from purple sweet potato (PSPP); CEHP, 5% cellulose + 1% PSPP; IN, 5% inulin; INLP, 5% inulin + 0.2% PSPP; INHP, 5% inulin + 1% PSPP. Statistical significance amongst the groups was determined by two-way ANOVA analysis to assess the effect of fiber (CE and IN), PSPP, and their combination. P < 0.05 was considered to be statistically significant.*

CHAPTER 4 - General Conclusions and implications

As have been mentioned in chapter 1, the main aim or general focus of the present study was to examine the biological activity of polyphenols from commonly consumed root crop, particularly the purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) with a particular attention to the fermentation characteristics to elucidate its potential as a healthy substrate for colonic microbiota, and the results obtained might contribute to a better understanding of the potential health attributes of colored foods. In doing this, an *in vitro* study was conducted, which was discussed in chapter 2, and following this, an animal study was conducted and was discussed in chapter 3 to examine the fermentation characteristics of polyphenols.

In the *in vitro* study, the purple sweet potato polyphenol modulates the colonic microbiota, particularly, with high impact when combined with less fermentable dietary fiber (cellulose), which implies that polyphenol might be a potential material for the fermentation of less- or non-fermentable fibers. In this case, a distinctively separated cluster formation between the polyphenol-combined and without polyphenol. Besides, polyphenol extract differentially proliferated and inhibited some beneficial and pathogenic bacterial growth respectively, and has the potential to reduce the unwanted cecal fermentation products. Additionally, an *in vivo* study showed the modulatory effect of polyphenol on cecal microbiota by positively affecting the biomarkers of colonic health. Like in *in vitro* study, the animal study also showed the similar trend in the modulatory effects of polyphenol with more impact in association with less fermentable fiber.

Therefore, with respect to the main aim or focus of the present study regarding the biological activity of polyphenols, particularly the fermentation characteristics and its potential health attributes, it was clear that the polyphenol isolated from purple sweet potato positively affecting

the colonic microbiota and biomarkers of colonic health during fermentation and could be a potential material for improving the fermentation characteristics of less- or non-fermentable dietary fiber during colonic fermentation that will simultaneously confer beneficial health attributes to the host. Besides, purple sweet potato polyphenols might be a potential material for functional food ingredients with health-associated benefits to the host.

Summary

Introduction and Objective

The growing interest in polyphenols has led many researchers to study and confirmed its potential health attributes. Studies have reported that polyphenols are strong antioxidants, which means that they (polyphenols) can counteract oxidative stress caused by reactive oxygen species (ROS). Besides, polyphenols can alleviate the risk factors associated with heart diseases such as lowering of blood pressure and low-density lipoprotein (LDL)-bad cholesterol level. Polyphenols are very low (less than 5%) in terms of absorption in the upper gastrointestinal tract, while more than 90% exited the upper gastrointestinal tract into the large intestine and becomes a substrate for microbial fermentation. Speculation surrounding the non-absorbable polyphenols could be attributed to a certain role in the large intestine which requires a thorough investigation. Therefore, the main objective of the study was to investigate the biological activity of antioxidants (polyphenols) and prebiotics particularly the fermentation characteristics of purple sweet potato to elucidate its potential as a healthy substrate for colonic microbiota.

Materials & Methods

In Chapter 2, *in vitro* fermentation study was conducted by using a small-scale laboratory fermenter. Fermentation materials used were polyphenol extracts prepared from purple sweet potato. The samples were collected at 0, 6, 12, 24, and 48 h time points for the analysis of SCFA (HPLC), putrefactive products, intestinal immunity-related substances, and bacterial abundance (plate count and 16S rRNA gene sequencing). In chapter 3, *in vivo* study was conducted using Fischer 344 male rats (6 rats / group) aiming to investigate the fermentation characteristics of polyphenols. The rats were fed the experimental diets (5% cellulose (CEL), 5% CEL + 0.2% purple

sweet potato polyphenol extract (CELP), 5% CEL + 1% purple sweet potato polyphenol extract (CEHP), 5% inulin (INU), 5% INU + 0.2% purple sweet potato polyphenol extract (INLP), and 5% INU + 1% purple sweet potato polyphenol extract (INHP)) based on a modified AIN-93G diet. Organ weights, serum, and cecal digesta were collected for biochemical, cecal biomarkers, and microbiological analyses.

Results & Discussion

Chapter 2: The *in vitro* fermentation study using sweet potato polyphenols (PSP) displayed the modulatory effect on the colonic microbiota in this study. The differential beneficial bacterial growth and the reduction of pathogenic bacteria were depended on the fermentability of dietary fiber, which was more effective with cellulose and could be a potential material conducive for improving the fermentation characteristics of less-fermentable dietary fiber. PSP also reduces the level of putrefactive product (*p*-cresol). Thus, PSP might be a potential material for functional food ingredients that will confer health benefits to the host.

Chapter 3: Purple sweet potato polyphenol extract (PSPP) increased the serum triglyceride (TG) and HDL-C level while reducing the NH_3 level. Additionally, the β-diversity of bacterial species for inulin groups (IN, INLP, and INHP) were similar to the previous study in chapter 2 by clustering together while that of cellulose groups (CE, CELP, and CEHP) were not distinctively separated as seen in the previous study in chapter 2. Besides, PSPP reduces the relative abundance of *Oscillospira* and *Bacteroidetes* while increasing the relative abundance of *Dorea*. The production of acetate and succinate were increased particularly with lower PSPP concentration, while iso-butyrate was reduced. Further, the level of pH was reduced by PSPP while mucin was increased by PSPP in the CELP group. In this chapter, it seems PSPP was more effective with the

less fermentable dietary fiber (cellulose) as seen with the previous study in chapter 2. Finally, the fermentation characteristics of PSPP may have different effects on the microbiota depending on the fermentability of dietary fiber associated with it. Therefore, this study demonstrated that dietary inclusion of polyphenol/anthocyanin from the purple sweet potato might confer positive health attributes to the host gut.

概要

はじめに

ポリフェノールへの関心の高まりにより、多くの研究者がその潜在的な健康属性を研究および確認するようになりました。研究によると、ポリフェノールは強力な抗酸化物質であり、これは、ポリフェノールが活性酸素種（ROS）によって引き起こされる酸化ストレスに対抗できることを意味します。さらに、ポリフェノールは、血圧の低下や低密度リポタンパク質（LDL）-悪玉コレステロール値などの心臓病に関連する危険因子を軽減することができます。ポリフェノールは上部消化管での吸収が非常に低く（5%未満）、90%以上が上部消化管を出て大腸に入り、微生物発酵の基質になります。非吸収性ポリフェノールを取り巻く推測は、徹底的な調査を必要とする大腸における特定の役割に起因する可能性があります。したがって、この研究の主な目的は、抗酸化剤（ポリフェノール）とプレバイオティクスの生物学的活性、特に紫色のサツマイモの発酵特性を調査して、結腸微生物叢の健康な基質としての可能性を解明することでした。

材料と方法

第 2 章では、小規模の実験室用発酵槽を使用して *in vitro* 発酵研究を実施しました。使用した発酵材料は、紫色のサツマイモから調製したポリフェノール抽出物でした。サンプルは、SCFA（HPLC）、腐敗生成物（アッセイキット）、腸の免疫関連物質（アッセイキット）、および細菌の存在量（プレート数）の分析のために、0、6、12、24、お

よび 48 時間の時点で収集されました。および 16SrRNA 遺伝子シーケンシング）。第 3 章では、ポリフェノールの発酵特性を調査することを目的として、Fischer 344 雄ラット（6 匹/群）を使用して *in vivo* 研究を実施しました。ラットに AIN-93G ダイエットに基づいて修正された実験食（5%セルロース（CEL）、5%CEL + 0.2%紫サツマイモポリフェノール抽出物（CELP）、5%CEL + 1%紫サツマイモポリフェノール抽出物（CEHP）、5%イヌリン（INU）5%INU + 0.2%紫芋ポリフェノール抽出物（INLP）、および 5% INU + 1%紫芋ポリフェノール抽出物（INHP））を与えました。臓器重量、血清、および盲腸消化物は、生化学的、盲腸バイオマーカー、および微生物学的分析のために収集されました。

結果と考察

第 2 章：サツマイモポリフェノール（PSP）を使用した in vitro 発酵研究では、この研究で結腸微生物叢に対する調節効果が示されました。有益な細菌の増殖と病原菌の減少の違いは、食物繊維の発酵性に依存していました。食物繊維は、セルロースより効果的であり、発酵性の低い食物繊維の発酵特性を改善するのに役立つ可能性があります。 PSP は、細胞分解生成物（p-クレゾール）のレベルも低下させます。したがって、PSP は、宿主に健康上の利益をもたらす機能性食品成分の潜在的な材料である可能性があります。

第 3 章：紫色のサツマイモポリフェノール抽出物（PSPP）は、血清トリグリセリド（TG）と HDL-C レベルを増加させ、NH3 レベルを減少させました。さらに、セルロースグループ（CE、CELP、および CEHP）のβ多様性が明確に分離されていないのに対し、イヌリングループ（IN、INLP、および INHP）の細菌種のβ多様性は、クラスター

化し、第 2 章の以前の研究と同様でした。さらに、PSPP は、ドレアの相対的な存在量を増やしながら、オシロスピラとバクテロイデスの相対的な存在量を減らします。酢酸塩とコハク酸塩の生成は、特に PSPP 濃度が低いほど増加しましたが、イソ酪酸塩は減少しました。さらに、CELP グループでは PSPP によって pH が低下し、PSPP によってムチンが上昇しました。この章では、PSPP は、第 2 章の前の研究で見られたように、発酵性の低い食物繊維（セルロース）より効果的だったようです。最後に、PSPP の発酵特性は、食物繊維の発酵性に応じて微生物叢に異なる影響を与える可能性があります。したがって、この研究は、紫色のサツマイモからのポリフェノール/アントシアニンの食事による含有が、宿主の腸に正の健康属性を与える可能性があることを示しました。

References

1. Vinson, J.A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: Fruits. *J. Agric.Food Chem*. **2001**, *49*, 5315–5321.
2. Tomás-Barberán, F.A.; Espin, J.C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric*. **2000**, *81*, 853–876.
3. Haslam, E. Plant polyphenols (syn. vegetable tannins) and chemical defense-A reappraisal. *J. Chem. Ecol*. **1998**, *14*, 1789–1805.
4. Scalbert, A. Antimicrobial properties of tannins. *Phytochemistry* **1991**, *30*, 3875–3883.
5. Harborne, J.B.; Williams, C.A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481-504.
6. Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev*. **1998**, *56*, 317-333.
7. Cheynier, V. Polyphenols in foods are more complex than often thought. *Am. J. Clin. Nutr*. **2005**, *81*, 223S-229S.
8. Duynhoven, J.V.; Vaughan, E.E.; Jacobs, D.M.; Kemperman, R.A.; Velzen, E.J.J.V.; Gross, G.; Roger, L.C.; Possemiers, S.; Smilde, A.K.; Doré, J.; Westerhuis, J.A.; de Wiele T.V. Metabolic fate of polyphenols in the human superorganism. *Proc. Natl. Acad. Sci. USA*. **2011**, *108*, 4531–4538.
9. Kawabata, K.; Mukai, R.; Ishisaka, A. Quercetin and related polyphenols: New insights and implications for their bioactivity and bioavailability. *Food Funct.* **2015**, *6*, 1399–1417.
10. Balentine, D.A.; Albano, M.C.; Nair, M.G. Role of medicinal plants, herbs and spices in protecting human health. *Nutrition Reviews*. **1999**, *7*: S41-S45.
11. Kurulich, A.C.; Tsau, G.J.; Brown, A.; Howard, L.; Klein, B.P.; Jeffery, E.H.; Kushad, M.; Wallig, M.A.; Juvik, J.A. Carotene, tocopherol and ascorbate contents in subspecies of Brassical oleracea. *J. Agric. Food Chem*. **1999**, *47*, 1576-1581.
12. Milner, J.A. Reducing the risk of cancer. In functional foods: Designer foods, pharmafoods, nutraceuticals; Goldberg, I, Ed.; Chapman & Hall: New York, NY, USA, **1994**, 39-70.
13. Duthie, G.G.; Brown, K.M. Reducing the risk of cardiovascular disease. In Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals; Goldberg, I., Ed.; Chapman & Hall: New York, NY, USA, **1994**, 19-38.
14. Gu, Y.Y.; Yu, S.; Park, J.Y.; Kevin, H.; Joshua, D.L. Dietary cocoa reduces metabolic endotoxemia and adipose tissue inflammation in high-fat fed mice. *J Nutr Biochem.* **2014**, *25*, 439–445.
15. Manach, C.; Scalbert, A.; Morand, C.; Jiménez, L.; Rémésy, C. Polyphenols: Food sources and bioavailability. *Am J Clin Nutr*. **2004**, *79*, 727–747.
16. Peng, Y.; Yan, Y.; Wan, P.; Chen, D.; Ding, Y.; Ran, L.; Mi, J.; Lu, L.; Zhang, Z.; Li, X.; Zeng, X.; Cao, Y. Gut microbiota modulation and anti-inflamatory properties of anthocyanins from the fruits of *Lycium ruthenicum* Murray in dextran sodium sulfate-induced colitis in mice. *Free Radical Biol. Med*. **2019**, *136*, 96-108

17. Jiao, X.; Wang, Y.; Lin, Y.; Lang, Y.; Li, E.; Zhang, X.; Zhang, Q.; Feng, Y.; Meng, X.; Li, B. Blueberry polyphenols extract as a potential prebiotic with anti-obesity effects on C57BL/6 J mice by modulating the gut microbiota. *J Nutr Biochem*. **2019**, *64*, 88-100.
18. Lee, H.C.; Jenner, A.M.; Low, C.S.; Lee, Y.K. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Res. Microbiol*. **2006**, *157*, 876–884.
19. Goto, K.; Kanaya, S.; Nishikawa, T.; Hara, H.; Terada, A.; Ishigami, T.; Hara, Y. The effects of tea catechins on fecal flora of elderly residents in long-term care facilities. *J. Nutr. Sci. Vitaminol.* (Tokyo). **1999**, *45*, 135–141.
20. Taira, T.; Yamaguchi, S.; Takahashi, A.; Okazaki, Y.; Yamaguchi, A.; Sakaguchi, H.; Chiji, H. Dietary polyphenols increase fecal mucin and immunoglobulin A and ameliorate the disturbance in gut microbiota caused by a high fat diet. *J Clin Biochem Nutr*. **2015**, *57*, 212-216.
21. Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *J Diabetes Metab Disord*. **2013**, *12*, 43.
22. D'Archivio, M.; Filesi, C.; Di Benedetto, R.; Gargiulo, R.; Giovannini, C.; Masella, R. Polyphenols, dietary sources and bioavailability. *Ann. Ist. Super. Sanita*. **2007**, *43*, 348-361.
23. Goutzourelas, N.; Stagos, D.; Spanidis, Y.; Liosi, M.; Apostolou, A.; Priftis, A.; Haroutounian, S.; Spandidos, D.A.; Tsatsakis, A.M.; Kouretas, D. Polyphenolic composition of grape stem extracts affects antioxidant activity in endothelial and muscle cells. *Mol. Med. Rep*. **2015**, *12*, 5846-5856
24. M. Leopoldini, M.; Russo, N.; Toscano, M. The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem*. **2011**, *125*, 288-306.
25. Martin, K.; Appel, C.L. Polyphenols as dietary supplements: a double-edged sword. *Nutr. Diet. Suppl.* **2010**, *2*, 1-12.
26. Sahpazidou, D.; Geromichalos, G.D.; Stagos, D.; Apostolou, A.; Haroutounian, S.A.; Tsatsakis, A.M.; Tzanakakis, G.N.; Hayes, A.W.; Kouretas, D. Anticarcinogenic activity of polyphenolic extracts from grape stems against breast, colon, renal and thyroid cancer cells. *Toxicol. Lett*. **2014**, *230*, 218-224.
27. Kim, Y.; Keogh, J.B.; Clifton, P.M. Polyphenols and Glycemic Control. *Nutrients* **2016**, *8*, 17.
28. Azzini, E.; Giacometti, J.; Russo, G.L. Antiobesity effects of anthocyanins in preclinical and clinical studies. *Oxid Med Cell Longev*. **2017**, *2017*, 2740364.
29. Grosso, G.; Stepaniak, U.; Micek, A.; Kozela, M.; Stefler, D.; Bobak, M.; Pajak, A. Dietary polyphenol intake and risk of type 2 diabetes in the Polish arm of the Health, Alcohol and Psychosocial factors in Eastern Europe (HAPIEE) study. *Br J Nutr*. **2017**, *118*, 60-68.
30. Xiao, J.B.; Högger, P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. *Curr Med Chem*. **2015**, *22*, 23-38.

31. Khoo, H.E.; Azlan, A.; Tang, S.T.; Lim, S.M. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res*. **2017**, *61*, 1361779.
32. Hunter, P. The inflammation theory of disease. The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment. *EMBO Rep*. **2012**, *13*, 968-970.
33. Tangney, C.C.; Rasmussen, H.E. Polyphenols, inflammation, and cardiovascular disease. *Curr Atheroscler Rep*. **2013**, 15, 324.
34. Hussain, T.; Tan, B.; Yin, Y.; Blachier, F.; Tossou, M.C.; Rahu, N. Oxidative stress and inflammation: What polyphenols can do for us? *Oxid Med Cell Longev*. **2016**, *2016*, 7432797.
35. Potì, F.; Santi, D.; Spaggiari, G.; Zimetti, F.; Zanotti, I. Polyphenol health effects on cardiovascular and neurodegenerative disorders: A review and meta-analysis. *Int J Mol Sci*. **2019**, 20, 351.
36. Cheng, Y.C.; Sheen, J.M.; Hu, W.L.; Hung, Y.C. Polyphenols and Oxidative Stress in Atherosclerosis-Related Ischemic Heart Disease and Stroke. *Oxid Med Cell Longev*. **2017**, *2017*, 8526438.
37. Zhou, Y.; Zheng, J.; Li, Y.; Xu, D.P.; Li, S.; Chen, Y.M.; Li, H.B. Natural polyphenols for prevention and treatment of cancer. *Nutrients* **2016**, *8*, 515.
38. Madigan, M.; Karhu, E. The role of plant-based nutrition in cancer prevention. *J Unexplored Med Data*. **2018,** *3*, 9.
39. Dinu, M.; Abbate, R.; Gensini, G.F.; Casini, A.; Sofi, F. Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies. *Crit Rev Food Sci Nutr*. **2017**, 57, 3640-3649.
40. Zhang, H.; Tsao, R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Curr. Opin. Food Sci*. **2016**, *8*, 33-42.
41. Hui, C.; Qi, X.; Qianyong, Z.; Xiaoli, P.; Jundong, Z.; Mantian, M. Flavonoids, flavonoid subclasses and breast cancer risk: A meta-analysis of epidemiologic studies. *PLoS ONE* **2013**, *8*, e54318.
42. Niedzwiecki, A.; Roomi, M.W.; Kalinovsky, T.; Rath, M. Anticancer efficacy of polyphenols and their combinations. *Nutrients* **2016**, *8*, 552.
43. Wang, J.; Tang, L.; Wang, J.S. Biomarkers of dietary polyphenols in cancer studies: Current evidence and beyond. *Oxid Med Cell Longev*. **2015**, *2015*, 732302.
44. Laparra, J.M.; Sanz, Y. Interactions of gut microbiota with functional food components and nutraceuticals. *Pharmacol Res*. **2010**, *61*, 219-225.
45. Marín, L.; Miguélez, E.M.; Villar, C.J.; Lombó, F. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. *Biomed Res Int*. **2015**, *2015*, 905215.

46. Pacheco-Ordaz.; Wall-Medrano, A.; Goñi, M.G.; Ramos-Clamont-Montfort, G.; Ayala-Zavala, J.F.; González-Aguilar, G.A. Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. *Lett Appl Microbiol*. **2018**, *66*, 25-31.
47. Dryden, G.W.; Song, M.; McClain, C. Polyphenols and gastrointestinal diseases. *Curr Opin Gastroenterol*. **2006**, 22, 165-170.
48. Parkar, S.G.; Trower, T.M.; Stevenson, D.E. Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. *Anaerobe* **2013**, *23*, 12-19.
49. Eckburg, P.B.; Bik, E.M.; Bernstein, C.N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S.R.; Nelson, K.E.; Relman, D.A. Diversity of the human intestinal microbial flora. *Science* **2005**, *308*, 1635–1638.
50. Guinane, C.M.; Cotter, P.D. Role of the gut microbiota in health and chronic gastrointestinal disease: Under-standing a hidden metabolic organ. *Ther. Adv. Gastroenterol*. **2013**, *6*, 295–308.
51. Gronlund, M.M.; Lehtonen, O.P.; Eerola, E.; Kero, P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr*. **1999**, 28, 19–25.
52. Mountzouris, K.C.; McCartney, A.L.; Gibson, G.R. Intestinal microflora of human infants and current trends for its nutritional modulation. *Br J Nutr*. **2002**, *87*, 405–420.
53. Penders, J.; Thijs, C.; Vink, C.; Stelma, F.F.; Snijders, B.; Kummeling, I.; van den Brandt, P.A.; Stobberingh, E.E. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* **2006**, 118, 511–521.
54. Salminen, S.; Gibson, G.R.; McCartney, A.L.; Isolauri, E. Influence of mode of delivery on gut microbiota composition in seven-year-old children. *Gut* **2004**, *53*, 1388–1389.
55. Dominguez-Bello, M.G.; Costello, E.K.; Contreras, M.; Magris, M.; Hidalgo, G.; Fierer, N.; Knight, R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA*. **2010**, *107*, 11971–11975.
56. Xu, J.; Gordon, J.I. Inaugural Article: Honor thy symbionts. *Proc Natl Acad Sci USA*. **2003**, *100*, 10452–10459.
57. Saarela, M.; Lähteenmäki, L.; Crittenden, R.; Salminen, S.; MattilaSandholm, T. Gut bacteria and health foods—the European perspective. *Int J Food Microbiol*. **2002**, *78*, 99–117.
58. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (poly) phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal*. **2013**, *18*, 1818–1892.
59. Vaiserman, A.M.; Koliada, A.K.; Marotta, F. Gut microbiota: a player in aging and a target for anti-aging intervention. *Ageing Res. Rev*. **2017**, *35*, 36–45.
60. Sonnenburg, J.L.; Bäckhed, F. Diet-microbiota interactions as moderators of human metabolism, *Nature* **2016**, *535*, 56–64.

61. Pompei, A.; Cordisco, L.; Raimondi, S.; Amaretti, A.; Pagnoni, U.M.; Matteuzzi, D.; Rossi, M. In vitro comparison of the prebiotic effect of two inulin-type fructans. *Anaerobe* **2008**, *14*, 280–286.
62. van de Wiele, T.; Boon, N.; Possemiers, S.; Jacobs, H; Verstraete, W. Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. *J Appl Microbiol*. **2007**, *102*, 452-460.
63. De Filippo, C.; Cavalieri, D.; Di Paola, M.; Ramazzotti, M.; Poullet, J.B.; Massart, S.; Collini, S.; Pieraccini, G.; Lionetti, P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. USA*. **2010**, *107*, 14691–14696.
64. Chen, T.; Chen, D.; Tian, G.; Zheng, P.; Mao, X.; Yu, J.; He, J.; Huang, Z.; Luo, Y.; Luo, J.; Yu, B. Soluble fiber and insoluble fiber regulate colonic microbiota and barrier function in a piglet model. *Biomed Res Int*. **2019**, *2019*, 7809171.
65. Kawabata, K.; Yoshioka, Y.; Terao, J. (2019). Role of intestinal microbiota in the bioavailability and physiological functions of dietary polyphenols. *Molecules* **2019**, *24*, 1–25.
66. Mojzer, E.B.; Knez Hrnčič, M.; Škerget, M.; Knez, Ž.; Bren, U. Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules* **2016**, *21*, 1–38.
67. Marsilio, V.; Lanza, B. Characterization of an oleuropein degrading strain of Lactobacillus plantarum. Combined effects of compounds present in olive fermenting brines (phenols, glucose and NaCl) on bacterial activity. *J Sci Food Agric*. **1998**, *76*, 520–524.
68. Rozès, N.; Peres, C. Effects of phenolic compounds on the growth and the fatty acid composition of Lactobacillus plantarum. *Appl Microbiol Biotechnol.* **1998**, *49*, 108–111.
69. Salih, A.G; Le Quéré, J.M; Drilleau, J.F. Action des acides hydroxycinnamiques libres et esterifies sur la croissance des bactéries lactiques. *Sci des Aliments*. **2000**, 20, 537–560.
70. Rodríguez-Daza, M.C.; Daoust, L.; Boutkrabt, L.; Pilon, G.; Varin, T.; Dudonné, S.; Levy, É.; Marette, A.; Roy, D.; Desjardins, Y. Wild blueberry proanthocyanidins shape distinct gut microbiota profile and influence glucose homeostasis and intestinal phenotypes in high-fat high-sucrose fed mice. *Sci Rep*. **2020**, 10, 2217.
71. Lee, S.; Keirsey, K.I.; Kirkland, R.; Grunewald, Z.I.; Fischer, J.G.; de La Serre, C.B. Blueberry supplementation influences the gut microbiota, inflammation, and insulin resistance in high-fat-diet-fed rats. *J Nutr*. **2018**, 148, 209-219.
72. Neyrinck, A.M.; Catry, E.; Taminiau, B.; Cani, P.D; Bindels, L.B.; Daube, G.; Dessy, C.; Delzenne, N.M. Chitin–glucan and pomegranate polyphenols improve endothelial dysfunction. *Sci Rep*. **2019**, *9*, 14150.
73. Fotschki, B.; Juskiewicz, J.; Jurgonski, A.; Kolodziejczyk, K.; Milala, J.; Kosmala, M.; Zdunczyk, Z. Anthocyanins in strawberry polyphenolic extract enhance the beneficial effects of diets with fructooligosaccharides in the rat cecal environment. *PLoS ONE 2016*, *11*, e0149081

74. Peng, Y.; Yan, Y.; Wan, P.; Dong, W.; Huang, K.; Ran, L.; Mi, J.; Lu, L.; Zeng, X.; Cao, Y. Effects of long-term intake of anthocyanins from Lycium ruthenicum Murray on the organism health and gut microbiota in vivo. *Food Res Int*. **2019**, *130*, 108952.
75. Patterson, E.; Cryan, J.F.; Fitzgerald, G.F.; Ross, R.P.; Dinan, T.G.; Stanton, C. Gut microbiota, the pharmabiotics they produce and host health. *Proc Nutr Soc*. **2014**, *73*, 477–89.
76. Hague, A.; Elder, D.J.; Hicks, D.J.; Paraskeva, C. Apoptosis in colorectal tumour cells: Induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate, *Int. J. Cancer*. **1995**, *60*, 400–406.
77. Abrahamse, S.L.; Pool-Zobel, B.L.; Rechkemmer, G. Potential of short chain fatty acids to modulate the induction of DNA damage and changes in the intracellular calcium concentration by oxidative stress in isolated rat distal colon cells, *Carcinogenesis* **1999**, 20, 629–634.
78. Wong, J.M.; de Souza, R.; Kendall, C.W.; Emam, A.; Jenkins, D.J. Colonic health: Fermentation and short chain fatty acids. J. *Clin. Gastroenterol*. **2006**, *40*, 235–243.
79. Robles Alonso, V.; Guarner, F. Linking the gut microbiota to human health, *Br. J. Nutr*. **2013**, *109*, 21–26.
80. Li, J.; Wu, T.; Li, N.; Wang, X.; Chen, G.; Lyu, X. Bilberry anthocyanin extract promtes intestinal barrier function and inhibits digestive enzyme activity by regulating the gut microbiota in aging rats. *Food Funct*. **2019**, *22*, 333–343.
81. Liu, J.; Hao, W.; He, Z.; Kwek, E.; Zhao, Y.; Zhu, H.; Liang, N.; Ma K.Y.; Lei, L.; He, W.S.; Chen, Z. Y. Beneficial effects of tea water extracts on the body weight and gut microbiota in C57BL/6J mice fed with a high-fat diet. *Food Funct*. **2019**, *10*, 2847–2860.
82. Liu, J.; Yue, S.; Yang, Z.; Feng, W.; Meng, X.; Wang, A.; Peng, C.; Wang, C.; Yan, D. Oral hydroxysafflor yellow A reduces obesity in mice by modulating the gut microbiota and serum metabolism. *Pharmacol Res*. **2018**, *134*, 40–50.
83. Henning, S.M.; Yang, J.; Hsu, M.; Lee, R.P.; Grojean, E.M.; Ly, A.; Tseng, C.H.; Heber, D.; Li, Z. Decaffeinated green and black tea polyphenols decrease weight gain and alter microbiome populations and function in diet-induced obese mice. *Eur J Nutr*. **2018**, *57*, 2759–2769.
84. Zhu, X.L; Zhang, X; Sun, Y; Su, D.; Sun, Y.; Hu, B.; Zeng, X.X. Purification and fermentation in vitro of sesaminol triglucoside from sesame cake by human intestinal microbiota. *J Agric Food Chem*. **2013**, *61*, 1868–1877.
85. Larrosa, M.; Yañéz-Gascón, M.J.; Selma, M.V.; González-Sarrías, A.; Toti, S.; Cerón, J.J.; Tomas-Barberán, F.; Dolara, P.; Espín, J.C. Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. *J Agr Food Chem*. **2009**, *57*, 2211–2220.
86. Sanz, M.L.; Côté, G.L.; Gibson, G.R.; Rastall, R.A. Prebiotic properties of alternansucrase maltose-acceptor oligosaccharides. *J Agric Food Chem*. **2005**, *53*, 5911–5916.

87. Sanz, M.L.; Polemis, N.; Morale,s V.; Corzo, N.; Drakoularakou, A.; Gibson, G.R.; Rastall, R.A. In vitro investigation into the potential prebiotic activity of honey oligosaccharides. *J Agric Food Chem*. **2005**, *53*, 2914–2921.
88. Dominika, S.; Arjan, N.; Karyn, R.P.; Henryk, K. The study on the impact of glycated pea proteins on human intestinal bacteria. *Int J Food Microbiol*. **2011**, *145*, 267–272.
89. Kosmala, M.; Zduńczyk, Z.; Kołodziejczyk, K.; Klimczak, E.; Juśkiewicz, J.; Zduńczyk, P. Chemical composition of polyphenols extracted from strawberry pomace and their effect on physiological properties of diets supplemented with different types of dietary fibre in rats. *Eur J Nutr*. **2014**, *53*, 521–532.
90. Negi, P.S.; Jayaprakasha, G.K. Antibacterial activity of grapefruit (Citrus paradisi) peel extract. *Eur Food Res Technol*. **2001**, *213*, 484–487.
91. Zduńczyk, Z.; Juśkiewicz, J.; Estrella, I. Cecal parameters of rats fed diets containing grapefruit polyphenols and inulin as single supplements or in a combination. *Nutrition* **2006**, *22*, 898–904.
92. Wallace, A.J.; Eady, S.L.; Hunter, D.C.; Skinner, M.A.; Huffman, L.; Ansell, J.; Blatchford, P.; Wohlers, M.; Herath, T.D.; Hedderley, D.; Rosendale, D.; Stoklosinski, H.; McGhie, T.; Sun-Waterhouse, D.; Redman, C. No difference in fecal levels of bacteria or short chain fatty acids in humans, when consuming fruit juice beverages containing fruit fiber, fruit polyphenols, and their combination. *Nutr Res*. **2015**, *35*, 23–34.
93. Etxeberria, U.; Arias, N.; Boqué, N.; Macarulla, M.; Portillo, M.; Martínez, J.; Milagro, F. Reshaping faecal gut microbiota composition by the intake of trans‐resveratrol and quercetin in high‐fat sucrose diet‐fed rats. *J Nutr Biochem*. **2015**, *26*, 651–660.
94. An, C.; Kuda, T.; Yazaki, T.; Takahashi, H.; Kimura, B. Caecal fermentation, putrefaction and microbiotas in rats fed milk casein, soy protein or fish meal. *Appl Microbiol Biotechnol*. **2014**, *98*, 2779–2787.
95. Macfarlane, G.T.; Macfarlane, S. Human colonic microbiota: Ecology, physiology and metabolic potential of intestinal bacteria. *Scand. J. Gastroenterol. Suppl.* **1997**, *222*, 3–9.
96. Windey, K.; Preter, D.; Verbeke, K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res*. **2012**, *56*, 184–196.
97. Davila, A.M.; Blachier, F.; Gotteland, M.; Andriamihaja, M.; Benetti, P.H.; Sanz, Y.; Tomé, D. Intestinal luminal nitrogen metabolism: Role of the gut microbiota and consequences for the host. *Parmacol. Res*. **2013**, *68*, 95–107.
98. Vanholder, R.; Schepers, E.; Pletinck, A.; Nagler, E.V.; Glorieux, G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: A systematic review. *J. Am. Soc. Nephrol.* **2014**, *25*, 1897–1907.
99. Brocca, A.; Virzì, G.M.; de Cal, M.; Cantaluppi, V.; Ronco, C. Cytotoxic effects of p-cresol in renal epithelial tubular cells. *Blood Purif.* **2013**, *36*, 219–225.
100. Ma, N.; Tian, Y.; Wu, Y.; Ma, X. Contributions of the Interaction between Dietary Protein and Gut Microbiota to Intestinal Health. Curr. *Protein Pept. Sci*. **2017**, *18*, 1.

101. Zhao, J.; Zhang, X.; Liu, H.; Brown, M.A.; Qiao, S. Dietary Protein and Gut Microbiota Composition and Function. Curr. *Protein Pept. Sci*. **2019**, *20*, 145–154.
102. Hertog, M.G.; Feskens, E.J.; Hollman, P.C.; Katan, M.B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet* **1993**, *342*, 1007–1011.
103. Arts I.C.W.; Hollman, P.C.H. "Polyphenols and disease risk in epidemiologic studies," *Am J Clin Nutr*. **2005**, *81*, 317S–325S.
104. Yamakoshi, J.; Tokutake, S.; Kikuchi, M.; Kubota, Y.; Konishi, H.; Mitsuoka, T. Effect of proantocyanidin -rich extract from grape seed on human fecal flora and fecal odor. *FASEB Journal*. **2001**, *15*, 633.
105. Jurgoński, A.; Juśkiewicz, J.; Zduńczyk, Z.; Matusevicius, P.; Kołodziejczyk, K. Polyphenol-rich extract from blackcurrant pomace attenuates the intestinal tract and serum lipid changes induced by a high-fat diet in rabbits. *Eur. J. Nutr*. **2014**, *53*, 1603–1613.
106. Nagata, R.; Echizen, M.; Yamaguchi, Y.; Han, K.H.; Shimada, K.; Ohba, K.; Kitano-Okada, T.; Nagura, T.;Uchino, H.; Fukushima, M. Effect of a combination of inulin and polyphenol containing adzuki bean extract on intestinal fermentation In Vitro and In Vivo. Biosci. *Biotechnol. Biochem*. **2018**, *82*, 489–496.
107. Bilić-Šobot, D.; Zamaratskaia, G.; Rasmussen, M.K.; Čandek-Potokar, M.; Škrlep, M.; Maja Prevolnik Povše, M.; Dejan Škorjanc, D. Chestnut wood extract in boar diet reduces intestinal skatole production, a boar taint compound. *Agron. Sustain. Dev*. **2016**, *36*, 62.
108. Belkaid, Y.; Harrison, O.J. Homeostatic immunity and the microbiota. *Immunity* **2017**, *46*, 562-576.
109. Chen, Y.E.; Fischbach.; MA.; Belkaid, Y. Skin microbiota-host interactions. *Nature* **2018**, 553, 427-436.
110. Wells, J.M.; Rossi, O.; Meijerink, M.; van Baarlen, P. Epithelial crosstalk at the microbiota-mucosal interface. *Proc Natl Acad Sci U S A*. **2011**, 108, 4607-4614.
111. Tanabe, H.; Morita, T.; Sugiyama, K.; Kiriyama, S. Dietary high-amylose cornstarch enhances mucin and mmunoglobulin A secretions in rat gastrointestinal tract. *J. Jpn. Assoc. Dietary Fiber Res.* **2004**, *8*, 31-42.
112. Beukema, M.; Faas, M.M.; de Vos, P. The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells. *Exp Mol Med*. **2020**, *52*, 1364–1376.
113. Desai, M.S; Seekatz, A.M.; Koropatkin, N.M.; Kamada, N.; Hickey, C.A.; Wolter, M.; Pudlo, N.A.; Kitamoto, S.; Terrapon, N.; Muller, A.; Young, V.B.; Henrissat, B.; Wilmes, P.; Stappenbeck, T.S.; Núñez, G.; Martens, E.C. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell.* **2016**, *167*, 1339-1353.
114. Szliszka, E.; Krol, W. The role of dietary polyphenols in tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis for cancer chemoprevention. *Eur J Cancer Prev*. **2011**, *20*, 63–69.

115. Johansson, M.E.; Phillipson, M.; Petersson, J.; Velcich, A.; Holm, L.; Hansson, G.C. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A*. **2008**, *105*, 15064-15069.
116. Johansson, M.E.; Hansson, G.C. Mucus and the goblet cell. *Dig Dis*. **2013**, 31, 305-309.
117. Pelaseyed, T.; Bergström, J.H.; Gustafsson, J.K.; Ermund, A.; Birchenough, G.M.; Schütte, A.; van der Post, S.; Svensson, F.; Rodríguez-Piñeiro, A.M.; Nyström, E.E.; Wising, C.; Johansson, M.E.; Hansson, G.C. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol Rev*. **2014**, *260*, 8–20.
118. Sakata, T.; Setoyama, H. Local stimulatory effect of short chain fatty acids on the mucus release from the hindgut mucosa of rats (Rattus norvegicus). *Comp Biochem Physiol A Physiol.* **1995**, *111*, 429–432.
119. Shimotoyodome, A.; Meguro, S.; Hase,T.; Tokimitsu, I.; Sakata, T. Short chain fatty acids but not lactate or succinate stimulate mucus release in the rat colon. *Comp Biochem Physiol A Mol Integr Physiol*. **2000**, *125*, 525–531.
120. Dohrman, A.; Miyata, S.; Gallup, M.; Li, J.D.; Chapelin, C.; Coste, A.; Escudier, E.; Nadel, J.; Basbaum, C. *Biochim. Biophys*. **1998**, *1406*, 251–259.
121. Carasi, P.; Racedo, S.M.; Jacquot, C.; Romanin, D.E.; Serradell, M.A.; Urdaci, M.C. Impact of kefir derived lactobacillus kefiri on the mucosal immune response and gut microbiota. *J Immunol Res*. **2015**, *2015*, 1–12.
122. Rosillo, M.A.; Sanchez-Hidalgo, M.; Cardeno, A.; ´Alarcon de la Lastra, C. Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease. *Biochem Pharmacol*. **2011**, *82*, 737–745.
123. Guri, A.; Li, Y.; Corredig, M. Interfacial dilational properties of tea polyphenols and milk proteins with gut epithelia and the role of mucus in nutrient adsorption. *Food Funct*. **2015,** *6*, 3642–3651.
124. Georgiades, P.; Pudney, P.D.A.; Rogers, S.; Thornton, D.J.; Waigh, T.A. Tea derived galloylated polyphenols crosslink purified gastrointestinal mucins. *PLoS ONE* **2014**, *9*, e105302.
125. Lakhanpal, D.P.; Rai, D.D.K. Quercetin: a versatile flavonoid. *EJOURNAL*. **2007**, *2*, 20–35.
126. Selma, M.V.; Espín, J.C.; Tomás-Barberán, F.A. Interaction between phenolics and gut microbiota: role in human health. *J Agric Food Chem*. **2009**, *57*, 6485–6501.
127. Fotschki, B.; Milala, J.; Jurgoński, A.; Karlińska, E.; Zduńczyk, Z.; Juśkiewicz, J. Strawberry ellagitannins thwarted the positive effects of dietary fructooligosaccharides in rat cecum. *J Agric Food Chem*. **2014**, *62*, 5871–5880.
128. Breedveld, A.; van Egmond, M. IgA and FcαRI: Pathological roles and therapeutic opportunities. *Front Immunol*. 2**019**, *10*, 553.

129. Mkaddem, S.B.; Christou, I.; Rossato, E.; Berthelot, L.; Lehuen, A.; Monteiro, R.C. IgA, IgA receptors, and their anti-inflammatory properties. *Curr Top Microbiol Immunol*. **2014**, *382*, 221-235.
130. Mestecky, J.; McGhee, R. Immunoglobulin A (IgA): molecular and cellular interactions involved in IgA biosynthesis and immune response. *Adv Immunol*. **1987**, *40*, 153-245.
131. Woof, J.M.; Mestecky J. Mucosal immunoglobulins. *Immunol Rev*. **2005**, 64-82.
132. Mora, J.R.; von Andrian, U.H. Differentiation and homing of IgA-secreting cells. *Mucosal Immunol*. **2008**, *1*, 96–102.
133. Corthésy, B. Role of secretory immunoglobulin A and secretory component in the protection of mucosal surfaces. *Future Microbiol*. **2010**, *5*, 817-29.
134. Yazdani, R.; Azizi, G.; Abolhassani, H.; Aghamohammadi, A. Selective IgA deficiency: epidemiology, pathogenesis, clinical phenotype, diagnosis, prognosis and management. *Scand J Immunol*. **2017**, *85*, 3-12.
135. Vo Ngoc, D.T.; Krist, L.; van Overveld, F.J.; Rijkers, G.T. The long and winding road to IgA deficiency: causes and consequences. *Expert Rev Clin Immunol*. **2017**, *13*, 371-382.
136. Ballow, M. Primary immunodeficiency disorders: antibody deficiency. *J Allergy Clin Immunol*. **2002**, *109*, 581-591.
137. Aghamohammadi, A.; Cheraghi, T.; Gharagozlou, M.; Movahedi, M.; Rezaei, N.; Yeganeh, M.; Parvaneh, N.; Abolhassani, H.; Pourpak, Z.; Moin, M. IgA deficiency: correlation between clinical and immunological phenotypes. *J Clin Immunol*. **2009**, *29*, 130-136.
138. Kawamoto, S.; Maruya, M.; Kato, L.M.; Suda, W.; Atarashi, K.; Doi, Y.; Tsutsui, Y.; Qin, H.; Honda K.; Okada, T.; Hattori, M.; Fagarasan, S. Foxp3(+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. *Immunity*, **2014**, *41*, 152-165.
139. Okazaki, Y.; Han, Y.; Kayahara, M.; Watanabe, T.; Arishige, H.; Kato, N. Consumption of curcumin elevates fecal immunoglobulin A, an index of intestinal immune function, in rats fed a high-fat diet. *J Nutr Sci Vitaminol (Tokyo)*. **2010**, *56*, 68-71.
140. Williams, A.R.; Krych, L.; Ahmad, H.F.; Nejsum, P.; Skovgaard, K.; Nielsen, D.S.; Thamsborg, S.M. A polyphenol-enriched diet and Ascaris suum infection modulate mucosal immune responses and gut microbiota composition in pigs. *PLoS ONE* **2017**, *12*, e0186546.
141. Alvarez-Cilleros, D.; Ramos, S.; Lopez-Oliva, M.E.; Escriva, F.; Alvarez, C.; Fernandez-Millan, E.; Martin, M.A. Cocoa diet modulates gut microbiota composition and improves intestinal health in Zucker diabetic rats. *Food Res. Int*. **2020**, *132*, 109058.
142. Xu, J.; Su, X.; Lim, S.; Griffin, J.; Carey, E. Characterization and stability of anthocyanins in purple-fleshed sweet potato P40. *Food Chem*. **2015**, *186*, 90-96.
143. Yildirim, Z.; Tokusoglu, O.; Ozturk, G. Determination of sweet potato (Ipomoea batatas (L.) Lam) genotypes suitable to the Aegean region of Turkey. *Turkish J F Crop*. **2011**. *16*, 48-53.

144. Yildirim, T.Z.; Durucasu, I. Nutraceutical phenolics (total polyphenols, chlorogenic (5-O-Caffeoylquinic) acid) in tubers, leaves, stalks and stems of new developed sweet potato (*Ipomea Batatas L*): Alterations in tubers during short-term storage. *J Food Technol*. **2005**, *3*, 444-448.
145. Austin, D.F. The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. In P. Gregory (ed.), Exploration, maintenance and utilization of sweet potato genetic resources. *CIP, Lima, Peru*, **1988**, 27-60.
146. Yen, D.E. Sweet potato in historical perspective. In: R.L. Villareal and T.D. Griggs, eds. Sweet potato: Proceedings of the first international symposium. *AVRDC, Tainan, Taiwan*. **1982**, 17-30.
147. Bottema, T. Rapid market appraisal, issues and experience with sweet potato in Vietnam. In Scott, G., S. Wiersema, and P. I. Ferguson (eds.) Product Development for root and tuber crops. International Potato Center, Lima, Peru. **1992**, *1*.
148. Miranda, J.E.C. 2002. Batata-doce. Available from: www.cnph.embrapa.br/cultivares/bat-doce.htm.
149. Goodman.; Grant, K. Japan and the Dutch 1600-1853. *London: Routledge*. **2013**, 66–67.
150. Gunn.; Geoffrey, C. First Globalization: The Eurasian Exchange, 1500-1800. *The Sixteenth Century Journal*. **2003**, *36*, 932–933.
151. Obrien.; Patricia, J. The sweet potato: Its origin and dispersal. *Am. Anthropol*. **1972**, *74*, 342–365.
152. Itoh, M. The storied history of the potato in Japanese cooking. *The Japan times*. **2017**.
153. Jung, J.K.; Lee, S.U.; Kozukue, N.; Levin, C.E.; Friedman, M. Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (Ipomoea batata L.) plants and in-home processed roots. *J Food Compos Anal*. **2011**, *24*, 29-37.
154. Yamakawa, O.; Suda, I.; Yoshimoto, M. Development and utilization of sweet potato cultivars with high anthocyanin content. *Foods Food Ingredients J. Jpn*. **1998**, *178*, 69–78.
155. Gould, K.; Davies, K.; Winefield, C. Anthocyanins: biosynthesis, functions, and applications. *Springer* **2008**, *1*, 1-345.
156. Truong, V.D.; Deighton, N.; Thompson, R.L.; McFeeters, R.F.; Dean, L.O.; Pecota, K. V., Yencho, G.C. Characterization of anthocyanins and anthocyanidins in purple fleshed sweet potatoes by HPLC-DAD/ESI-MS/MS. *J Agric Food Chem*. **2010**, *58*, 404–410.
157. Castañeda-Ovando, A.; de Lourdes Pacheco-Hernández, M.; Páez-Hernández, E.; Rodríguez, J.A.; AndrésGalán-Vidal, C. Chemical studies of anthocyanins: a review. *Food Chem*. **2009**, *113*, 859–871.
158. Ikuo, S; Tomoyuki, O.; Mami, M.; Mio, K.; Yoichi, N.; Shu, F. Physiological functionality of purple-fleshed sweet potatoes containing anthocyanins and their utilization in foods. *JARQ*. **2003**, *37*, 167-173.
159. Srinivasan, V.S. Bioavailability of nutrients: A practical approach to in vitro demonstration of the availability of nutrients in multivitamin-mineral combination products. *J Nutr*. **2001**, *131*, 1349–1350.

160. McGhie, T.K.; Walton, M.C. The bioavailability and absorption of anthocyanins: Towards a better understanding. *Mol Nutr Food Res*. **2007**, *51*, 702-713.
161. Gomez-Rico, A.; Salvador, M.D.; La Greca, M.; Fregapane, G. Phenolic and volatile compounds of extra virgin olive oil (Olea europaea L. Cv. Cornicabra) with regard to fruit ripening and irrigation management. *J. Agric. Food Chem*. **2006**, *54*, 7130–7136.
162. Gomez-Alonso, S.; Fregapane, G.; Salvador, M.D.; Gordon, M.H. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *J. Agric. Food Chem*. **2003**, *51*, 667–672.
163. Andrikopoulos, N.K.; Dedoussis, G.V.; Falirea, A.; Kalogeropoulos, N.; Hatzinikola, H.S. Deterioration of natural antioxidant species of vegetable edible oils during the domestic deep-frying and pan-frying of potatoes. *Int. J. Food Sci. Nutr*. **2002**, 53, 351–363.
164. Gliszczynska-Swiglo, A.; Tyrakowska, B. Quality of commercial apple juices evaluated on the basis of the polyphenol content and the TEAC antioxidant activity. *J. Food Sci*. 2003, 68, 1844–1849.
165. Mullen, W.; Stewart, A.J.; Lean, M.E.; Gardner, P.; Duthie, G.G.; Crozier, A. Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. *J. Agric. Food Chem*. **2002**, *50*, 5197–5201.
166. Vallejo, F.; Tomas-Barberan, F.; Garcia-Viguera, C. Health-promoting compounds in broccoli as influenced by refrigerated transport and retail sale period. *J. Agric. Food Chem.* **2003**, *51*, 3029–3034.
167. Donovan, J.L.; Kasim-Karakas, S.; German, J.B.; Waterhouse, A.L. Urinary excretion of catechin metabolites by human subjects after red wine consumption. *Br. J. Nutr*. **2002**, *87*, 31–37.
168. Nurmi, T.; Mursu, J.; Heinonen, M.; Nurmi, A.; Hiltunen, R.; Voutilainen, S. Metabolism of berry anthocyanins to phenolic acids in humans. *J. Agric. Food Chem*. **2009**, *57*, 2274–2281.
169. Frank, T.; Netzel, M.; Strass, G.; Bitsch, R.; Bitsch, I. Bioavailability of anthocyanidin-3-glucosides following consumption of red wine and red grape juice. *Can J Physiol Pharmacol*. **2003**, *81*, 423–435.
170. Bub, A.; Watzl, B.; Heeb, D.; Rechkemmer, G.; Briviba, K. Malvidin-3-glucoside bioavailability in humans after ingestion of red wine, dealcoholized red wine and red grape juice. *Eur J Nutr*. **2001**, *40*, 113–120.
171. Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. **2005**, *81*, 230-242.
172. Kay, C.D.; Pereira-Caro, G.; Ludwig, I.A.; Clifford, M.N.; Crozier, A. Anthocyanins and flavanones are more bioavailable than previously perceived: A review of recent evidence. *Annu Rev Food Sci Technol*. **2017**, *8*, 35.
173. Wang, H.; Cao, G.; Prior, R.L. Oxygen radical absorbing capacity of anthocyanins. *J Agric Food Chem*. **1997**, *45*, 304 –309.

174. Tsuda, T.; Horio, F.; Osawa, T. The role of anthocyanins as an antioxidant under oxidative stress in rats. *Biofactors* **2000**, *13*, 133 –139.
175. Hagiwara, A.; Yoshino, H.; Ichihara, T.; Kawabe, M.; Tamano, S.; Aoki, H.; Koda, T.; Nakamura, M.; Imada, K.; Ito, N.; Shirai, T. Prevention by natural food anthocyanins, purple sweet potato color and red cabbage color, of 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP)-associated colorectal carcinogenesis in rats initiated with 1,2-dimethylhydrazine. *J. Toxicol. Sci*. **2002**, *27*, 57–68.
176. Matsumoto, H.; Nakamura, Y.; Tachibanaki, S.; Kawamura, S.; Hirayama, M. Stimulatory effect of cyanidin 3-glucosides on the regeneration of rhodopsin. *J. Agric. Food Chem*. **2003**, *51*, 3560–3563.
177. Ghiselli, A.; Nardini, M.; Baldi, A.; Scaccini, C. Antioxidant activity of different phenolic fractions separated from an Italian red wine. *J. Agric. Food Chem*. **1998**, *46*, 361–367.
178. Omenn, G.S. What accounts for the association of vegetables and fruits with lower incidence of cancers and coronary heart diseases? *Ann Epidemiol*. **1995**, *5*, 333 – 335.
179. Stintzing, F.C.; Stintzing, A.S.; Carle, R.; Frei, B.; Wrolstad, R.E. Color and antioxidant properties of cyanidin-based anthocyanin pigments. *J Agric Food Chem*. **2002**, *50*, 6172–6181.
180. Roewer, N.; Broscheit, J. Delphinidin for combating melanoma cells. U.S. Patent Application No. 14/651, **2013**, 262.
181. Tsuda, T.; Shiga, K.; Ohshima, K.; Kawakishi, S.; Osawa, T. Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from Phaseolus vulgaris L. *Biochemical Pharmacol*. **1996**, *52*, 1033–1039.
182. Kang, M.K.; Lim, S.S.; Lee, J.Y.; Yeo, K.M.; Kang, Y.H. Anthocyanin-rich purple corn extract inhibits diabetes-associated glomerular angiogenesis. *Plos One* **2013**, *8*, e79823.
183. Roy, S.; Khanna, S.; Alessio, H.M.; Vider, J.; Bagchi, D.; Bagchi, M.; Sen, C.K. Anti-angiogenic property of edible berries. *Free Rad Res*. **2002**, *36*, 1023–1032.
184. Matsunaga, N.; Tsuruma, K.; Shimazawa, M.; Yokota, S.; Hara, H. Inhibitory actions of bilberry anthocyanidins on angiogenesis. *Phytother Res*. **2010**, *24*, 42–47.
185. Stein, J.H.; Keevil, J.G.; Wiebe, D.A.; Aeschlimann, S.; Folts, J.D. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* **1999**, *100*, 1050–1055.
186. Keevil, J.G.; Osman, H.E.; Reed, J.D.; Folts, J.D. Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. *J. Nutr*. **2000**, *130*, 53–56.
187. Heinonen, I.M.; Lehtonen, P.J.; Hopia, A.I. Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem*. **1998**, *46*, 25–31.
188. Mazza, G.; Kay, C.D.; Cottrell, T.; Holub, B.J. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *J. Agric. Food Chem*. **2002**, *50*, 7731–7737.
189. Liu, M.; Li, X.Q.; Weber, C.; Lee, C.Y.; Brown, J.; Liu, R.H. Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food Chem*. **2002**, *50*, 2926–2930.

190. Yoshimoto, M.; Okuno, S.; Yoshinaga, M.; Yamakawa, O.; Yamaguchi, M.; Yamada, J. Antimutagenicity of sweetpotato (Ipomoea batatas) roots. *Biosci. Biotechnol. Biochem*. **1999**, *63*, 537–541.
191. Suda, I.; Furuta, S.; Nishiba, Y.; Yamakawa, O.; Matsugano, K.; Sugita, K. Reduction of liver injury induced by carbon tetrachloride in rats administered PSP juice. *J. Jpn. Soc. Food Sci. Technol.* **1997**, *44*, 315–318.
192. Tsukui, A.; Suzuki, A.; Nagayama, S.; Terahara, N. Stability of anthocyanin pigments from purple leaves of Perilla ocimoides L. var. crispa. *Nippon Shokuhin Kagaku Kogaku Kaishi*. **1996**, *43*, 451–457.
193. Tsukui, A.; Suzuki, A.; Komaki, K.; Terahara, N.; Yamakawa, O.; Hayashi, K. Stability and composition ratio of anthocyanin pigments from Ipomoea batatas. Poir. *Nippon Shokuhin Kagaku Kogaku Kaishi*. **1999**, *46*, 148–154.
194. Kano, M.; Takayanagi, T.; Harada, K.; Makino, K.; Ishikawa, F. Antioxidative activity of anthocyanins from purple sweet potato, Ipomoera batatas cultivar Ayamurasaki. *Biosci Biotechnol Biochem*. **2005**, *69*, 979-88.
195. Han, K.H.; Matsumoto, A.; Shimada, K.; Sekikawa, M.; Fukushima, M. Effects of anthocyanin-rich purple potato flakes on antioxidant status in F344 rats fed a cholesterol-rich diet. *Br J Nutr*. **2007**, *98*, 14-21.
196. Han, K.H.; Sekikawa, M.; Shimada, K.; Hashimoto, M.; Hashimoto, N.; Noda, T.; Tanaka, H.; Fukushima, M. Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats. *Br J Nutr*. **2006**, *96*, 1125–1133.
197. Keppler, K.; Humpf, H.U. Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora. *Bioorg Med Chem*. **2005**, *13*, 5195–5205.
198. Jawi, M.; Wita, W.; Suprapta, D.N. Extract of purple sweet potato tuber increases Sod and decreases VCAM-1 Expression by increasing Nrf2 expression in the aortic endothelia of hypercholesterolemic rabbits. *J. Biol. Agric. Healthcare*. **2014**, *4*, 76–84.
199. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total polyphenol and other oxidation Fsubstrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol*. **1999**, *299*, 152–178.
200. Yu, Z.; Morrison, M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques* **2004**, *36*, 808–812.
201. Warren, F.J.; Fukuma, N.M.; Mikkelsen, D.; Flanagan, B.M.; Williams, B.A.; Lisle, A.T.; Cuív, P.Ó.; Morrison, M.; Gidley, M.J. Food starch structure impacts gut microbiome composition. *Msphere* **2018**, *3*, 86–118.
202. Lozupone, C.; Knight, R. UniFrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol*. **2005**, *71*, 8228–8235.
203. Zakrzewski, M.; Proietti, C.; Ellis, J.J.; Hasan, S.; Brion, M.J.; Berger, B.; Krause, L. Calypso: A user-friendly web-server for mining and visualizing microbiome-environment interactions. *Bioinformatics* **2017**, *33*, 782–783.

204. Ikeda, T.; Tanaka, Y.; Yamamoto, K.; Morii, H.; Kamisako, T.; Ogawa, H. Geranium Dielsianum extract powder (Miskamiska) improves the intestinal environment through alteration of microbiota and microbial metabolites in rats. *J. Funct. Foods*. **2014**, *11*, 12–19.
205. Dolara, P.; Luceri, C.; De Filippo, C.; Femia, A.P.; Giovannelli, L.; Caderni, G.; Cecchini, C.; Silvi, S.; Orpianesi, C.; Cresci, A. Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutat Res*. **2005**, *591*, 237-246.
206. Tuohy, K.M.; Conterno, L.; Gasperotti, M.; Viola, R. Up-regulating the human intestinal microbiome using whole plant foods, polyphenolas and/or fiber. *J Agric Food Chem*. **2012**, *60*, 8776–8782.
207. Tabasco, R.; Sánchez-Patán, F.; Monagas, M.; Bartolomé, B.; Moreno-Arribas, M.V.; Peláez, C.; Requena, T. Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: Resistance and metabolism. *Food Microbiol*. **2011**, *28*, 1345-1352.
208. Canard, B.; Cole, S.T. Genome organization of the anaerobic pathogen Clostridium perfringens. *Proc Natl Acad Sci*. **1989**, *86*, 6676-6680.
209. Lopez-Legarrea, P.; Fuller, N.; Zulet, M.; Martinez, J.A.; Caterson, I.D. The influence of Mediterranean, carbohydrate and high protein diets on gut microbiota composition in the treatment of obesity and associated inflammatory state. *Asia Pac J Clin Nutr*. **2014**, *23*, 360-368.
210. FRO linger, T.; Sims, S.; Smith, C.; Wang, J.; Cheng, H.; Faith, J.; Ho, L.; Hao, K.; Pasinetti, G.M. The gut microbiota composition affects dietary polyphenols-mediated cognitive resilience in mice by modulating the bioavailability of phenolic acids. *Sci. Rep*. **2019**, *9*, 3546.
211. Espley, R.V.; Butts, C.A.; Laing, W.A.; Martell, S.; Smith, H.; McGhie, T.K.; Zhang, J.; Paturi, G.; Hedderley, D.; Bovy, A.; Schouten, H.J.; Putterill, J.; Allan, A.C.; Hellens, R.P. Dietary flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice. *J. Nutr*. **2014**, *144*, 146–154.
212. De, F.C.; Cavalieri, D.; Di, P.M.; Ramazzotti, M.; Poullet, J.B.; Massart, S.; Collini, S.; Pieraccini, G.; Lionetti, P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. U. S. A*. **2010**, *107*, 14691–14696.
213. Wu, G.D.; Chen, J.; Hoffmann, C.; Bittinger, K.; Chen, Y.Y.; Keilbaugh, S.A.; Bewtra, M.; Dan, K.; Walters, W.A.; Knight, R. Linking long-term dietary patterns with gut microbial enterotypes. *Science* **2011**, *334*, 105–108.
214. Kovatcheva-Datchary, P.; Nilsson, A.; Akrami, R.; Lee, Y.S.; De Vadder, F.; Arora, T.; Hallen, A.; Martens, E.; Björck, I.; Bäckhed, F. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of Prevotella. *Cell Metab*. **2015**, *22*, 971–982.

215. Zhang, X.; Yang, Y.; Wu, Z.; Weng, P. The modulatory effect of anthocyanins from purple sweet potato on human intestinal microbiota in vitro. *J. Agric. Food Chem*. **2016**, *64*, 2582–2590.
216. Koropatkin, N.M.; Cameron, E.A.; Martens, E.C. How glycan metabolism shapes the human gut microbiota. *Nat. Rev. Microbiol*. **2012**, *10*, 323–335.
217. Yang, Q.; Lin, S.L.; Kwok, M.K.; Leung, G.M.; Schooling, C.M. The roles of 27 genera of human gut microbiota in ischemic heart disease, Type 2 diabetes mellitus, and their risk factors: A Mendelian randomization study. *Am J Epidemiol*. **2018**, *187*, 1916–1922.
218. Öco, U.; Frank, J.A.; Fangel, J.U.; Oostindjer, M.; da Silva, C.S.; Bolhuis, E.J.; Bosch, G.; Willats, W.G.; Pope, P.B.; Diep, D.B. Resistant starch diet induces change in the swine microbiome and a predominance of beneficial bacterial populations. *Microbiome* **2015**, *3*, 1–15.
219. Molan, A.L.; Liu, Z.; Kruger, M. The ability of blackcurrant extracts to positively modulate key markers of gastrointestinal function in rats. *World, J. Microbiol. Biotechnol*. **2010**, *26*, 1735–1743.
220. Kim, H.J.; White, P.J. In vitro fermentation of oat flours from typical and high β-glucan oat lines. *J. Agric. Food Chem*. **2009**, *57*, 7529–7536.
221. Yoshinaga, M. New cultivar "Ayamurasaki" for colorant production. Sweet potato Research. *Frontiers (Boulder)* **1995**, 1–2.
222. Lim, S.; Xu, J.; Kim, J.; Chen, T.Y.; Su, X.; Standard, J.; Carey, E.; Griffin, J.; Herndon, B.; Katz, B.; Tomich, J.; Wang, W. Role of anthocyanin-enriched purple-fleshed sweet potato p40 in colorectal cancer prevention. *Mol. Nutr. Food Res*. **2013**, *57*, 1908–1917.
223. Furuta, S.; Suda, I.; Nishiba, Y.; Yamakawa, O. High tert-butylperoxyl radical scavenging activities of sweet potato cultivars with purple flesh. *Food Sci. Technol. Int*. **1998**, *4*, 33–35.
224. Miyazaki, M.; Makino, K.; Iwadate, E.; Deguchi, Y.; Ishikawa, F. Anthocyanins from purple sweet potato Ipomoea batatas cultivar Ayamurasaki suppress the development of atherosclerotic lesions and both enhancements of oxidative stress and soluble vascular cell adhesion molecule-1 in apolipoprotein E-deficient mice. *J. Agric. Food Chem*. **2008**, *56*, 11485–11492.
225. Suda, I.; Oki, T.; Masuda, M.; Kobayashi, M.; Nishiba, Y.; Furuta, S. Physiological functionality of purple-fleshed sweet potatoes containing anthocyanins and their utilization in foods. *JARQ-Jpn. Agr. Res. Q*. **2003**, *37*, 167–173.
226. Stahl, W.; van den Berg, H.; Arthur, J.; Bast, A.; Dainty, J.; Faulks, R.M.; Gärtner, C.; Haenen, G.; Hollman, P.; Holst, B.; Kelly, F.J.; Polidori, M.C.; Rice-Evans, C.; Southon, S.; van Vliet, T.; Viña-Ribes, J.; Williamson, G.; Astleyet, S.B. Bioavailability and metabolism. *Mol. Aspects. Med*. **2002**, *23*, 39–100.
227. Han, K.H.; Ohashi, S.; Sasaki. K.; Nagata, R.; Pelpolage, S.; Fukuma, N.; Reed, J.D.; Shimada, K.; Kadoya, N.; Fukushima, M. Dietary adzuki bean paste dose-dependently reduces visceral fat accumulation in rats fed a normal. *Food Res. Int*. **2020**, *130*, 1088902.

228. National Research Council. Guide for the care and use of laboratory animals. Available from: http://newton.nap.edu/html/labrats/. **1996**.
229. Bovee-Oudenhoven, I.M.; Termont, D.S.; Heidt, P.J.; Van der Meer, R. Increasing the intestinal resistance of rats to the invasive pathogen Salmonella enteritidis: additive effects of dietary lactulose and calcium. *Gut* **1997**, *40*, 497–504.
230. Kilua, A.; Chihiro, H.; Han, K.H.; Homma, K.; Fukuma, N.; Kamitani, T.; Suzuki, T.; Fukushima, M. Whole kidney bean (Phaseolus vulgaris) and bean hull reduce the total serum cholesterol, modulate the gut microbiota and affect the caecal fermentation in rats. *Bioact. Carbohydr. Diet. Fibre*. **2020**, *24*, 100232.
231. Cox, R.A.; García-Palmieri, M.R. Cholesterol, triglycerides, and associated lipoproteins. (3rd ed.). Boston: Butterworths, (Chapter 31). **1990**.
232. Kosmala, M.; Jurgoński, A.; Juśkiewicz, J.; Karlińska, E.; Macierzyński, J.; Rój, E.; Zduńczyk, Z. Chemical composition of blackberry press cake, polyphenolic extract, and defatted seeds, and their effects on cecal fermentation, bacterial metabolites, and blood lipid profile in rats. *J Agric Food Chem*. **2017**, *65*, 5470-5479.
233. Zern, T.L.; West, K.L.; Fernandez, M.L. Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. *J Nutr*. **2003**, *133*, 2268-2272.
234. Pokimica, B.; García-Conesa, M.T.; Zec, M.; Debeljak-Martačić, J.; Ranković, S.; Vidović, N.; Petrović-Oggiano, G.; Konić-Ristić, A.; Glibetić, M. Chokeberry juice containing polyphenols does not affect cholesterol or blood pressure but modifies the composition of plasma phospholipids fatty acids in individuals at cardiovascular risk. *Nutrients* **2019**, *11*, 850.
235. Nakagawa, K.; Maruyama, Y.; Miyazawa, T. Anthocyanin administration elevates plasma homocysteine in rats. *J Nutr Sci Vitaminol (Tokyo).* **2002**, *48*, 530-535.
236. Alberti, K.G.M.M.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.C.; James, W.P.; Loria, C.M.; Smith, S.C., Jr. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. *Circulation* **2009**, *120*, 1640–1645.
237. Miranda, A.M.; Steluti, J.; Fisberg, R.M.; Marchioni, D.M. Association between polyphenol intake and hypertension in adults and older adults: A population-based study in Brazil. *PLoS ONE* **2016**, *11*, e0165791.
238. Castro-Barquero, S.; Tresserra-Rimbau, A.; Vitelli-Storelli, F.; Doménech, M.; Salas-Salvadó, J.; Martín-Sánchez, V.; Rubín-García, M.; Buil-Cosiales, P.; Corella, D.; Fitó, M.; Romaguera, D.; Vioque, J.; Alonso-Gómez, Á.M.; Wärnberg, J.; Martínez, J.A.; Serra-Majem, L.; Tinahones, F.J.; Lapetra, J.; Pintó, X.; Tur, J.A.; Garcia-Rios, A.; García-Molina, L.; Delgado-Rodriguez, M.; Matía-Martín, P.; Daimiel, L.; Vidal, J.; Vázquez, C.; Cofán, M.; Romanos-Nanclares, A.; Becerra-Tomas, N.; Barragan, R.; Castañer, O.; Konieczna, J.; González-Palacios, S.; Sorto-Sánchez, C.; Pérez-López, J.; Zulet, M.A.; Bautista-Castaño, I.; Casas, R.; Gómez-Perez, A.M.; Santos-Lozano, J.M.; Rodríguez-

Sanchez, M.Á.; Julibert, A.; Martín-Calvo, N.; Hernández-Alonso, P.; Sorlí, J.V.; Sanllorente, A.; Galmés-Panadés, A.M.; Cases-Pérez, E.; Goicolea-Güemez, L.; Ruiz-Canela, M.; Babio, N.; Hernáez, Á.; Lamuela-Raventós, R.M.; Estruch, R. Dietary polyphenol intake is associated with HDL-Cholesterol and a better profile of other components of the metabolic syndrome: A PREDIMED-Plus Sub-Study. *Nutrients* **2020**, *12*, 689.

239. Clemmesen, J.O.; Larsen, F.S.; Kondrup, J.; Hansen, B.A.; Ott, P. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. *Hepatology*, **1999**, *29*, 648-53.
240. Jaroslawska, J.; Juskiewicz, J.; Wroblewska, M.; Jurgonski, A.; Krol, B.; Zdunczyk, Z. Polyphenol-rich strawberry pomace reduces serum and liver lipids and alters gastrointestinal metabolite formation in fructose-fed rats. *J Nutr*. **2011**, *141*, 1777-1783.
241. Collins, B.; Hoffman, J.; Martinez, K.; Grace, M.; Lila, M.A.; Cockrell, C.; Nadimpalli, A.; Chang, E.; Chuang, C.C.; Zhong, W.; Mackert, J.; Shen, W.; Cooney, P.; Hopkins, R.; McIntosh, M. A polyphenol-rich fraction obtained from table grapes decreases adiposity, insulin resistance and markers of inflammation and impacts gut microbiota in high-fat-fed mice, *J. Nutr. Biochem*. **2015**, *31*, 150–165.
242. Zhang, NN.; Guo, W.H.; Hu, H.; Zhou, A.R.; Liu, Q.P.; Zheng, B.D.; Zeng, S.X. Effect of a polyphenol-rich canarium album extract on the composition of the gut microbiota of mice fed a high-fat diet. *Molecules*, **2018**, *23*, 2188.
243. Konikoff, T.; Gophna, U. Oscillospira, A central, enigmatic component of the human gut microbiota. *Trends Microbiol*. **2016**, *24*, 523–524.
244. Keren, N.; Konikoff, F.M.; Paitan, Y.; Gabay, G.; Reshef, L.; Naftali, T.; Gophna, U. Interactions between the intestinal microbiota and bile acids in gallstones patients. *Environ. Microbiol. Rep*. **2015**, *7*, 874–880.
245. Marques, C.; Fernandes, I.; Meireles, M.; Faria, A.; Spencer, J.P.E.; Mateus, N.; Calhau, C. Gut microbiota modulation accounts for the neuroprotective properties of anthocyanins. *Sci. Rep*. **2018**, *8*, 11342.
246. Slobodníková, L.; Fialová, S.; Rendeková, K.; Kováč, J.; Mučaji, P. Antibiofilm activity of plant polyphenols. *Molecules* **2016**, *21*, 1717.
247. Forester, S.C.; Waterhouse, A.L. Gut metabolites of anthocyanins, gallic acid, 3-O-methylgallic acid, and 2,4,6-trihydroxybenzaldehyde, inhibit cell proliferation of Caco-2 cells. *J. Agric. Food Chem*. **2010**, *58*, 5320–5327.
248. Sun, H.; Zhang, P.; Zhu, Y.; Lou, Q.; He, S. Antioxidant and prebiotic activity of five peonidin-based anthocyanins extracted from purple sweet potato (Ipomoea Batatas (L.) Lam.). *Sci. Rep*. **2018**, *8*, 5018.
249. Le Chatelier, E.; Nielsen, T.; Qin, J.; Prifti, E.; Hildebrand, F.; Falony, G.; Almeida, M.; Arumugam, M.; Batto, J.M.; Kennedy, S.; Leonard, P.; Li, J.; Burgdorf, K.; Grarup, N.; Jørgensen, T.; Brandslund, I.; Nielsen, H.B.; Juncker, A.S.; Bertalan, M.; Levenez, F.; Pons, N.; Rasmussen, S.; Sunagawa, S.; Tap, J.; Tims, S.; Zoetendal, E.G.; Brunak, S.; Clément, K.; Doré, J.; Kleerebezem, M.; Kristiansen, K.; Renault, P.; Sicheritz-Ponten, T.; de Vos, W.M.; Zucker, J.D.; Raes, J.; Hansen, T.; MetaHIT consortium.; Bork, P.; Wang, J.;

Ehrlich, S.D.; Pedersen, O. Richness of human gut microbiome correlates with metabolic markers. *Nature*, **2013**, *500*, 541–546.
250. Yang, C.; Deng, Q.; Xu, J.; Wang, X.; Hu, C.; Tang, H.; Huang, F. Sinapic acid and resveratrol alleviate oxidative stress with modulation of gut microbiota in high-fat diet-fed rats. *Food Res. Int*. **2019**, *116*, 1202–1211.
251. Cheng, J.R.; Liu, X.M.; Chen, Z.Y.; Zhang, Y.S.; Zhang, Y.H. Mulberry anthocyanin biotransformation by intestinal probiotics. *Food Chem*. **2016**, *213*, 721–727.
252. Nohynek, L.J.; Alakomi, H.L.; Kähkönen, M.P.; Heinonen, M.; Helander, I.M.; Oksman-Caldentey, K.M.; Puupponen-Pimiä, R.H. Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. *Nutr. Cancer*. **2006**, *54*, 18–32.
253. Hidalgo, M.; Oruna-Concha, M.J.; Kolida, S.; Walton, G.E.; Kallithraka, S.; Spencer, J.P.; de Pascual-Teresa, S. Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. *J. Agric. Food Chem*. **2012**, *60*, 3882–3890.
254. Scharlau, D.; Borowicki, A.; Habermann, N.; Hofmann, T.; Klenow, S.; Miene, C.; Munjal, U.; Stein, K.; Glei, M. Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutat. Res*. **2009**, *682*, 39–53.
255. Louis, P.; Flint, H.J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett*. **2009**, *294*, 1–8.
256. Pedersen, H.K.; Gudmundsdottir, V.; Nielsen, H.B.; Hyotylainen, T.; Nielsen, T.; Jensen, B.A.; Forslund, K.; Hildebrand, F.; Prifti, E.; Falony, G.; Le Chatelier, E.; Levenez, F.; Doré, J.; Mattila, I.; Plichta, D.R.; Pöhö, P.; Hellgren, L.I.; Arumugam, M.; Sunagawa, S.; Vieira-Silva, S.; Jørgensen, T.; Holm, J.B.; Trošt, K.; MetaHIT Consortium.; Kristiansen, K.; Brix, S.; Raes, J.; Wang, J.; Hansen, T.; Bork, P.; Brunak, S.; Oresic, M.; Ehrlich, S.D.; Pedersen, O. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* **2016**, *535*, 376–381.
257. Kanakis, C.D.; Hasni, I.; Bourassa, P.; Tarantilis, P.A.; Polissiou, M.G.; Tajmir-Riahi, H.A. Milk beta-lactoglobulin complexes with tea polyphenols. *Food Chem*. **2011**, *127*, 1046–1055.
258. Siebert, K.J.; Troukhanova, N.V.; Lynn, P.Y. Nature of polyphenol-protein interactions. *J. Agric. Food. Chem*. **1996**, *44*, 80–85.
259. Smith, E.A.; Macfarlane, G.T. Dissimilatory amino acid metabolism in human colonic bacteria. *Anaerobe* **1997**, *3*, 327–337.
260. Azuma, T.; Shigeshiro, M.; Kodama, M.; Tanabe, S.; Suzuki, T. Supplemental naringenin prevents intestinal barrier defects and inflammation in colitic mice. *J. Nutr*. **2013**, *143*, 827–834.
261. Childers, N.K.; Bruce, M.G.; McGhee, J.R. Molecular mechanisms of immunoglobulin A defense. *Annu. Rev. Microbiol*. **1989**, *43*, 503–536.