FORMULATION OF RESISTANT STARCH-ENRICHED FRESH WHEAT NOODLES AND INSTANT NOODLES

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ABSTRACT

While a high fiber diet helps to prevent colon cancer, lower the risk of heart disease, and influences metabolic and inflammatory bowel disease, such as diabetes and diverticulitis, the general population still consumes dietary fiber at a rate below recommended levels. Home-made meals are rarely seen and are being replaced by ready-to-cook and ready-to-eat foods. Wheat noodle products have become one of the food products that are consumed among people of all socioeconomic levels in both urban and rural areas of Thailand particularly in the form of instant noodles and fresh wheat noodles. However, instant noodles, especially the fried type, are sometimes classified by academics as “not nutritious” due to their high salt, fat and carbohydrate contents and inadequate fiber. Therefore, many attempts have been made to add dietary fiber to various noodle products with different fiber sources such as soy bean hulls, green and yellow peas, lentils, garbanzo bean flour, etc. However, high levels of natural fiber in foods impart poor texture and mouthfeel and unpleasant flavor and odor to the product. At present, commercial resistant starch (RS) has captured the attention of food industries for both functional properties and potential benefits that are similar to dietary fiber. This study aims to formulate resistant starch-enriched fresh wheat noodles and instant noodles by using commercial resistant starch (Hi-maize®) in order to increase resistant starch content to provide health benefits as well as to add value to fresh wheat noodles and instant noodles. RS was applied by partially substituting it for wheat flour in the noodles formulation. High levels of RS incorporation in noodle products caused adverse effects to the rheological property of noodle dough as well as the quality of finished product. To improve the quality of RS-enriched noodle products, it was necessary to add gluten and an additional amount of water. Results from product application testing and sensory evaluation indicated that RS could partially substitute for wheat flour up to 30%. RS-enriched fresh wheat noodles and instant noodles in this study had total dietary fiber (TDF) values of 16.8 and 16.7 g/ 100g, respectively. This was markedly higher than the control noodles (about 9 times). The TDF content of RS-enriched fresh wheat noodles and instant noodles was about 8 g per reference amount which is equals to 33% Thai RDI.

The cost of Hi-maize® was relatively higher than the commercial wheat flour. However, due to the high TDF content of RS-enriched noodles, they could be recommended as an alternative food choice for health conscious consumers. This, in turn, will enhance dietary fiber intake towards the recommended level.

KEY WORDS: FRESH WHEAT NOODLES / INSTANT NOODLES / RESISTANT STARCH / DIETARY FIBER / HI-MAIZE®

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การพัฒนาผลิตภัณฑ์บะหมี่สดและบะหมี่กึ่งสำเร็จรูปเสริมรีซิสแต๊นท์สตาร์ช (FORMULATION OF RESISTANT STARCH-ENRICHED FRESH WHEAT NOODLES AND INSTANT NOODLES)

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บทคัดย่อ

เป็นที่ทราบดีว่าใยอาหารมีประโยชน์มากมาย สามารถช่วยป้องกันและบรรเทาอาการรุนแรงของโรคหลายชนิดได้ เช่น มะเร็งลำไส้ใหญ่, โรคหัวใจ, โรคเบาหวาน และโรคเกี่ยวกับทางเดินอาหารต่างๆ แต่แนวโน้มการบริโภคใยอาหารกลับลดลงและมีอัตราการบริโภคต่ำกว่าค่าที่แนะนำอยู่ ทั้งนี้เนื่องจากในปัจจุบันพบว่าการประกอบอาหารรับประทานเองในครัวเรือนมีน้อยลง และถูกแทนที่ด้วยอาหารสำเร็จรูปหรืออาหารที่ปรุงได้ง่าย ซึ่งในจำนวนนี้อาหารประเภทเส้นจัดเป็นอาหารยอดนิยมชนิดหนึ่งที่มีปริมาณการบริโภคจากคนทั่วไปสูงทั้งในเขตเมืองและต่างจังหวัดโดยเฉพาะอย่างยิ่งในรูปแบบของบะหมี่สดและบะหมี่กึ่งสำเร็จรูป แต่ถึงกระนั้นบะหมี่กึ่งสำเร็จรูปโดยเฉพาะชนิดทอดในน้ำมันกลับถูกจัดไว้ในกลุ่มอาหารที่มีคุณค่าทางโภชนาการต่ำเนื่องจากเป็นอาหารที่มีปริมาณของเกลือ ไขมัน และคาร์โบไฮเดรตที่สูงแต่มีปริมาณใยอาหารต่ำ ดังนั้นจึงมีความพยายามจากหลายบุคคลหลายกลุ่มที่จะเพิ่มปริมาณใยอาหารเข้าไปในอาหารประเภทเส้นทั้งหลายนี้ โดยใช้แหล่งของใยอาหารที่แตกต่างกัน เช่น เปลือกถั่วเขียว เปลือกถั่วเหลือง ต่างๆ เป็นต้น แต่การทดแทนในปริมาณมากกลับส่งผลเสียในเรื่อง รสสัมผัส รสชาติ กลิ่น รวมถึงโครงสร้างของเส้นบะหมี่จึงทดแทนได้ในปริมาณไม่มากนัก ซึ่งปัจจุบันรีซิสแต๊นท์สตาร์ชกำลังได้รับความสนใจจากภาคอุตสาหกรรมอาหารเนื่องจากคุณสมบัติเชิงหน้าที่ที่ดีกว่าแหล่งใยอาหารทั่วไปและให้ผลการทดลองทางคลินิกว่ามีคุณประโยชน์ทางร่างกายที่คล้ายคลึงกับใยอาหาร ดังนั้นวัตถุประสงค์ของงานวิจัยนี้คือการพัฒนาสูตรผลิตภัณฑ์บะหมี่สดและบะหมี่กึ่งสำเร็จรูปเสริมรีซิสแต๊นท์สตาร์ช (Hi-maize®) เพื่อเพิ่มปริมาณใยอาหารและเพิ่มคุณค่าให้กับผลิตภัณฑ์บะหมี่สดและบะหมี่กึ่งสำเร็จรูป ซึ่งการทดแทนแป้งสาลีด้วยรีซิสแต๊นท์สตาร์ชในปริมาณมากส่งผลเสียต่อโดของบะหมี่และคุณภาพของผลิตภัณฑ์บะหมี่ทั้งสองซึ่งในการแก้ไขปัญหานี้ได้ทำการเติมกลูเตนและน้ำเพิ่มเข้าไปและเมื่อทำการตรวจสอบด้านกายภาพของโดและของเส้นบะหมี่ทั้งสองรวมถึงการประเมินทางประสาทสัมผัสแล้วพบว่าบะหมี่สดและบะหมี่กึ่งสำเร็จรูปสามารถเสริมรีซิสแต๊นสตาร์ชได้มากถึง 30% ผลการวิเคราะห์ทางเคมีพบว่าปริมาณรีซิสแต๊นท์สตาร์ชในผลิตภัณฑ์บะหมี่ทั้งสองชนิดมีปริมาณสูงขึ้น ซึ่งเมื่อนำมาคำนวณเป็นค่าใยอาหารพบว่า มีปริมาณใยอาหารเท่ากับ16.8 และ 16.7 กรัม/100 กรัม ในบะหมี่สดและบะหมี่กึ่งสำเร็จ ตามลำดับ ซึ่งมากกว่าบะหมี่ที่ไม่ได้เสริมรีซิสแต๊นท์สตาร์ชถึง 9 เท่า หรือเท่ากับมีปริมาณ 8 กรัมต่อหนึ่งหน่วยบริโภค ซึ่งเทียบเท่ากับ 33% Thai RDI.

ถึงแม้ว่าราคาของ Hi-maize® เมื่อเทียบกับแป้งสาลีทั่วไปจะถือว่ามีราคาสูง แต่หากคำนึงถึงคุณประโยชน์ต่อสุขภาพจากใยอาหารปริมาณมาก ก็ถือได้ว่า บะหมี่สดและบะหมี่กึ่งสำเร็จรูปเสริมรีซิสแต๊นท์สตาร์ช เป็นอีกทางเลือกหนึ่งสำหรับผู้ที่ใส่ใจในการเลือกอาหารเพื่อสุขภาพ และผู้ที่ต้องการใช้ประโยชน์จากอาหารที่มีปริมาณใยอาหารสูงได้

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LIST OF ABBREVIATIONS

RS	resistant starch
TDF	total dietary fiber
TS	total starch
RDS	rapidly digestible starch
SDS	slowly digestible starch
min	minute
h	hour
sec	second
°C	degree Celsius
AOAC	The Association of Official Analytical Chemists
SCFA	short-chain fatty acid
KJ	kilojoules
g/d	gram/day
KOH	potassium hydroxide
HCl	hydrogen chloride
CFR	Code of Federal Regulation
ANOVA	analysis of variance
RDI	Recommended Dietary Intake
rpm	Revolutions Per Minute
ml	milliliter

CHAPTER I
INTRODUCTION

The concept of resistant starch (RS) has evoked new interest in the bioavailability of starch and in its usage as a source of dietary fiber, particularly in adults. RS is now considered to provide functional properties and find applications in a variety of foods (1, 36).

According to a number of studies, a lower dietary (DF) intake is reportedly associated with several disorders of the human body such as diverticular disease, colon cancer, constipation, ischemic heart disease and other diseases of the gastrointestinal tract (1-4). The survey on DF intake in Thai population presented varying results. According to the study on the DF consumption of Thai adolescents in Bangkok by Uthang, the average intake of DF in males was only 7.32 g/day and the average intake of DF in females was only 8.88 g/day (5). While the study on the daily DF consumption of nursing students in Kaukarun Nursing Collage by Supingklud was 8.05 g (6). Generally, adolescents and adults are recommended to eat food containing 25 to 30 g of DF daily (7-8).

Currently in modern Thai society, traditional Thai staples and side dishes are being replaced by diets containing larger proportions of fats and animal meat, and smaller proportions of vegetables and fruits. Concomitant with these trends is the selection of food that requires less time and skill to prepare. Home-made meals are rarely seen and are being replaced by ready-to-cook and ready-to-eat foods (9).

Wheat noodle products have become one of the food products that are consumed among people of all socioeconomic levels in both urban and rural areas of Thailand particularly in the form of instant noodles (10-12) and fresh wheat noodles. The annual production of instant noodles is 1.9 billion packs (13). However, instant noodles, especially fried type, are sometimes classified by academician as “not nutritious” due to their high salt, fat and carbohydrate contents and inadequate of protein (10). In addition, it contains 2 g of DF/100 g edible portion (14) or only 4% of the recommended level of TDF in one noodle package. Although, the label on the package suggests adding meat or egg and vegetables, however because of inconvenience, this suggestion is seldom practiced by consumers (11-12). Consequently, these products sustain limited nutritive value.

Many attempts have been made to add dietary fiber to various food products such as bakery products, noodles, snack bars with different fiber sources such as soy bean hull, pineapple core extract, guava pomace, apple pomace, etc. (15-18). However, high levels of natural fiber in foods impart poor texture and mouthfeel, unpleasant flavor and odor to the product (19-21).

At present, commercial resistant starch (RS) is one of the most interested fiber ingredients. Initial clinical studies demonstrated that resistant starch has similar properties to dietary fiber and shows promising physiological benefits in human, which may result in disease prevention (1, 22). Application of resistant starch into food has shown an improvement in eating quality such as crispness, appearance, texture, mouthfeel, color and flavor over products produced with traditional fiber sources (23-24).

Therefore, this study aims to formulate resistant starch-enriched fresh wheat noodles and instant noodles by using commercial resistant starch (Hi-maize®) in order to increase resistant starch content for providing health benefits as well as adding value to fresh wheat noodles and instant noodles.

CHAPTER II
OBJECTIVES

General objective

To develop resistant starch-enriched fresh wheat noodles and instant noodles with acceptable sensory characteristics.

Specific objectives

This study aims:

(1) To formulate resistant starch-enriched fresh wheat noodles and instant noodles with acceptable characteristics by using commercial resistant starch (Hi-maize®) as the source of resistant starch.

(2) To determine the physical and chemical properties of the resistant starch-enriched fresh wheat noodles and instant noodles.

(3) To evaluate nutritional values and sensory acceptability of the resistant starch-enriched fresh wheat noodles and instant noodles.

(4) To measure the content of resistant starch in developed resistant starch-enriched noodle products.

CHAPTER III
LITERATURE REVIEW

Introduction

Starch, which is the major dietary source of carbohydrates, is the most abundant storage polysaccharide in plants, and occurs as granules in the chloroplast of green leaves and the amyloplast of seeds, pulses, and tubers (25). The relatively recent recognition of incomplete digestion and absorption of starch in the small intestine as a normal phenomenon has raised interest in nondigestible starch fractions (26, 27). These are called “resistant starches,” and extensive studies have shown them to have physiological functions similar to those of dietary fiber (28, 29). The diversity of the modern food industry and the enormous variety of food products it produces require starches that can tolerate a wide range of processing techniques and preparation conditions (30). These demands are met by modifying native starches with chemical, physical, and enzymatic methods (31), which may lead to the formation of indigestible residues. The availability of such starches therefore deserves consideration.

3.1 Structure of starch

Chemically, starches are polysaccharides, *i.e.* they are composed of a number of monosaccharides or sugar (glucose) molecules linked together with α 1–4 and/or α 1–6 linkages. Two main structural types of starch exist: amylose which is a linear α 1–4 molecule and typically constitutes 15–20% of starch, and amylopectin which is a larger branched molecule with α 1–4 and α 1–6 linkages and is a major component of starch (32). Two crystalline structures of starch have been identified (an ‘A’ and ‘B’ type), which contain differing proportions of amylopectin. A type starches are found in cereals, while B type starches are found in tubers and amylose-rich starches. A third type called ‘C type’ appears to be a mixture of both A and B forms and is found in legumes (33). In general, digestible starches are broken down (hydrolysed) by the enzymes α -amylases, glucoamylase and sucrase-isomaltase in the small intestine to yield free glucose that is then absorbed (24).

3.2 Structure of resistant starch

The resistance of starch to digestion is influenced by the nature of the association between starch polymers, with higher amylose levels in the starch being associated with slower digestibility rates. Both B and C type starches appear to be more resistant to digestion with high-amylose maize producing RS which has been particularly useful in the preparation of foods (34). Retrograded starches refer to certain structural forms of RS. Retrogradation occurs when starch is cooked in water beyond its gelatinization temperature and then cooled. Amylose is found in the amorphous parts of the starch crystal, while amylopectin gives starch its crystalline structure. Upon heating with excess water and at sufficiently high temperatures, the starch crystalline regions 'melt'. The starch granules gelatinize and the starch is subsequently more easily digested. However, these starch gels are unstable and upon cooling re-form crystals that are resistant to hydrolysis by amylases (*i.e.* are resistant to digestion). Slow cooling of the gelatinized starch favors Type A crystallization while slow cooling in excess water favors Type B crystallization. This process is known as retrogradation (33). In general, starches rich in amylose are naturally more resistant to digestion and also more susceptible to retrogradation (24).

3.3 Various ways to classify native starches

3.3.1 X-ray diffraction

Three types of starches, designated as type A, type B, and type C, have been identified based on X-ray diffraction patterns. These depend partly on the chain lengths making up the amylopectin lattice, the density of packing within the granules, and the presence of water (35). Although type A and type B are real crystalline modifications, type C is a mixed form. The important features of the types of starches are as follows (36).

Type A. The type A structure has amylopectin of chain lengths of 23 to 29 glucose units. The hydrogen bonding between the hydroxyl groups of the chains of amylopectin molecules results in the formation of outer double helical structure. In between these micelles, linear chains of amylose moieties are packed by forming hydrogen bonds with outer linear chains of amylopectin. This pattern is very common in cereals.

Type B. The type B structure consists of amylopectin of chain lengths of 30 to 44 glucose molecules with water inter-spread. This is the usual pattern of starches in raw potato and banana.

Type C. The type C structure is made up of amylopectin of chain lengths of 26 to 29 glucose molecules, a combination of type A and type B, which is typical of peas and beans. An additional form, called type V, occurs in swollen granules. X-ray diffraction diagrams of these starches are shown in **Figure 1.**

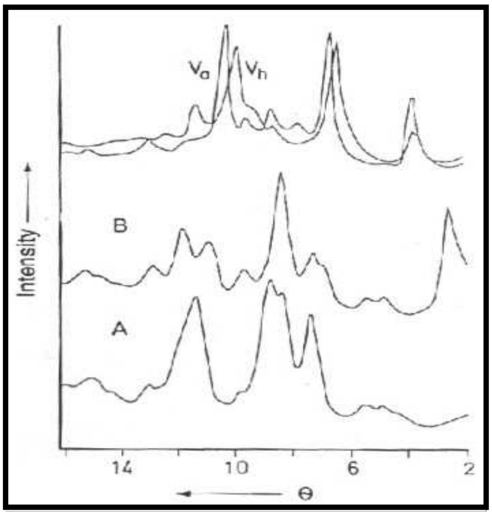

Figure 1
X-ray diffraction diagrams of starches:
type A (cereals), type B (legumes), and type V (swollen starch, V_a: water- free, V_h : hydrated)
Source: Sajilata 2006 (36)

3.3.2 Based on the action of enzymes

According to Berry (37), starches can be classified according to their behavior when incubated with enzymes without prior exposure to dispersing agents as follows.

Rapidly digestible starch (RDS). RDS consists mainly of amorphous and dispersed starch and is found in high amounts in starchy foods cooked by moist heat, such as bread and potatoes. It is measured chemically as the starch, which is converted to the constituent glucose molecules in 20 min of enzyme digestion.

Slowly digestible starch (SDS). Like RDS, SDS is expected to be completely digested in the small intestine, but for 1 reason or another, it is digested more slowly. This category consists of physically inaccessible amorphous starch and raw starch with a type A and type C crystalline structure, such as cereals and type B starch, either in granule form or retrograded form in cooked foods. It is measured chemically as starch converted to glucose after a further 100 min of enzyme digestion.

Resistant starch. The term "resistant starch" was first coined by Englyst and others (38) to describe a small fraction of starch that was resistant to hydrolysis by exhaustive α-amylase and pullulanase treatment in vitro. RS is the starch not hydrolyzed after 120 min of incubation (27). However, because starch reaching the large intestine may be more or less fermented by the gut microflora, RS is now defined as that fraction of dietary starch, which escapes digestion in the small intestine. It is measured chemically as the difference between total starch (TS) obtained from homogenized and chemically treated sample and the sum of RDS and SDS, generated from non-homogenized food samples by enzyme digestion.

RS = TS – (RDS + SDS)

3.3.3 Based on the nutritional characteristics

This classification is based on the extent of digestibility of the starch as follows (36).

Digestible starches. These include the starches digestible by body enzymes, namely the rapidly digestible starches (RDS) and the slowly digestible starches (SDS). RDS consists mainly of amorphous and dispersed starch, found in high amounts in starchy foods cooked by moist heat. Like RDS, SDS is expected to be completely digested in the small intestine, but for 1 reason or another, it is digested more slowly.

Resistant starch. RS is indigestible by body enzymes. It has been classified into four general subtypes called RS1–RS4 (27, 39). **Table 1** outlines a summary of the different types of RS, their classification criteria and food sources.

RS1 is the term given to RS where the starch is physically inaccessible to digestion, *e.g.* due to the presence of intact cell walls in grains, seeds or tubers.

RS2 describes native starch granules that are protected from digestion by the conformation or structure of the starch granule as in raw potatoes and green bananas. A particular type of RS2 is unique as it retains its structure and resistance even during the processing and preparation of many foods; this RS2 is called high-amylose maize starch.

RS3 refers to non-granular starch-derived materials that resist digestion. RS3 forms are generally formed during the retrogradation of starch granules. Some examples of RS3 are cooked and cooled potatoes and cornflakes.

RS4 describes a group of starches that have been chemically modified and include starches which have been etherized, esterified or cross-bonded with chemicals in such a manner as to decrease their digestibility. RS4 may be further subdivided into four subcategories according to their solubility in water and the experimental methods by which they can be analyzed (34).

Although RS is found naturally in all starch-containing foods, factors which influence the net amount of RS present include the initial quantity and type of starch present, how the starchy food is processed, cooked and stored and how it is ingested (40). As evident in **Table 1**, RS1 is made less resistant to digestion by milling and chewing, RS2 by food processing techniques (*e.g.* cooking) and RS3 by the conditions of food processing used (*e.g.* during the preparation of bread and cornflakes). RS4, as a result of chemical modification, can resist hydrolysis after food processing; however, this is dependent upon the starch base, and the type and level of modification. In addition to the structural factors mentioned above whereby the presence of water and the chemical structure of starch can influence the amount of RS present, other factors intrinsic to starchy foods can affect α-amylase activity and therefore starch breakdown. These include the formation of amylose-lipid complexes, the presence of native α-amylase inhibitors and also nonstarch polysaccharides, all of which can directly affect a-amylase activity (27). Extrinsic additives may also bind to starch making it more or less susceptible to degradation, *e.g.* phosphorus (41). In addition, physiological factors can impact the amount of RS in a food – increased chewing decreases particle size (smaller particles being more easily digested in the gut), while intra-individual variations in transit time and biological factors (*e.g.* menstrual cycle) also affect the digestibility of starch. At present, it is not known how the various types of RS4 are affected by digestion *in vivo* (24).

Table 1 Classification of types of resistant starch (RS), food sources, and factors affecting their resistance to digestion in the colon.

Type of RS	Description	Food sources	Resistance minimized by
RS1	Physically protected	Whole- or partly milled grains and seeds, legumes	Milling, chewing
RS2	Ungelatinized resistant granules with type B crystallinity, slowly hydrolyzed by α-amylase	Raw potatoes, green bananas, some legumes, high amylose corn	Food processing and cooking
RS3	Retrograded starch	Cooked and cooled potatoes, bread, cornflakes, food products with repeated moist heat treatment	Processing conditions
RS4	Chemically modified starches due to cross-linking with chemical reagents	Foods in which modified starches have been used (for example, breads, cakes)	Less susceptible to digestibility in vitro

Source: Nugent 2005 (24)

3.4 Functionality of RS

RS has a small particle size, white appearance, and bland flavor. RS also has a low water-holding capacity. It has desirable physicochemical properties (42) such as swelling, viscosity increase, gel formation, and water-binding capacity, making it useful in a variety of foods. These properties make it possible to use most resistant starches to replace flour on a 1-for-1 basis without significantly affecting dough handling or rheology. RS not only fortifies fiber but also imparts special characteristics not otherwise attainable in high-fiber foods (43). The functional properties and advantages of commercial sources of RS2 and RS3 (24) have been summarized as follows. They are natural sources, bland in flavor, white in color, with fine particle size (which causes less interference with texture). They have high gelatinization temperature, good extrusion and film-forming qualities, and lower water-holding properties than traditional fiber products. They allow the formation of low-bulk high-fiber products with improved texture, appearance, and mouth feel (such as better organoleptic qualities) compared with traditional high-fiber products; they increase coating crispness of products and the bowl life of breakfast cereals. They are functional food ingredients lowering the calorific value of foods and useful in products for coeliacs, as bulk laxatives and in products for oral rehydration therapy. Some of these properties of RS have been successfully used in a range of baked and extruded products as described subsequently (36).

3.4.1 RS as a texture modifier in baked goods

One way to ensure that the general population receives adequate amounts of fiber in the diet is to fortify good-tasting foods that normally do not come to mind with fiber fortification but are often eaten as breakfast items or snacks. RS were incorporated in a variety of baked goods, many of which include batter systems, such as in cakes, cake-like muffins, or brownies. In general, application tests showed that RS acts as texture modifier, imparting a favorable tenderness to the crumb. A low-fat, loaf cake was formulated with RS and various fibers to obtain approximately 3% TDF or 2.5 g of fiber per 80 g serving. These included a 40% TDF RS (Novelose 240 starch), oat fiber, a blend of oat fiber with Novelose 240 starch in a 50/50 ratio based on TDF contribution, and a 23% TDF RS (Hylon VII starch). The baked cakes made with RS were similar to that containing oat fiber and the control in the amount of moisture loss after baking, height, specific volume, and density. A panel rated the 40% TDF RS loaf cakes as the best for flavor, grittiness, moisture perception, and tenderness 24 h after baking (36).

3.4.2 RS as a crisping agent

Among other functional properties, RS can be used as an ingredient that improves crispness in foods where high heat is applied to a product’s surface during processing. French toast and waffles, especially frozen reheated types, represent foods in which surface crispness is desired. Tests were conducted to compare the functionality of RS and various fibers in a buttermilk waffle formulation. Based on the evaluation of the toasted waffles for initial crispness, crispness after 3 min, moistness, and overall texture by a trained sensory panel RS waffle indicated greater crispness than control or traditional fiber (36).

3.4.3 RS as a functional ingredient in other foods

Along with textural enhancement, RS can improve expansion in extruded cereals and snacks. Various cereals were formulated to contain 40% TDF RS (Novelose 240 starch) alone and in combination with oat fiber in ratios of 50/50 and

25/75 based on weight. The cereal with RS and no oat fiber had greater volumetric expansion than the control. In blends with oat fiber, the cereal containing 75% of RS had better expansion than the one containing only 50%. Dried pasta products containing up to 15% RS can be made with little or no effect on dough rheology during extrusion. Although the resultant pasta was lighter in color, a firm “al dente” texture was obtained in the same cooking time as a control that had no added fiber. RS may also be used in thickened, opaque health beverages in which insoluble fiber is desired. Insoluble fibers generally require suspension and add opacity to beverages. Compared with insoluble fibers, RS imparts a less gritty mouthfeel and masks flavors less (36).

3.5 Measurement of resistant starch

The main step of any method to measure the content of RS in foods must first remove all of the digestible starch from the product using thermostable α–amylases (44). At present, the method of McCleary & Monaghan (2002 and AOAC method 2002.02) is considered the most reproducible and repeatable measurement of RS in starch and plant materials, but it has not been shown to analyze all RS as defined (45). It is based on the principle of enzymic digestion and measures the portions of starch resistant to digestion at 37° C that are typically not quantitated due to the gelatinization at 100° C followed by digestion at 60° C. **Table 2** lists the RS contents from a number of sample foods using this method and sourced from McCleary & Rossiter (44). Two general methods specifically proposed to determine RS (27,37) remove digestible starch using different amylases, and the residual fraction is quantified after solubilization in 2M KOH. The Siljestrom and Asp (46) procedure includes preparation and quantification of dietary fiber residue before RS determination. This is usually done by drying the samples at 105 °C. As heating influences the RS content in foods, results may be modified by this step. A modified method for measuring RS in dietary fiber residues from various sources developed by Saura-Calixto and others (47) involves mixing fiber residues with KOH, acetate buffer, and HCl. After incubation with amyloglucosidase samples are centrifuged and diluted with distilled water. RS is calculated as glucose (mg) × 0.9. Advantages of the method are the use of small amount of sample, less reagents and elimination of drying.

Table 2 Resistant starch contents of a number of sample foods and commercially manufactured sources of resistant starch

Food sample	RS content (as assessed using AOAC 2002.02)
Wheat bran	0.42
Rye crispbread	1.20
Kidney beans	5.30
Corn flakes	2.80
Native potato starch	78.10
Cooked and cooled potato starch	3.80
HYLON VII	53.70
Hi-maize 1043	45.70
NOVELOSE 240	46.90
ActiStar	58.00
CrystaLean	40.90

Source: Nugent 2005 (24)

***In vivo* methods**

Different methods are used to analyze RS in vivo. One of the ways to assay RS physiologically is to determine starch in the undigested ileal content. Terminal ileal samples can be recovered by intubation or from ileostomy bags. The classic way to substantiate starch digestion is by measuring the glycemic index as described by Jenkins and others (48). This implies measuring the area under the curve (AUC) of the serum glucose concentration over the first 2 h after administering a starch and dividing this by the serum glucose response after consumption of an equal amount of glucose. Determination of breath hydrogen (breath tests) can also be used as a semiquantitative measurement for RS. In a study on effect of RS on human colon, increased fermentation was verified by elevated breath hydrogen excretion (49). From the different animal models, the antibiotic-treated rat model is the one commonly used (50).

3.6 Digestibility, energy value, and RDA of RS

RS is highly resistant to mammalian enzymes. In cereal products, the RS fraction is not digestible both in vitro and in vivo (51). Four different RS fractions have been identified in cereal products: native starch, retrograded amylose, the amylo-lipid complex, and encapsulated gelatinized starch. After reaching the large intestine, the RS fractions are fermented by the colonic flora, resulting in short-chain fatty acids (SCFA). SCFA profiles derived from RS are lower in acetate and higher in butyrate than those of conventional fibers. The SCFA are an energy source for colonic cells (butyrate) and to the body as a whole (acetate and propionate). Maize starch acylated with acetic, propionic, or butyric anhydride are also RS and raise large bowel SCFA, apparently through bacterial release of the esterified fatty acid and fermentation of the residual starch (52). Some sources of RS seem to be less available for fermentation, as has been observed in some chemically modified starches as well as RS in arepa (high corn meal bread). Feeding trials on Himalaya 292, a hulless barley cultivar with a higher RS content, also have resulted in high levels of SCFA in feces (53). Most studies indicate that 30% to 70% of RS is metabolized (23, 54-57), while the balance is excreted in the feces. The variability is largely due to effects caused by the malabsorption of the ingested starch. In human subjects, replacement of 27 g of digestible starch by RS (raw potato starch) in a single meal lowered diet-induced thermogenesis by an average of 90 KJ/5 h (58). A study was designed to compare the metabolizable energy of 2 starch sources, standard cornstarch and high amylase cornstarch (56). Based on energy intake and fecal excretion from all subjects, the partial digestible energy value for the RS averaged 11.7 KJ/g RS, which was 67.3% of the energy of standard cornstarch. Control and hyperinsulinemic subjects differed in their ability to digest RS, averaging 81.8% and 53.2%, respectively. RS averaged 2.8 kcal/g for all subjects but only 2.2 kcal/g in the hyperinsulinemic subjects. This enables the use of RS in reducing the energy value of foods. Approximately 20 g/d is recommended to obtain the beneficial health benefits of RS.

However, worldwide, dietary intakes of RS are believed to vary considerably. It is estimated that intakes of RS in developing countries with high starch consumption rates range from approximately 30 to 40 g/d (59). Dietary intakes in India and China were recently estimated at 10 and 18 g/d (60, 61). Intakes in the EU are believed to lie between 3 and 6 g/d. Dietary intakes of RS in the U.K. are estimated at 2.76 g/d and are believed to range from 5 to 7 g/d in Australia (59). In Sweden, the daily RS intake is estimated to be 3.2 g (62). In New Zealand, RS intakes have been approximated to be 8.5 g/d and 5.2 g/d in 15- to 18-year-old males and females, respectively (63).

3.7 Physiological effects of resistant starch

A number of physiological effects have been ascribed to RS and are listed in **Table 3** and will be described below. RS, by escaping digestion in the small intestine, has few interactions with other components of the upper gastrointestinal tract. It is fermented in the large intestine resulting in the production of such fermentation products as carbon dioxide, methane, hydrogen, organic acids (*e.g.* lactic acid) and SCFA. However, RS is believed to result in only a modest production of these gases compared with other non-digestible oligosaccharides, fructo-oligosaccharides and lactulose (64). SCFA produced include butyrate, acetate and propionate, and it is thought that these SCFA in particular mediate the effects of RS, rather than RS exerting a physical bulking effect (65).

Table 3 Physiological effects of resistant starch

Potential physiological effects	Conditions where there may be a protective effect
- Improve glycemic and insulinemic responses	- Diabetes, impaired glucose and insulin responses, the metabolic syndrome
- Improved bowel health	- Colorectal cancer, ulcerative colitis, inflammatory bowel
- Improved blood lipid profile	- Cardiovascular disease, lipid metabolism, the metabolic syndrome disease, diverticulitis, constipation
- Prebiotic and culture protagonist	- Colonic health
- Increased satiety and reduced energy intake	- Obesity
- Increased micronutrient absorption	- Enhanced mineral absorption, osteoporosis
- Adjunct to oral rehydration therapies	- Treatment of cholera, chronic diarrhea
- Synergistic interactions with other dietary components, *e.g.* dietary fibers, proteins, lipids	- Improved metabolic control and enhanced bowel health
- Thermogenesis	- Obesity, diabetes

Source: Nugent 2005 (24)

3.7.1 The effects of resistant starch on short chain fatty acid production

Resistant starch can increase the production of SCFA and therefore may help improve colonic health. Animal studies in pigs and rats have reported that feeding

RS increased the caecal and fecal production of total SCFA and also the individual concentrations of propionate, butyrate and acetate (66, 67). In most human studies, increased fecal excretion and/or fecal concentrations of SCFA were reported following supplementation with RS (57, 68-71). However, discrepancies have been observed with respect to effects on the individual SCFA and indeed no effect was observed in the study of Hylla *et al*. (49). These differences are most likely due to the experimental method used, the source, type and amount of RS, interindividual variations in length of transit time and on the duration of feeding. In particular, RS2 (from raw potato starch) is reported to increase the concentration of butyrate in humans and rats (57, 66, 67, 72), while RS3 (retrograded starch) is reported to increase the concentration of acetate in pigs (72), but not in humans (57). It has also been reported that sufficient time for microbial adaptation is necessary before changes in SCFA will be observed (33).

3.7.2 Experimental measures used in studies of resistant starch and colonic function

In the interim, a large number of animal and human studies have attempted to investigate the effects of RS on colonic function. In general, these studies have tended to look at two main areas: outcomes of colorectal neoplasia and markers of colonic function and colorectal cancer (36). Commonly measured outcomes of colorectal neoplasia include:

- tumor formation;
- tumor size and incidence;
- cell proliferation;
- formation of DNA adducts;
- the presence of aberrant crypt foci;
- apoptosis.

Aberrant crypt foci (ACF) are precursor lesions of colorectal cancer, which can be identified under a microscope and have been found to correlate with colon cancer risk, and adenoma size and number in humans. DNA adducts are complexes formed from the reaction of toxic chemicals or their metabolites with cellular DNA. The presence of DNA adducts reflects exposure to toxic chemicals and their bioaccumulation throughout life: in the colonic mucosa increased levels are thought to result in increased cancer risk (73). However, concerns have been raised concerning the sensitivity and specificity of the analytical techniques for detecting these DNA adducts. Maintenance of epithelial mass is important for regulation of normal colonic function and hyperproliferation (cell overgrowth) may result in an increased risk of colon cancer development. Epithelial cell proliferative activity is thought to be an intermediate risk marker for colorectal cancer (74) but its exact usefulness as a marker of colonic cell function is unclear and results are often difficult to interpret.

Other measured markers of colorectal cancer and colonic function are:

- SCFA production (particularly butyrate);
- fecal pH;
- ammonia and phenol concentrations;
- fecal weight and output;
- secondary bile acid excretion;
- cytotoxicity of fecal water;
- transit time;
- activity of bacterial enzymes and microbial populations.

In general, improved colonic function is associated with increased SCFA production, lower pH, lower production of ammonia and phenol, decreased secondary bile acid excretion, reduced cytotoxicity of fecal water, reduced transit time and altered bacterial activity. The benefits imparted by SCFA have already been discussed. A lower pH is thought to depress the conversion rate of primary to secondary bile acids and lower their carcinogenic potential. Furthermore, a low (acid) pH in combination with high concentrations of SCFA is thought to prevent the overgrowth of pH-sensitive pathogenic bacteria (33). Phenol and ammonia are products of protein fermentation and reduced concentrations indicate a decreased reliance on protein for colonic fermentation and possibly a shortened transit time (73). Reduced activity of certain bacterial enzymes (*e.g.* b-glucuronidase) depresses the formation of toxic and carcinogenic metabolites from dietary and endogenous compounds (73). The effect of RS on the activity of microbial populations will be discussed later (prebiotics).

3.7.3 Animal studies of resistant starch and colonic function

Studies examining the effect of RS on colonic function and colon cancer development in animals have generally focused on pigs, mice and rats, and have used experimentally induced colon cancer (usually using dimethylhydrazine, azoxymethane) or colitis (using dextran sodium sulphate) or genetic models of intestinal tumors (*e.g. Min* mice). As presented in **Table 4**, animal studies would suggest that RS appears to have a protective effect on markers of colonic function (*e.g.* SCFA concentrations, pH, etc.)(66, 75-82). Results are less clear with respect to tumor formation, size, cellular proliferation and DNA damage (78, 83-86). Differences in results may be in part due to the animal models and types of carcinogens used, the different types of RS (RS used was mainly RS2 or RS3) or even the different feeding regimens. Further research is needed using different types and mixes of RS, and examining any potential interactions between RS and other macronutrients commonly found in the diet (*e.g.* protein and fat). Interestingly it would appear that feeding RS at high levels, in combination with other dietary macronutrients may also directly affect outcomes (87, 88)

3.7.4 Human studies of resistant starch and colonic function

A limited number of studies have investigated the effects of different types of RS and colonic function in humans; summaries of the major studies are presented in **Table 5**. Positive effects of supplementation with RS have been observed in most studies examining transit time (49, 71) and fecal output and/or bulk (49, 57, 71, 75, 89-91). In addition, most authors reported a stool-softening effect. A limited number of studies have examined the effect of RS on cellular proliferation and DNA damage. Only Van Munster *et al*. (89) reported a small decrease in cellular proliferation, while three other studies have showed no effect (74, 92, 93). Clearly, there still remains a need for further research into the effects of RS on human colonic function and markers of colon cancer risk. It is difficult to explain the discrepancies between the studies; however, there were large variations in study sample size, duration, dose of RS and even form of RS.

In conclusion, it would appear that RS can improve certain markers of colonic function in humans (*e.g.* increase fecal output, fecal bulk and transit time, decrease pH and ammonia levels, increase SCFA and decrease bile salts in fecal water) (57, 68, 71, 74, 89, 91, 92, 94, 96).More research is needed to elucidate the exact effects of RS on cellular and molecular functions before a direct protective effect can be determined.

Table 4 Animal intervention studies examining the effects of resistant starch on colonic function

Author	**Animal model**	**Intervention**	**Parameters measured = Outcome**
Thorup *et al*. (1995)	Wistar rats (azoxymethane)	Carbohydrate content of diet replaced by: sucrose, cornstarch or RPS (RS2; 67 g/100 g)	ACF = ↓ RPS total and larger ACF
Caderni *et al*. (1996)	Sprague Dawley rats (Dimethylhydrazine)	Sucrose, glucose, fructose, cornstarch or HYLON VII (RS2)	Cell proliferation = NSD Caecal pH = ↓ Caecal concentrations SCFA = ↓
Sakamoto *et al*. (1996)	Sprague Dawley rats (Dimethylhydrazine)	3 or 10 g/100 g cellulose or 3 or 10 g/100 g RS3 (high amylose maize starch hydrolyzed with pancreatin)	Tumor incidence = NSD SCFA and butyrate production = ↑ Fecal output = ↑
Young *et al*. (1996)	Sprague Dawley rats (Dimethylhydrazine)	Low RS, low fiber diet or 14.4 g/100 g diet RPS (RS2) or 14.4 g/100 g RPS and 14.4 g/100 g wheat bran	Tumor incidence = NSD Tumor size and multiplicity = ↑ ACF = ↑ density Cell proliferation = ↑ Fecal output = ↑
Pierre *et al*. (1997)	C57BL/6 J min mice	RS-free diet (2% cellulose, no RS) or Wheat bran (18.8 g/100 g) or RS3 (high amylose cornstarch; 18.8 g/100 g)	Tumor incidence = NSD
Maziere *et al*. (1998)	Sprague Dawley rats (Dimethylhydrazine)	RS-free diet (2% cellulose) or 25 g/100 g RS3 (high-amylose maize starch)	ACF = ↓ Caecal pH = ↓ Fecal weight and output = ↑ Bacterial enzyme activity = ↑ β-glucuronidase activity
Cassand *et al*. (1997)	Sprague Dawley rats	Retrograded high-amylose cornstarch (RS3)	ACF = ↓ Fecal output = ↑ Fecal pH = ↓ SCFA = ↑ total and butyrate

Table 4 Animal intervention studies examining the effects of resistant starch on colonic function (Continued)

Kleeson *et al*. (1997)	Wistar rats	RPS, or retrograded potato starch (RS2) 10 g/100 g	SCFA = ↑ = ↑ butyrate, RS2
Eibhara *et al*. (1998)	Wistar rats	Potato starch or CMS	Fecal output = ↑ Caecal SCFA = ↓butyrate with CMS Caecal bile acids = ↑with CMS
Silvi *et al*. (1999)	Fisher rats	RS- and cellulose-free diet (2.1%) or Retrograded amylose starch (15 g/100 g	Caecal SCFA = ↑ butyrate Bacterial enzyme activity =↓ β-glucuronidase activity Ammonia production = Cell proliferation = NSD↓
Williamson *et al*. (1999)	*Min* mouse	RS- and NSP-free diet or 1:1 RPS (RS2) and high-amylose maize diet (RS3)	Tumor incidence = ↑with RS diet
Bird *et al*. (2000b)	Pigs	Brown rice or white rice and bran	SCFA excretion = ↑ Large bowel digesta mass = ↑
Ferguson *et al*. (2000)	Wistar rats	RS- and NSP-free diet or Potato starch or High amylose maize starch or a-amylase treated Hi-maize (35 g/100 g)	Fecal output = ↑ SCFA = ↑ including butyrate Transit time =↑ by potato starch and α -amylase treated Hi-maize
Wang *et al*. (2002)	Balb/C mice	Amylomaize starch Modified amylomaize starch (40 g/100 g diet)	SCFA = ↑butyrate in feces
Ferguson *et al*. (2003)	Wistar rats	RS- and NSP-free diet Potato starch and high amylose maize starch (35g/100g)	Fecal output = ↑ Excretion of the food carcinogen, IQ=↑carcinogen bioavailability
Le Leu *et al*. (2003)	Sprague Dawley rats	High amylose maize starch	pH =↓

Table 4 Animal intervention studies examining the effects of resistant starch on colonic function (Continued)

Conlon & Bird (2003)	Sprague Dawley rats	10 g/100 g fish oil or sunflower oil And 10 g/100 g dietary fiber (wheat bran or cellulose) Or 10 g/100 g RS (Hi-maize or NOVELOSE)	Colonic DNA damage = ↑ DNA damage with RS and fish oil vs. RS and sunflower oil Reverse with dietary fiber.
Toden *et al*. (2003)	Sprague Dawley rats	15 or 25g/100g casein with or without 48% Hi-maize	DNA damage = ↓ with RS diet Thinning of mucosal layer = ↓ with RS diet
Kestell *et al*. (2004)	Wistar rats	RS- and fiber-free diet Potato starch Hi-maize Apple pectin Wheat straw	Metabolism and disposal of the food carcinogen, IQ = RS ↑ number of intact IQ and ↓ level of metabolites

ACF, aberrant crypt foci; CMS, chemically modified starch; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; NSD, non-significant difference; RS, resistant starch; RPS, raw potato starch; SCFA; short chain fatty acid.
Source: Nugent 2005 (24)

Table 5 Human intervention studies examining the effects of resistant starch on colonic function

Author	Sample size and study length	Intervention	Parameters measured = Outcome
Tomlin & Read (1990)	8 subjects	6 large bowls Cornflakes (10.33 g RS) or 6 large bowls Rice Krispies (0.86 g RS)	Breath hydrogen = ↑
Van Munster *et al*.(1994)	14 healthy subjects fed the diets for 3 weeks	45 g HYLON VII (32%) RS or low RS, 20 g natural fiber	Cell proliferation = ↓ Fecal output = ↑ pH = NSD Fecal SCFA = ↑total SCFA and butyrate Breath hydrogen = ↑ Bile acid excretion = ↓secondary bile acids and concentrations of soluble bile acids Cytotoxicity = ↓
Phillips *et al*. (1995)	11 healthy subjects in a crossover study for 3 weeks	High RS (Hi-maize or cooked or uncooked green banana flour; 26–50 g RS/day) or low RS diet (3–8 g RS/day)	Fecal output = ↑ Fecal pH = ↓ SCFA = ↑ butyrate and acetate Excretion of starch = ↑ pH = ↓ by 0.6 units
Birkett *et al*. (1996)	11 subjects in randomized controlled cross-over for 3 weeks	High RS (39 g/day, RS1, RS2, RS3 mix) or low RS (5 g/day)	Fecal nitrogen excretion = ↑ Fecal ammonia = ↓ Fecal phenols = ↓ Fecal pH = ↓
Cummings *et al*. (1996)	12 healthy subjects fed each diet for 15-day periods	RS2 – potato and banana starch RS3 – maize and wheat starch RS-free diet (wheat starch) RS diets contained 17–30 g RS	Fecal weight = ↑ SCFA = ↑ NSP breakdown = ↓ breakdown and ↑ fecal NSP

Table 5 Human intervention studies examining the effects of resistant starch on colonic function (Continued)

Noakes *et al*. (1996)	23 hypertriglycerimd emics for 4 weeks	High amylose maize starch (17–25 g RS/day) or Oat bran	Bile acids = ↓ secondary bile acids in fecal water pH = ↓ SCFA= ↑ fecal total SCFA and fecal butyrate
Heijnen *et al*. (1998)	24 healthy volunteers fed each diet for 1-week periods	Uncooked HYLON VII (32 g RS2/day) Retrograded high amylose cornstarch (32 g RS2/day) Glucose syrup	Fecal output = ↑ pH = NSD SCFA= NSD Bile acids = NSD Cytotoxicity = NSD
Hylla *et al*. (1998)	12 healthy volunteers for 4 weeks	High RS – high amylose maize (55.2 g RS/day) Low RS – corn starch (7.7 g RS/day)	Fecal weight = ↑ SCFA = NSD Bile acids = ↓ total and secondary concentrations Transit time =↓ Bacterial enzymes = ↓ β- glucosidase activity Sterols = ↑ fecal total sterols
Jenkins *et al*. (1998)	24 healthy subjects fed each diet for 2 weeks with a 2-week washout period	RS2 (21.5 g RS/day) RS3 (27.9 g RS/day) Wheat bran (1.5 g RS/day) Low fiber diet (2.3 g RS/day)	Fecal bulk = ↑ SCFA = ↑ butyrate SCFA ratio
Grubben *et al*. (2001)	23 patients with recently removed colonic adenomas for 4 weeks	45 g amylomaize (28 g RS/day as a capsule) or 45 g maltodextrin	Cell proliferation = NSD Fecal weight = NSD pH = NSD SCFA excretion =↑ fecal butyrate Bile acids = ↑ primary and secondary bile acids in fecal water

Table 5 Human intervention studies examining the effects of resistant starch on colonic function (Continued)

Van Gorkom *et al*. (2002)	111 sporadic adenoma patients	High RS: 30 g HYLON VII (19 g RS) or Controlled placebo	Cell proliferation = NSD
Wacker *et al*. (2002)	12 healthy subjects for 4 weeks (only 8 volunteers for DNA data)	High RS: HYLON VII (50.7–59.7 g/day) or Low RS: cornstarch	Cell proliferation = NSD DNA adducts = ↑ adduct levels in colonic mucosa
Muir *et al*. (2004)	20 volunteers for 3 weeks	Wheat bran (12 g fiber) RS and Wheat bran (22 g RS and 12 g fiber/day)	Fecal output = ↑ Transit time = ↓ Fecal pH = ↓ SCFA = ↑ fecal concentration Phenol = ↓ Ammonia = ↓

NSD, non-significant difference; RS, resistant starch; SCFA, short chain fatty acid.
Source: Nugent 2005 (24)

3.7.5 Resistant starch and inflammatory bowel disease, diverticulitis and constipation

A limited number of studies have examined the potential benefits of RS in ameliorating the symptoms of inflammatory bowel diseases such as ulcerative colitis. SCFA enemas can be used to treat ulcerative colitis in human patients therefore in principle, if RS increases SCFA production it may prove a useful adjunct to traditional treatment regimens. Based on this hypothesis, RS has been studied (and is sometimes used) as a treatment for ulcerative colitis. This relies on the *in vivo* generation of SCFA and butyrate to treat the ulcerations. In the study of Jacobasach *et al*. (97), RS-fed rats showed earlier improvements in histological markers of inflammation and normalization of cell functions such as activation of colonic cell proliferation, restoration of apoptotic responses and uptake of SCFA. In addition, RS also enhanced the growth of intestinal bacteria presumed to promote health (97). Similarity in the study of Moreau *et al*. (98), RS-rich diet improved caecal and distal macroscopic and histological observations and increased caecal levels of butyrate compared with a fructo-oligosaccharide-rich diet and the control diet (RS and fructo-oligosaccharide-free) (98). Therefore, it would appear that RS can confer some healing properties in the management of inflammatory bowel disease, at least in rats, however, data in humans are lacking. There is a lack of studies testing the potential benefits of RS in the management of diverticulitis and constipation; however, due to the beneficial effects of RS supplementation on stool bulk, stool consistency and transit time for example, it is possible that increasing dietary intakes of RS may help ameliorate these conditions. Indeed a number of over the counter products for bowel health are now available. It is possible that by combining RS with other forms of dietary fiber, it may have more favorable effects on bowel health than consuming RS or dietary fiber alone. A recent study by Muir *et al*. (71) suggests that the health benefits of RS can be maximized when given in conjunction with different types of dietary fibers.

3.7.6 Resistant starch and colonic microflora: prebiotics and probiotics

RS appears to function as a prebiotic and symbiotic (99, 100). Studies in humans and pigs have revealed that consumption of high-RS diets result in a time-dependent shift in fecal and large-bowel SCFA profiles, suggesting a change in the autochthonous (local) microbial population and that RS could interact with gut bacteria (65, 101). It is thought that RS may act as a feeding substrate for Bifidobacteria *in vitro* (100) and that it may provide protection to these bacteria *in vivo* as they travel through the upper gastrointestinal tract (100-103). However, there is a lack of data relating to the efficacies of the individual types of RS. There are shortcomings with using probiotics to promote gut health: only a small proportion of ingested organisms reach the colon intact and once probiotic consumption decreases the organisms are washed out of the gastrointestinal tract (65). RS may safeguard against these losses by providing physical protection and by slowing the rate at which the bacteria are lost once probiotic consumption ceases (101). However, more research in humans is needed, particularly with respect to the doses of RS needed and the differences in efficacy between the different types of RS. In addition to these prebiotic effects, RS also appears to exert other health-promoting actions on gut health. RS (high amylose starch) supplementation, in association with oral hydration therapy, is reported to reduce fluid loss and halve recovery time when fed to people with cholera-induced diarrhea (104-106). It is thought that RS may confer these benefits through increased fluid absorption as a result of greater SCFA production (65). SCFA stimulate water and cation (sodium, potassium, calcium) uptake in the proximal colon and, through their action on muscular activity and blood flow in the colon, may directly reduce the severity of diarrhea. However, more research is needed to clarify the exact role of RS in the treatment of diarrhea and its mechanisms of action. Furthermore, the efficacy of the various types of RS needs to be established.

3.7.7 Resistant starch and metabolic responses

Consumption of soluble fiber can confer benefits to heart health, influencing both lipid and glucose metabolism. RS shares some common properties with soluble dietary fibers insofar as it is poorly digested in the small intestine and largely digested and metabolized (fermented) in the colon releasing SCFA. However, unlike soluble fiber, the fraction of RS arriving at the colon is not viscous, it can easily be incorporated into most starchy foods in the diet and is considered more palatable (107). A significant number of studies have examined whether RS affects lipid and glucose metabolism (including glycemic index), energy expenditure and macronutrient oxidation.

3.7.8 Resistant starch and lipid metabolism

As is evident in **Table 6**, RS appears to particularly affect lipid metabolism based on studies in rats where reductions in a number of measures of lipid metabolism have been observed. These include total lipids, total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), triglycerides, triglyceride-rich lipoproteins. In these studies, reductions of up to 22–32% in plasma cholesterol levels and 29–42% in plasma triglyceride levels were noted. In the study of Younes *et al*. (108), RS was more effective than the drug cholestyramine (a bile sequestrant) in lowering plasma cholesterol and triglyceride levels. RS has also been shown to be effective in lowering plasma cholesterol levels in genetically obese and lean rats (109) and in diabetic rats (110). Some earlier studies in humans reported a beneficial effect of feeding RS on fasting plasma triglyceride and cholesterol levels (94, 111, 112), however, it would appear that RS does not affect total lipids (55, 91, 94, 113), triglycerides (91, 94, 113-116); HDL or LDL (91, 94, 113) or VLDL levels in humans (55, 94). Therefore, on balance RS does not appear to influence these markers of lipid metabolism in humans.

3.7.9 Resistant starch and insulin and glucose metabolism

RS-rich foods release glucose slowly and therefore one would expect this to result in a lowered insulin response, greater access to and use of stored fat and, potentially, a muted generation of hunger signals. Not only would these conditions help in the management of clinical conditions, such as diabetes and impaired glucose tolerance, but also possibly in the treatment of obesity and in weight management. There have been a number of studies examining the effects of various forms and doses of RS on glucose (glycemic) and insulin (insulinemic) responses. Most studies in humans have focused on postprandial glycemic and/or insulinemic responses and have varied in quality (see below). There is a lack of consensus regarding the precise effects of RS on insulin and glucose responses: 15 studies have reported an improvement in these measures following the consumption of a RS-rich test-meal (115, 117-129), while 10 have showed no, or a physiologically irrelevant effect (58, 91, 94, 112, 114, 130-133). It is noteworthy that, to date, there are no reports of RS worsening insulin and glucose responses.

Table 6 Summary of the effects of resistant starch (RS) on markers of lipid metabolism in animals (A) and humans (H)*

Parameter	RS exerted a positive effect	RS had no effect
Triglycerides	De Deckere *et al*.(1993,1995)(A) Verbeek *et al*. (1995) (A) Younes *et al*. (1995) (A) Cheng & Lai (2000) (A) Lopez *et al*. (2001) (A) Kishida *et al*. (2001) (A) Han *et al*. (2003a, 2003b) (A) Behall *et al*. (1989) (H) Reiser *et al*. (1989) (H) Noakes *et al*. (1996) (H)	Van Ameslvoort&Westrate(1992)(H) Kim *et al*. (2003) (A) Raben *et al*. (1994) (H) Behall & Howe (1995) (H) Heijnen *et al*. (1996) (H) Raben *et al*. (1997) (H) Jenkins *et al*. (1998) (H)
Total cholesterol (total lipids)	Mathe *et al*. (1993) (A) De Deckere *et al*. (1993) (A) Verbeek *et al*. (1995) (A) Younes *et al*. (1995) (A) Cheng & Lai (2000) (A) Kim *et al*. (2003) (A) Kishida *et al*. (2001) (A) Lopez *et al*. (2001) (A) Kim *et al*. (2003) (A) Han *et al*. (2003a, 2003b) (A) Reiser *et al*. (1989) (H) Behall *et al*. (1989) (H)	De Deckere *et al*. (1995) (A) Kishida *et al*. (2001) (A) Behall & Howe (1995) (H) Noakes *et al*. (1996) (H) Heijnen *et al*. (1996) (H) Jenkins *et al*. (1998) (H)
High density lipoproteins	Han *et al*. (2003a, 2003b) (A) Younes *et al*. (1995) (A) Kishida *et al*. (2001) (A) Noakes *et al*. (1996) (H) Heijnen *et al*. (1996) (H) Jenkins *et al*. (1998) (H)	Cheng & Lai (2000) (A) Lopez *et al*. (2001) (A) Kim *et al*. (2003) (A)
Low density lipoproteins	Han *et al*. (2003a, 2003b) (A) Younes *et al*. (1995) (A) Kishida *et al*. (2001) (A)	
Intermediate density and/or very low density lipoproteins	Han *et al*. (2003a, 2003b) (A)	
Triglyceride-rich lipoproteins	Kishida *et al*. (2001) (A) Younes *et al*. (1995) (A) Lopez *et al*. (2001) (A)	

*A positive effect refers to an increase in the concentrations of high density lipoproteins but a decrease in the concentrations of all other parameters.
Source: Nugent 2005 (24)

In general, positive effects were usually observed shortly (*i.e.* within the first 2–8 h) after the high RS-meal (134). It would also appear that RS consumption may confer a small decrease in postprandial glycemia, but is associated with more physiologically significant reductions in postprandial insulinemia. From these studies it was concluded that RS must contribute at least 14% of total starch intake in order to confer any benefits to glycemic or insulinemic responses (127, 134, 135). **Table7** lists the studies analyzed. Difficulties arise when trying to compare these studies as the composition of the test and control meals often vary in terms of amount of digestible starch, total dietary fiber and macronutrients present. Most food sources contain digestible starch as well as RS, yet often the content of digestible starch is overlooked (136). Problems also arise when studies fail to match the test meals for total dietary fiber content, fat content of a meal, physico-chemical properties of foods (can directly affect the amount of RS present), the effects of eating diets rich in RS on long-term glucose responses and insulin sensitivity etc (14, 114, 137-139). There is also a lack of information available regarding the influence of chemically modified RS on insulin and glucose metabolism (116).

3.7.10 Resistant starch and macronutrient oxidation, satiety and weight loss

A number of authors have examined the potential of RS to alter macronutrient and in particular fat oxidation. It is proposed that eating a diet rich in RS may potentially increase the mobilization and use of fat stores as a direct result of any reduction in insulin secretion (140). Experimentally, this is indicated by a reduced respiratory quotient (RQ). RQ is a relative measure of oxygen uptake and is indicative of the use of fat/carbohydrate as fuel whereby a high RQ is reflective of high carbohydrate oxidation. Studies to date in humans would indicate that diets rich in RS do not affect total energy expenditure, carbohydrate oxidation or fat oxidation (116, 132, 141, 142). Although in the study of Tagliabue *et al.* (141) the authors found that a RS-rich meal resulted in a short-term reduction in glucose oxidation and diet-induced thermogenesis and an increase in fat oxidation, these effects were lost after adjusting for total carbohydrate intake. Animal studies indicate that feeding high doses of RS may decrease adipocyte cell size (143-146) and lower fat pad weight (143). RS was also shown to reduce the activity of lipogenic enzymes such as fatty acid synthase (the rate limiting enzyme in fat synthesis) (108), and the expression of the protein responsible for insulin stimulated glucose uptake (GLUT-4) in these animals (144). As hypothesized by Higgins (146) this may imply that (at least in rats) a high-RS diet may reduce the initial increase in plasma glucose and nonesterified fatty acids levels, naturally observed after eating a food, and as a result attenuate glucose uptake and lipogenesis in the adipocytes.

Table 7 Summary of studies examining the effects of resistant starch (RS) on glucose and insulin responses in humans

Resistant starch decreased		Resistant starch had no effect on	
Glucose responses	Insulin responses	Glucose responses	Insulin responses
Krezowski *et al*. (1987)*‡	NSD	Goddard *et al*. (1984)*†	Goddard *et al*. (1984)*†
NSD	Behall *et al*. (1988)*	Reiser *et al*. (1989)*‡¶	Reiser *et al*. (1989)*‡¶
Holm & Bjorck (1992)*	Holm & Bjorck (1992)*	Van Amelsvoort& Westrate (1992)*	Van Amelsvoort& Westrate (1992)*
Liljeberg *et al*. (1994)*	Liljeberg *et al*. (1994)*	Westrate & van Amelsvoort (1993)*	Westrate & van Amelsvoort (1993)*
Raben *et al*. (1994)*	Raben *et al*. (1994)*	Ranganathan *et al*. (1994)*	Ranganathan *et al*. (1994)*
Byrnes *et al*. (1995)*	Byrnes *et al*. (1995)*	Heijnen *et al*. (1995)*	Heijnen *et al*. (1995)*
Granfeldt *et al*. (1995)*	Granfeldt *et al*. (1995)*	Noakes *et al*. (1996)*	Noakes *et al*. (1996)*
Lintas *et al*. (1995)†	—	Jenkins *et al*. (1998)*	Jenkins *et al*. (1998)*
—	De Roos *et al*. (1995)*§	Nestel *et al*. (2004)*	Nestel *et al*. (2004)*
Achour *et al*. (1997)*	Achour *et al*. (1997)*		
Raben *et al*. (1997)*	Raben *et al*. (1997)*		
Hoebler *et al*. (1999)*	Hoebler *et al*. (1999)*		
Vonk *et al*. (2000)*	—		
Skrabanja *et al*. (2001)*	Skrabanja *et al*. (2001)*		
Behall & Hallfrisch (2002)*‡	Behall & Hallfrisch (2002)*‡		
Anderson *et al*. (2002)*	—		
Robertson *et al*. (2003)*			

*Indicates a postprandial measurement.
†Small decreases in glucose and insulin were observed at early time-points, but overall there were no significant differences in glucose or insulin levels.
‡indicates that the study group were overweight (body mass index > 25), hyperinsulinemic or diabetic
§Insulin response was measured using a urinary markers of insulin secretion only. RS3 had an effect, whereas RS2 did not.
¶measured as glycemic index only.
— indicates parameter not measured or results not presented.
NSD; non-significant difference.
Source: Nugent 2005 (24)

In other words, RS may result in a smaller fat pad size and/or mass due to reduced glucose uptake and lipogenesis by the fat cells. However, no information exists regarding RS and adipocyte size and function in humans, and more animal studies are needed. Any food/food ingredient that can increase satiety may play a vital role in weight-loss diets. Some studies have examined the potential of RS as a satiety agent. These appear to show a weak or no association between RS and satiety over the course of several hours or an entire day. de Roos *et al*. (147) reported that long-term consumption of RS was more satiating than glucose, but the effect was small and did not affect daily caloric intake. Anderson *et al*. (128) reported that high-RS meals caused less satiety than low-RS meals at 1 hour post-ingestion, while in the study of Skrabanja *et al*. (126) human volunteers reported that breads rich in RS (sourced from buckwheat groats) imparted greater satiety than white bread, but only between 70 and 120 min post-meal. RS did not affect satiety in the studies of Holm & Bjorck (117), Westrate & van Amelsvoort (131) or Mèance *et al.* (148), but resulted in satiety in those of van Amelsvoort & Westrate (149), Raben *et al*. (115), Skrabanja *et al*. (126). It is noteworthy that these studies also showed a decrease in blood glucose levels following the consumption of a high-RS meal; therefore, it would appear that satiety is closely linked to blood glucose levels (134).

Future studies need to objectively measure satiety and account for changes in blood glucose levels using standardized test meals matched for macronutrients and fiber content but containing different levels of RS.

3.7.11 Other health benefits associated with resistant starch

Resistant starch is reported to enhance the ileal absorption of a number of minerals in rats and humans. Younes (108) reported an increased absorption of calcium, magnesium, zinc, iron and copper in rats fed RS-rich diets, in contrast Kishida (146) reported no effect. In humans, these effects appear to be limited to calcium (150, 151). RS may therefore improve the ileal absorption of a number of dietary minerals but any effect in humans is likely to be small.

More recently RS has been reported to influence immune function, particularly the production of a number of pro-inflammatory cytokines (*e.g.* tumor necrosis factor alpha) and the expression of a number of receptors on T- and B-lymphocytes and macrophages that are required for the initiation of immune responses [cluster of definition 3 (CD3), CD4, CD8, lymphocyte function associated antigen-1 (LFA-1), intercellular adhesion molecule-1 (ICAM-1), Mac-1] (152, 153). If RS can beneficially modulate immune function it could impart real benefits to patients with inflammatory bowel disease. Therefore, the immuno-modulatory potential of RS, particularly on gut-associated immune cells, warrants further research.

3.8 Labeling and legislation of fiber and resistant starch

At present, there is no legal definition for RS and with respect to labeling, RS falls under the remit of dietary fiber. Several countries including the USA, UK, Australia, Canada, Denmark, Finland, Italy, Sweden and Japan use the AOAC method 985.29 (154) for measuring and labeling the dietary fiber content of foods. As this method accounts for some of the RS present within foods, part of the published value for fiber will include RS, if present. The UK has traditionally used the Englyst method (27) for determining dietary fiber; this method does not measure RS present in foods. Although, the Food Standards Agency (UK) now recommends that industry use the AOAC method (which does measure some RS), problems arise as the UK government recommendations for population dietary fiber intakes are still based on the Englyst method. In addition, the existing UK food composition tables list dietary fiber values as measured by the Englyst method. These food composition tables are used by health professionals, some food manufacturers and catering outlets to determine the fiber content of foods and diets eaten. The agreed energy value for carbohydrates is 4 kcal /g. In Europe, the Nutrition Labeling Directive does not specify an energy value to be used for fiber, and the value is therefore considered to be zero (32). In Australia and Japan, dietary fibers have been assigned higher energy values (of 1.8 kcal/g and 2 kcal/g), respectively. In the USA, labeling of total dietary fiber is mandatory. The labeling of soluble and insoluble dietary fiber is optional and a labeling scheme has been defined whereby the energy value assigned to insoluble fiber is 0 kcal/ g and the energy value for soluble fiber is 4 kcal/ g.

Currently, non-modified resistant starches are considered safe under existing food classifications and legislations in the US and in Europe. In Europe and the EU, chemically modified starches are regulated as modified starches under the European Parliament and Council Directive 95/2/EC in Europe and under 21 CFR 172.892 in the

US. Canada has a distinct legislative process for all novel dietary fiber, however, as yet resistant starches have not been evaluated in Canada and cannot be claimed on the product label.

Globally, there is increasing interest amongst manufacturers of commercial sources of RS in labeling foods rich in RS. This is mainly due to the desirable properties associated with foods rich in RS: their potential health benefits and lowered food energy value in foods where RS replaces digestible starch. Both are of interest to food manufacturers who wish to include RS as an ingredient in low-energy and low-carbohydrate foodstuffs and slimming/'diet' products. The US allows manufacturers to label foods with terms such as 'resistant' or 'indigestible', but they must clearly label the legally approved name of the corresponding starches. The US also allows self-declared 'structure-function' claims without restriction (*e.g.* fiber maintains bowel regularity), but restrictions exist regarding 'health' claims linking food substances with diseases (*e.g.* antioxidants may reduce the risk of cancer).These claims must be supported by scientific factual evidence. In contrast in the EU, no distinction between structure-function and health claims is made, and under current regulations, no preventative, curative or disease treatment properties can be assigned to particular foods (32). Proposed EU legislation would allow nutrition and health claims, but only for certain categories. With respect to label claims on RS rich foods in Europe, much will depend on the final details of these new regulations.

For food labeling purposes, producers and regulators first need an agreed definition and method of analysis for both dietary fiber and RS. The use of different methods of analysis of dietary fiber makes comparisons of the fiber content of foodstuffs difficult between countries. With respect to RS, an agreed method is needed that is robust, reproducible, repeatable and simple to complete. Consensus on such an agreed method is likely to be hotly debate as the amount of RS in foods will continue to be affected by external influences such as degree of ripeness, transit time, extent of chewing (24).

3.9 Resistant starch & Food application

In 1998, Gomolmanee studied supplementary use of resistant starch (Novelose® 330, 30% total dietary fiber, RS3-type retrograded/non-granular by

National Starch & Chemical Co) in noodle products such as alkaline noodle, rice noodle and mungbean starch noodle in order to increase dietary fiber. Wheat flour noodles, rice noodles and mungbean noodles were substituted 15%, 15% and 20% respectively that were accepted by sensory test with no significant differences ($p > 0.05$) (155).

At the present, commercial resistant starch is available and is called Hi-maize® (60% total dietary fiber, RS2-type granule) which is developed by National Starch & Chemical Co to withstand not only attack, by human digestive enzymes but also food processing conditions (23, 24).

Hi-Maize® comes from a high amylose maize (80% amylose) developed and grown in Australia. Because this starch has a small and fine particle size, bland in flavor, white in color, and absorb less water than many other fibers, it can be formulated into many baked products without adversely affecting their organoleptic properties (taste, texture and appearance). Hi-maize® can be added to foods such as bakery products, bread, breakfast cereals, pasta and noodles, snack foods, soups, cereal drinks, yoghurt and certain dairy products (23, 24).

Positive results in applications suggest that a resistant starch can serve many purposes in development of healthy food products.

3.10 Noodle products

Definition

Noodles are one of the whole range of pasta products (e.g. spaghetti and macaroni) with the original dating back to the first B.C.(156) and are favored by Asian consumers for their simple preparation process, low cost and easy cooking (157). Noodle products are defined in Code of Federal Regulation (CFR) as “the products prepared by drying formed units of dough made from semolina, durum flour, farina flour, or any combination of two or more of these, with liquid eggs, frozen eggs, dried eggs, egg yolks, dried yolks, or any combination of two or more of these, with or without water, and with or without one or more of the optional ingredients specified in the CFR.”(158). Noodles can be categories by manufacturing process into 3 categories, fresh noodles, cooked noodles and dry noodles. According this study, fresh wheat noodles and instant noodles (deep-fried dry noodles) were investigated.

Fresh wheat noodles

Fresh noodle is the dough that passed the striped process but did not pass the preserved process, have a few days shelf-life.

Instant noodles

Instant noodles, mainly made from wheat flour or blend of rice and mungbean flours, are sold in single servings in pouches or cups, with a separate sachet containing the seasonings. Unlike traditional noodles which have to be boiled for 10-15 minutes to gelatinize the starch, instant noodles are prepared by adding boiling water. They are ready for consumption in 3-5 min. The steaming and frying process during instant noodle production gelatinize the starch and allow it to be consumed after rehydration (159-162).

3.11 Noodle ingredients and their function

- Flour and starch

Wheat flour is one of the main ingredients of noodles, and wheat quality greatly influences the final product. General flours used include dark northern spring (13% protein), hard red winter (11.5 to 13.3% protein) and Australian standard white (10% protein). For Thailand, all purpose wheat flour containing 10-11% protein is suitable for making noodle products. Noodles also contain starches that contribute to texture improvement. Potato, waxy corn, barley, rice and tapioca are often used. Modified starches are used in some applications because they are acid resistant, heat resistant, display less retrogradation, and exhibit good water-holding (160, 163).

a. Wheat flour

Wheat flour is a powdery substance produced by finely grinding wheat through a process called milling. It is used in many food products such as bakery and noodle products. Flour is composed of starch, protein, and small amounts of fat, sugar, and minerals. The protein in wheat flour is called gluten. It forms the framework of the baked products and makes the dough sticky. As the mixing of the dough proceeds, the gluten in the flour becomes elastic and pliable. When the dough is heated, the gluten coagulates, or become firm. It forms, along with the starch, the structure of the product. All flours do not have the same amount of gluten. Flour with very little gluten called soft flour is milled from soft white wheat. Soft flour is particularly good for making cookies and cakes. High protein contents lead to hardness of texture and coarseness of internal grain and surface appearance. On the other hand, if the flour is decreased too much, as when large amounts of enriching ingredients are added, the products will lack body and become too fragile (11, 162, 163).

b. Starch

Starch is the most important, abundant, digestible food polysaccharide. Common food starch are derived from seed and root. Starch have been modified to improve desired functional characteristics and are added in relatively small amounts to food as food additives. Starch is a homopolysaccharide composed only of glucose units and consists of mixture of two polymers, amylose (linearpolymer) and amylopectin (highly branched polymer). Starch granules are not water soluble but easily hydrate in an aqueous solution. When an aqueous suspension of granules is heated, additional swelling occurs until a temperature is reached where there is a transition from organization. This is known as the gelatinization temperature. Upon further heating, swelling continues and the amylose and portions of the amylopectin are leached from granule producing a viscous suspension. Cooling of this suspension leads to the formation of a gel. With further time, realignment of the linear chains of amylose and the short chains of amylopectin can occur in the process known as retrogradation. In food products based on starch gels, this can lead to liquid being expressed from the gel in the phenomenon known as syneresis, which is generally an undesirable occurrence (164, 165).

- Water

Water is essential in noodle making. Water is added to the wheat flour and kneaded to develop the gluten network, which contributes to the noodle structure. In addition, the water contributes to the viscoelastic properties of the dough and increases the smoothness of the noodle surface. When the dough becomes more cohesive after kneading, it also leads to the sheen and transparency of the noodles improve, the boiling time is decreased, and the size and shape of the noodles ate retained after boiling. If higher amounts of water are added, a very soft, uniform dough forms quickly. However, with less than 35% water, the dough exhibits resistance, requires more kneading, and takes muck longer to form (160, 163).

- Salt and Kunsui

a. Salt plays an important role in noodle making. It has direct effect on characteristic of gluten in dough by increasing dough strength and preventing dampness. It enhances flavor, improves texture and decreases the boiling time. In addition, salt also can preserve the noodles by inhibiting microbial growth. The salt is usually first dissolved in water and stored in a large tank in which the salt content is monitored.

b. Kunsui is an alkaline salt comprises potassium carbonate, sodium carbonate, and phosphates of sodium and potassium. It is diluted in hot water and added to the dough during kneading. It can be added directly into the dough at the levels of 0.5-1.7%. Kunsui interacts with the gluten, producing a gum-like texture that is typical of noodle texture. It also contributes to the development of the yellow color due to reaction of alkali with the flavanoid pigments and enhances the flavor (166).

- Egg

Egg increases the nutritional quality of noodle products and yellowness of noodles. Egg yolk color makes the color of noodle more attractive. It gives a firmer texture and also helps improving the texture, increases dough elasticity and extensibility (163, 166).

- Other ingredients

a. Gluten

The principal functional protein of wheat flour is gluten which consists of glutenins and gliadins. Generally, glutenins contribute to dough elasticity whereas gliadins contribute to dough viscosity. High molecular weight glutenin subunits join end-to-end through disulfide bonds to provide a sort of backbone to gluten complex. Low molecular weight glutenin subunits are also crosslinked through disulfide bonds into the protein network. The smaller spherical gliadin molecules are incorporated into gluten primarily through noncovalent (hydrogen and hydrophobic) bonds. When flour is hydrated and mixed to form dough, disulfide bonds may be rearranged as proteins align and as gluten forms. Gluten can be isolated from flour by making dough with water and then slowly manipulating this dough under a continuous stream of water (165, 167).

b. Sodium tripolyphosphate (STPP)

STPP inhibits enzyme protease in the flour. Enzyme protease affects elasticity of noodles making them too soft and fragile. Thus, STPP increases the strength and elasticity of dough (166)

c. Guar gum

Guar gum is a high molecular weight galactomannan derived from the seed of *Cyamopsis tetragonolobus*. It is a white to yellowish white, tasteless and odorless powder. It is desirable in either hot or cold water, forming a solution with a pH of between 5.4 and 7.0. Furthermore, it has a unique ability to hydrate rapidly in cold water to form very viscous colloidal dispersions. Guar gum is non-gelling and is used chiefly as a viscosity builder, stabilizer and water binder (14, 168).

3.12 Noodle production

Oriental style noodles are produced by three conventional steps including mixing, sheeting and cutting. The first product in line is fresh wheat noodles. Additional processing of fresh noodles yields other types of noodle such as dry noodles, fried noodles, instant dry noodles and instant fried noodles as outlined in **Figure 2**.

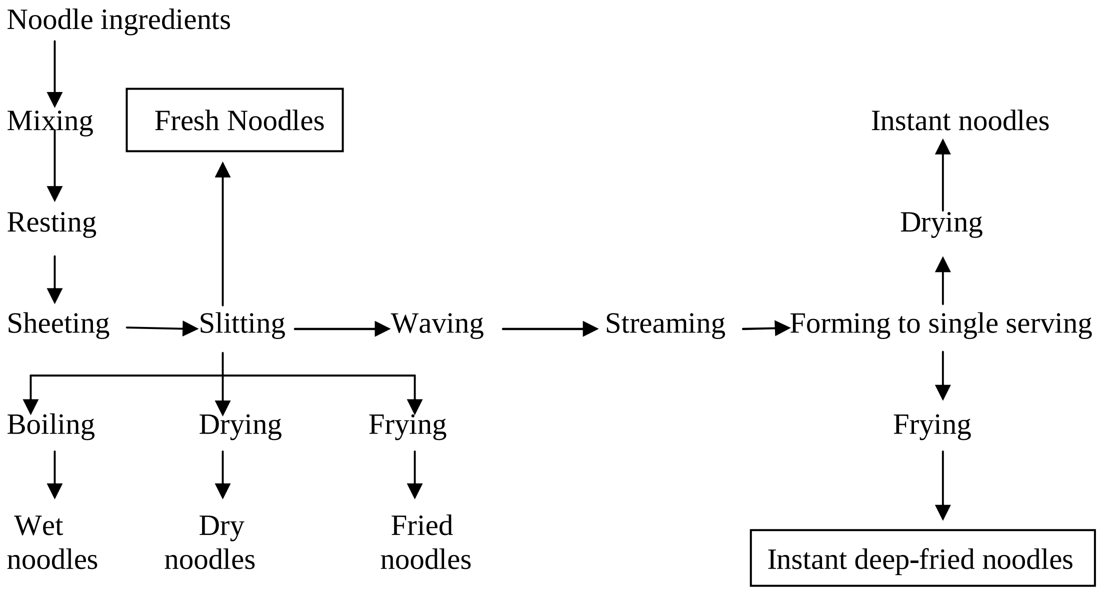

Figure 2 Processes for the production of different types of noodles

Source: Adapt from Oh NH. and others. (169)

3.12.1 Mixing ingredients

The first step in noodle manufacturing involves dissolving the salt or kansui in water and then this mixture is added to the flour. Mixing formula ingredients is often carried out in a horizontal or vertical mixer for 10-15 minutes. Since the horizontal mixer seems to have better mixing results, it is more commonly used than the vertical one in commercial noodle production. Mixing results in the formation of a crumbly dough with small and uniform particle sizes. Since the water addition level is relatively low (vs. bread dough), gluten development in noodle dough during mixing is minimized. This improves the dough sheet ability, sheeted dough smoothness and uniformity. Limited water absorption also slows down noodle discoloration and reduces the amount of water to be taken out during the final drying or frying processes. Flour proteins, pentosans and starch (especially damaged starch) determine the flour water absorption level. Even so, the water absorption level in noodle dough is not so sensitive to processing as is that in bread dough. Variation in noodle dough water absorption among different flours is generally within 2-3%, and this is usually determined by dough handling properties. Flour particle sizes and their distribution affect the time water penetrates into the flour. Large particle flours require a longer time for water to incorporate and tend to form larger dough lumps. It is desirable to have relatively fine and evenly distributed particle size flours to achieve optimum dough mixing (161, 166, 170).

3.12.2 Dough resting

After mixing, the dough pieces are rested for 20-40 minutes before compounding. Dough resting helps water penetrate into dough particles evenly, resulting in a smoother and less streaky dough after sheeting. In commercial production, the dough is rested in a receiving container while being stirred slowly (161, 166, 170).

3.12.3 Sheeting and Compounding

The rested, crumbly dough pieces are divided into two portions, each passing through a pair of sheeting rolls to form a noodle dough sheet. The two sheets are then combined (compounded) and passed through a second set of sheeting rolls to form a single sheet. The roll gap is adjusted so that the dough thickness reduction is between 20-40%. The sheet is repeatedly folded and passed through the rollers to facilitate gluten development, which gives the noodle its stringy and chewy texture. The combined dough sheet is often carried on a multi-layer conveyor belt located in a temperature and relative humidity controlled cabinet. This step is to relax the dough for easy reduction in the subsequent sheeting operation. The resting time takes about 30-40 minutes (161, 166, 170).

3.12.4 Sheeting, Slitting and Waving

Further dough sheeting is done on a series of 4-6 pairs of rolls with decreasing roll gaps. At this stage, roll diameter, sheeting speed and reduction ratio should be considered to obtain an optimum dough reduction. Noodle slitting is done by a cutting machine, which is equipped with a pair of calibration rolls, a slitter, and a cutter or a waver. The final dough sheet thickness is set on the calibration rolls according to noodle type and measured using a thickness dial gauge. Noodle width determines the size of noodle slitter to be used. The sheet is cut into noodle strands of desired width with a slitter. Noodles can be either square or round in shape by using various slitters. Noodle strands are cut into a desirable length by a cutter. At this stage, Chinese raw noodle, Japanese udon noodle, chuka-men and Thailand bamee noodle making is complete (fresh noodles). For making instant noodles, noodle strands are waved before steaming and cutting. The wavy noodles are produced by setting the conveyor belt at a slower pace than the cutting rolls above it. Alternatively, noodle strands emerging from the slitter are hindered by metal blocks (weights) resulting in the noodle waves (161, 166, 170).

3.12.5 Steaming

In making instant noodles, the liquid seasonings are sometimes added to the noodle strands prior to cutting and molding into blocks or another suitable shape. The wavy noodle-strands are conveyed to a steamer to cook the noodles at 100 ºC for 1–5 minutes. As mentioned earlier, the purpose of steaming is to gelatinize the starch, fix the noodle waves and improves the texture of the noodles. The steaming time varies according to noodle size, but can be determined by squeezing a noodle strand between two clear glass plates. If the white noodle core disappears, the noodles are well cooked. Steam temperature, steam pressure, and steaming time are key process factors affecting the product quality (161, 166, 170).

3.12.6 Drying noodles

The noodle drying can be achieved by deep frying in oil (fried instant noodles) and drying with hot air (non-fried instant noodles). Frying the noodles in oil at 140–160 ºC for 1–2 minutes decreases the moisture content of noodles from 30–50% at the steaming step to about 2–5%. While any edible oil is suitable for frying, palm oil or palm olein is often used in Asia and mixtures of canola, cottonseed, and palm oils are commonly used in North America. In hot air drying, the noodles are held at 70–90 ºC for 30–40 minutes to achieve 8–12% moisture content. The heating during frying or hot air drying further gelatinizes the starch and the noodles now obtain a porous texture. Frying is the preferred method of drying and more than 80% of instant noodles are fried because drying by frying is a very fast process. Water vaporizes quickly from the surface of the noodles upon dipping into the hot oil. Dehydration of the exterior surface drives water to migrate from the interior to the exterior of the noodle strands. Eventually, some of the water in the noodles is replaced by oil. Many tiny holes are created during the frying process due to the mass transfer, and they serve as channels for water to get in upon rehydration in hot water. It usually takes 3-4 minutes to cook or soak instant fried noodles in hot water before consumption. Air-dried instant noodles have a low fat content so some people prefer them. They also have a longer shelf-life because little fat rancidity is involved. Steaming appears to be very critical to this type of noodle since it affects the water rehydration rate of the product. While, the hot air drying can result in uneven drying that adversely affects the texture of the finished noodles, which slow output of the process and lack of pleasant shortening taste and mouth feel make the product less popular in Asia compared with instant fried noodles.

Non-fried instant noodles also require a longer cooking time. The disadvantage of frying, however, is that fried noodles contain about 15–20% oil (compared with a maximum of 3% fat in hot air–dried noodles) and are more susceptible to oxidation and spoilage; the use of antioxidants prolongs the shelf life of fried instant noodles. The dried noodles are then quickly cooled, checked for moisture, color, shape and other quality characteristics, and packaged with seasonings using films that are impermeable to water and air.

Instant noodles are commercially available in two packaged forms - in a cup with the seasoning sprinkled over the noodles or in a pouch (or bag) with the seasoning provided in a sachet inside the pouch. Two types of instant noodles are bag and cup or bowl types. Instant noodles come in several different flavors added to the seasoning – beef, chicken, pork, shrimp, oriental, creamy chicken, chicken mushroom, and others. In the cup style instant noodles, dehydrated vegetables and meats, as well as textured soy protein or flour are often added. Because of their low moisture content and a fairly high sodium content (about 2,100 mg per 100 g product), and the resulting low water activity, instant noodles are stable and have a shelf life of 4–6 months in tropical areas and 6–12 months in the northern hemisphere. They can be served after boiling in water for 1–2 minutes or soaking in hot water for 3–4 minutes (161, 166, 170).

CHAPTER IV
MATERIALS AND METHODS

4.1 Materials and instruments

4.1.1 Materials for fresh wheat noodles and instant noodles preparation

- All-purpose wheat flour, “Kite brand” (from United Flour Mill Public Co., Ltd.)
- Native potato starch, Modified potato starch (Perfectamyl AC.) (from Winner Group Enterprises Ltd.)
- Guar gum (from Thai Food and Chemical Co., Ltd)
- Sodium tripolyphosphate (STTP) and Sodium acid pyrophosphate (SAPP) (from Thai Food and Chemical Co., Ltd)
- Sodium bicarbonate (from Grand Chemical Co., Ltd)
- Potassium carbonate (from Grand Chemical Co., Ltd)
- Salt: Prungthip brand (from Saha Pathanapibul Public Co., Ltd)
- Vital wheat gluten 75% (from Nutrition Ltd., Part.)
- Palm oil; Emerald brand (from Morakot Industries Public Co., Ltd.)
- Seasoning powder: pork flavor (NST Food Ingredients Ltd and Thai President Foods Public Co., Ltd)
- Mono-sodium glutamate (MSG), “Ajinomoto brand” from Ajinomoto Co., (Thailand) Ltd.

4.1.2 Commercial Resistant Starch (RS)

Commercial resistant starch, Hi-maize® (**Figure 3**) was used as the source of resistant starch in this study. It was supplied by National Starch Chemical & Co., Thailand. The product specification sheet and nutritional information of Hi-maize® are shown in Appendix A and Appendix B, respectively.

Figure 3 Commercial resistant starch (Hi-maize®)

4.1.3 Chemical for analysis

- Enzyme assay kit for resistant starch analysis, Megazyme®, was purchased from Megazyme International (Ireland) Limited.

4.1.4 Instruments for physical analysis

- Spectro Colorimeter Model JS555 (Color Techno System Corporation, Japan)
- Texture Analyzer TA-XT2 (Stable Micro System, England)

4.2 Preliminary formulation trial

During the first stage of the study, attempts were made to determine the basic fresh wheat noodle and instant noodle formulas and process condition for using as the control formula.

4.2.1 Fresh wheat noodles formula

The formulation of fresh wheat noodles used in this study was chosen and applied from the basic formula of Loahavaleesant's study (171) and modified (no egg) Taeteang's basic formula (172). Both noodles formulas were tested in order to compare for noodle quality, sensory evaluation and effect of RS on noodle quality evaluation.

All ingredients (wheat flour, water, beaten whole egg, salt, sodium tripolyphosphate (STPP), sodium acid pyrophosphate (SAPP), sodium bicarbonate, and potassium carbonate), except wheat flour were mixed until homogeneous. The mixture was mixed with wheat flour in a dough mixer at a low speed for 1 min and then at a medium speed for 4 min until dry clumpy particles were formed. The particles were then kneaded in a polypropylene bag at room temperature for 20 min until a uniform dough was formed. The dough was sheeted by pressing through a pair of rollers on a noodle making machine (ATLAS 150, Marcoto, Italy) in 6 successive steps with decreasing roller gap, until the thickness of final dough sheets reached approximately 1.2 mm. The final dough sheets were cut with cutting rollers included with the noodle making machine to fresh wheat noodles of about 1 mm wide strips. Fresh noodle strips were packed in plastic bags until use. Fresh wheat noodles were produced as outlined in **Figure 4**.

4.2.2 Instant noodles formula

The formulation of fresh wheat noodles used in this study was applied and chosen from the basic formula of Prirahong's study (18) and Kounhawej's basic formula (173). Both noodles formulas were tested in order to compare for noodle quality, sensory evaluation and effect of RS on noodle quality evaluation.

The formula of instant noodle was made from the mixture of wheat flour, modified potato starch, water, beaten whole egg, salt, guar gum, STPP, SAPP, sodium bicarbonate, and potassium carbonate. Sodium bicarbonate and potassium carbonate were dissolved in water for making an alkaline solution. Dry mixture of salt, guar gum, SAPP and STPP and beaten egg were slowly poured into the alkaline solution and mixed using a magnetic stirrer until guar gum was fully swollen (at least 20 min). The mixture of wheat flour and modified starch was mixed with the above solution at a slow speed for 1 min and then at a medium speed for 4 min in a Kitchen aid ™ mixer. After forming, dough was then rested for 20 min in a plastic bag. The dough was sheeted by pressing through a pair of rollers on noodle making machine (ATLAS 150, Marcoto, Italy) in 6 successive steps with decreasing roller gap from 3.0 mm to 1.0 mm. The dough sheet was then cut into strips of about 1.0 mm wide and then steamed for 2 min in a steamer at atmospheric pressure until starch gelatinizes.

The steamed noodle strips were showered with soup containing 900 ml water, 20 g monosodium glutamate, and 60 g salt, drained, and then placed in a drilled stainless steel noodle block mold. The mold containing noodles were deep-fried in palm oil at 170 ºC for 45 sec. The fried noodles were laid on a stainless steel screen for 30 sec to remove excess oil, and cooled at room temperature for 30 min before storing in plastic bags at room temperature until use. Instant noodles were produced as outlined in **Figure 5.**

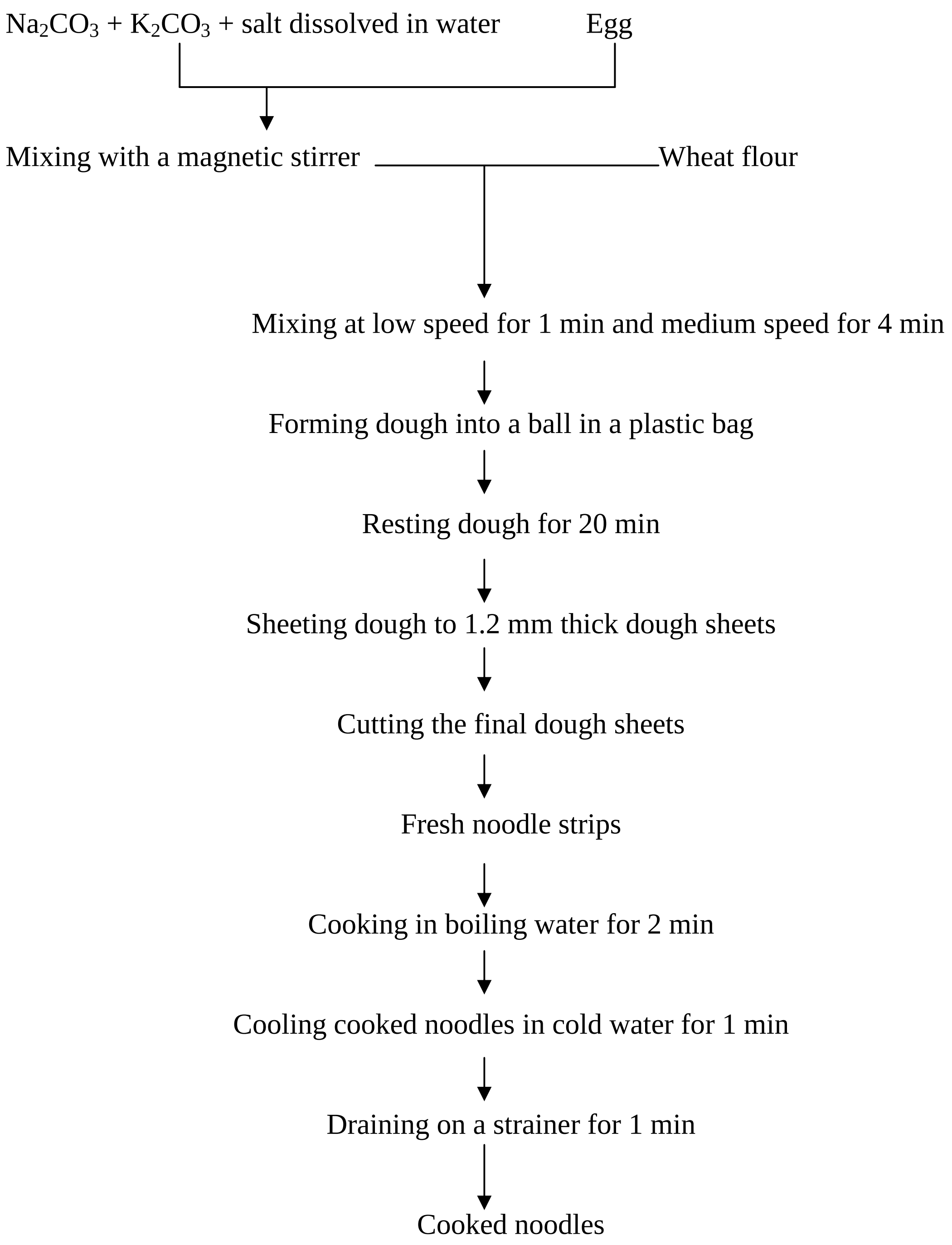

Figure 4 Procedure for making and cooking fresh wheat noodles

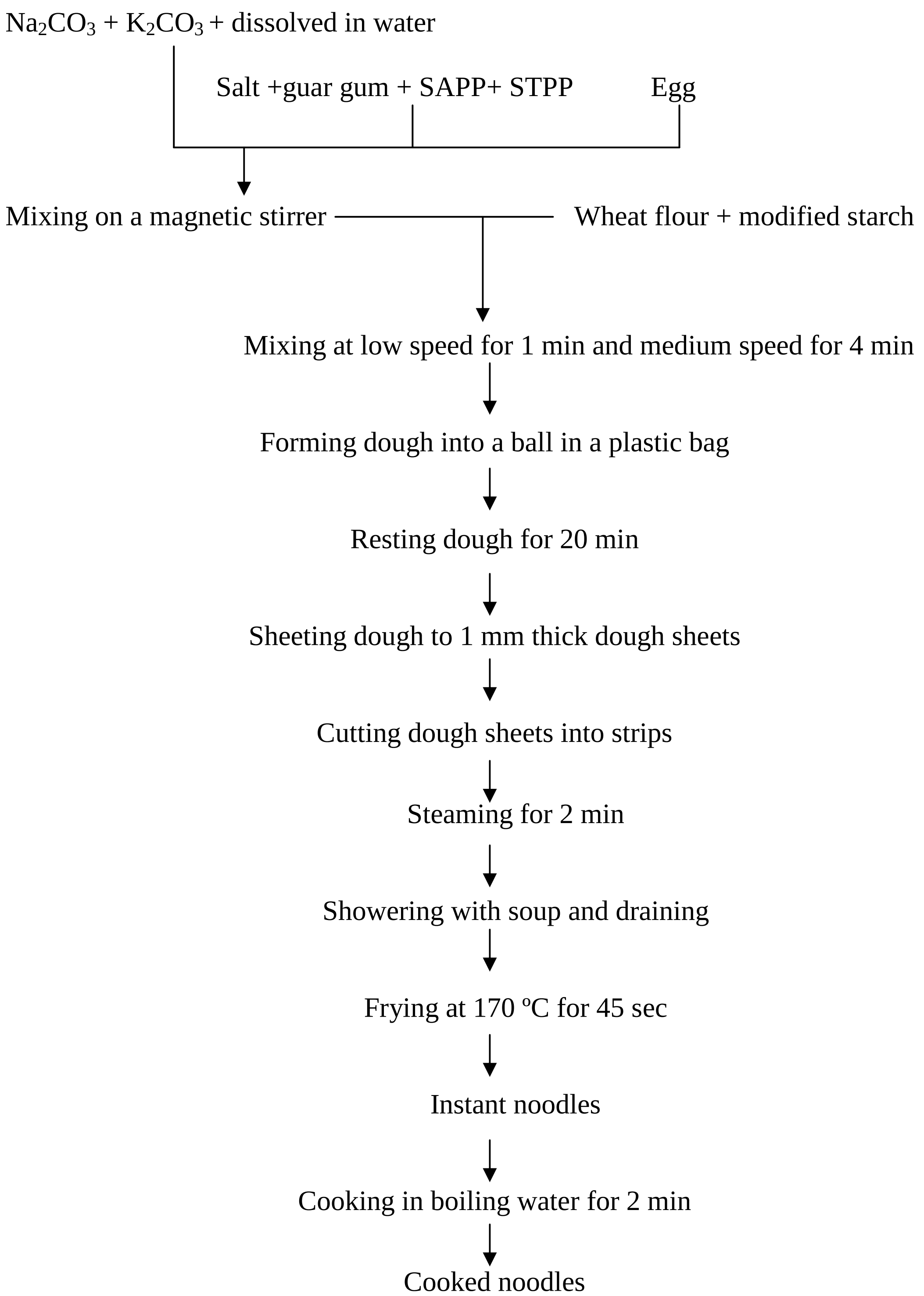

Figure 5 Procedure for making and cooking instant wheat noodles

4.2.3 Evaluation of noodles

Noodle quality was determined in terms of appearance, cooking time and eating quality (161).

a. Appearance: The appearance was determined by visual observation of noodle color, surface and shape.

b. Cooking time: Noodles were cooked in 1 L boiling water. Optimum cooking time (the time needed to obtain complete gelatinization of starch) was determined by periodically checking every 60 sec. To avoid overcooking, cooked noodles were soaked in 500 ml of cool water and drained on a strainer for 1 min. Optimum cooking time was recorded when the white core of noodles disappeared (174). The recorded cooking time was used throughout the study.

c. Eating quality: After cooking, the noodles were drained and placed in a stainless steel tray in order to minimize overcooking of the noodles. The weights of noodles were 10 g and flavored soup was added prior to tasting. The panelist rated the noodle for eating quality which emphasize on potential problem characteristic i.e. grittiness, texture of the noodles.

4.2.4 Sensory screening test (Pre-test)

The purpose of this part was to determine basic formulas of fresh wheat noodles and instant noodles for use as control formula by using sensory screening test (pre-test).

Acceptability of the control fresh wheat noodles and instant noodles determined by twenty panelists who were recruited from staff and graduate students of the Institute of Nutrition, Mahidol University.

The noodles were cooked in boiling water, then immediately cooled down on a stainless steel tray in order to minimize overcooking. The pork flavored soup was separately cooked and transferred into a double boiler, in order to maintain a constant temperature. Fifteen grams of the noodles were portioned into a white melamine bowl, diameter 3.5” labeled with three digit number codes selected from a random number table and then one-fourth cup of the hot soup was added before serving. The equilibrium temperature was about 50 ºC, which was the serving temperature. All samples were served to each panelist during the test, however, only one sample was evaluated at a time. The order of sample presentation for a panelist was randomized.

Panelist was asked to rinse his/her mouth with water between samples. The sensory evaluation was performed in the Food Science and Technology Laboratory under daylight fluorescent bulb, air-conditioned and free from cooking odor and noise. The panelists were not allowed to communicate with each other.

Sensory acceptability of each sample was evaluated for general appearance, color suitability, overall acceptability, elasticity suitability and softness suitability. The overall acceptability and general appearance were determined on nine-point hedonic scales. The scale ranked from “dislike extremely = 1” to “neither like nor dislike = 5” to like extremely = 9”. Other characteristics were determined by five-point just-about-right scales. The scale ranged from “much too elastic / hard / dark = 5” to “just-about-right = 3” to “much too brittle / soft / light = 1”. The questionnaires used in the fresh wheat noodles sensory test appear as **Appendix C** and the instant noodles sensory test as **Appendix D**. The formulas thereof which received an overall acceptability score above 5 would be selected for use as control formulas.

4.3 Formulation of RS-enriched noodle products

To formulate RS-enriched fresh wheat noodles and instant noodles wheat flour, was partially substituted with resistant starch which level starting at 15% wheat flour weight and then up to a possible maximum level. An additional amount of water was also needed to prevent drying out of the dough.

4.3.1 Effect of gluten

Gluten was added to improve the elasticity of noodles. The amount of gluten used in RS-enriched noodle formula started from a minimum level and then adjusted until the samples were acceptable in general appearance and texture. Additional amount of water was also needed to develop gluten network. The amount of gluten, which gave the most suitable quality, was selected for formulating RS-enriched noodles and the noodles were tested for noodle quality and sensory acceptability. The amount of gluten used in RS-enriched fresh wheat noodle and instant noodle formulas are shown in **Appendix E** and **Appendix F**, respectively.

4.3.2 Noodle quality evaluation

Noodle quality evaluation was performed in term of appearance, cooking time and eating quality.

4.3.3 Sensory evaluation

Sensory evaluation of developed noodle products was divided into 2 parts:

Part I: Sensory screening test (Pre-test)

Good quality RS-enriched fresh wheat noodle and instant noodle products made from each level of resistant starch substitution were selected for sensory screening test to determine the maximum level of resistant starch that could replace wheat flour. The experiment was designed as a completely randomized block (175).

The most appropriate formulas would be selected as the RS-enriched noodle formulas for further evaluation in an in-house consumer test.

Part II. In-house consumer test

In this part, sensory evaluation was carried out for fresh wheat noodles and instant noodles made with the highest possible level of resistant starch selected by the panelists in sensory test (Part I).

Fifty panelists included staff and graduate students from the Institute of Nutrition, Mahidol University. The experimental design and all evaluating techniques were the same as the evaluation performed in sensory evaluation for formula screening. The finished products accepted by panelists in the in-house consumer test should have the overall acceptability scores over 6.

4.4 Physical analysis

RS-enriched fresh wheat noodles and instant noodles were determined for physical characteristics in term of color and texture analysis.

4.4.1 Color measurement

The color of the noodle dough stored 15 min after sheeted was measured using a spectrocolorimeter Model JS 555. L*, a* and b* values were recorded. L* value represented darkness and lightness; a* value represented green and redness and b* value represented blue and yellow tones of noodle dough sheets. Three replicates for each sample were determined. Measurement was made 3 times, each at a different location on a consistent (same) side of the surface of the noodle sheet (157).

4.4.2 Texture analysis

Tensile strengths were determined using a texture analyzer (TA-XT2). One cooked noodles strand was clamped by two lobes (A/SPR probe), and increasing tensile load was applied until breakage occurred. Tensile forces at break and break length were recorded (157). The samples were measured in triplicates.

4.4.3 Chemical analysis

4.4.3.1 Proximate analysis

The samples were analyzed in duplicates according to the AOAC methods.

- Moisture content was determined by drying method at 100±5ºC (hot-air oven method; AOAC 1990, 952.45).

- Crude protein content was determined by using Macro Kjeldahl method; AOAC 2000, 979.09.

- Crude fat content was determined by using Soxhlet extraction; AOAC 2000, 920.39C.

- Ash content was determined by incinerating a sample in a muffle furnace at 550^{o}C.

- Carbohydrate content was calculated by subtracting the percentage of moisture, ash, crude protein and crude fat from 100.

- Total dietary fiber (TDF) was determined by calculation method based on 52% TDF of Hi-maize® and 2.7 % TDF of wheat flour.

- Energy content factors 4, 4 and 9 were used to calculate the energy provided by protein, carbohydrate and fat, respectively.

4.4.3.2 Resistant starch analysis

Resistant starch was analyzed following method AOAC 2002.02 (176) and the procedure is shown in **Appendix G.** The enzyme assay kit for resistant starch determination, Megazyme, consisted of pancreatic α–amylase, amyloglucosidase, glucose determination reagent (GOPOD), glucose reagent buffer, glucose standard solution and resistant starch control.

4.5 Cost estimation of products

Additional costs due to substitution of wheat flour with RS were estimated based on the prices of resistant starch (Hi-maize® 1043) substitution and wheat gluten used.

4.6 Statistical Analysis:

Data for physical properties of noodle products were analyzed by nonparametric test with SPSS for WINDOWS version 13.0 software. Significance among means was assessed by the Mann-Whitney U Test at the 5% level of probability. In the part of sensory evaluation, mean values of sensory acceptability scores of each product were evaluated for significant difference at 5% level of probability by using the Mann-Whitney U Test or one-way analysis of variance (One-way ANOVA). When significant differences in One-way ANOVA were detected, means were compared using the Duncan's multiple comparison test (175-177).

The experiment schematic is shown in **Figure 6.**

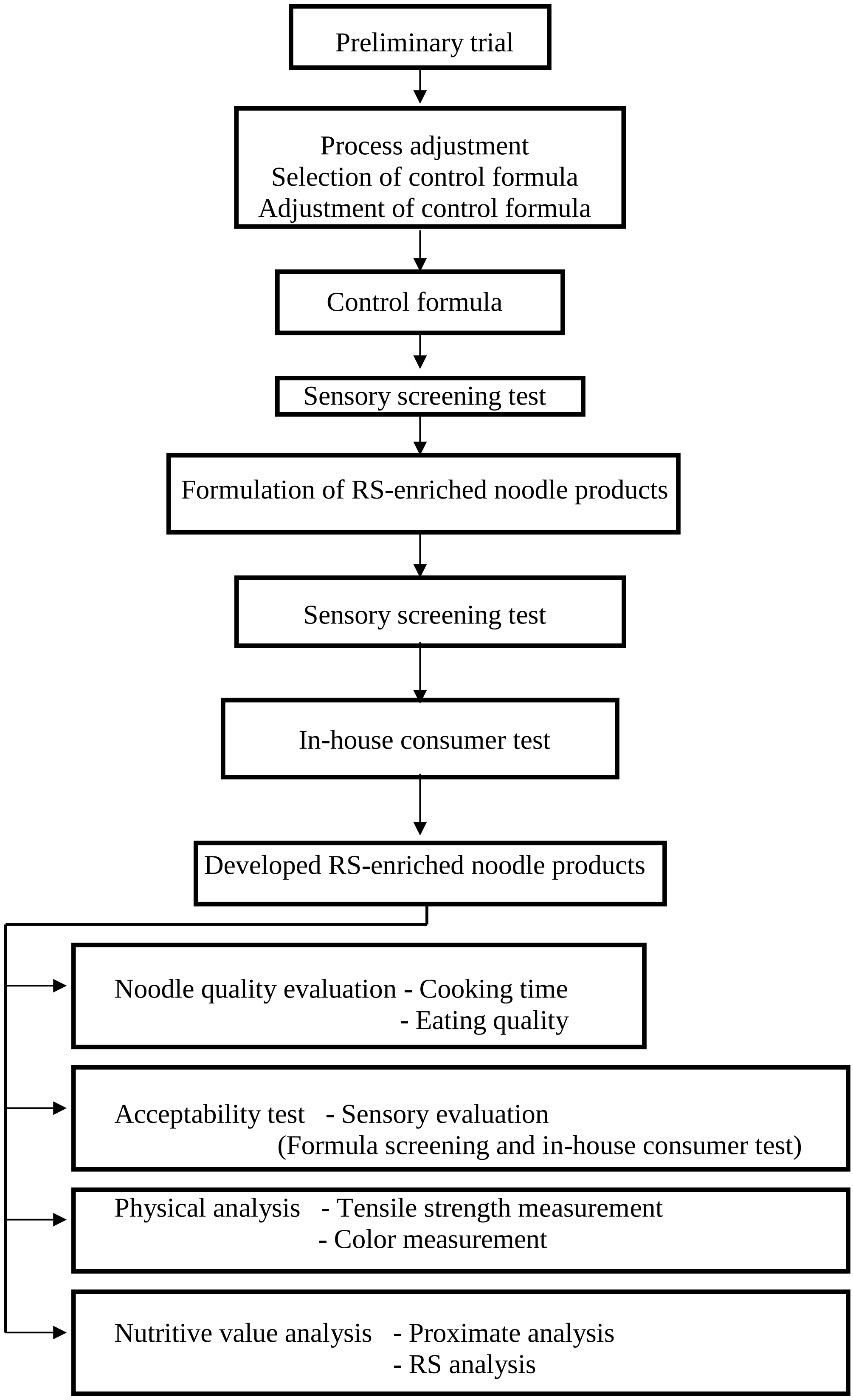

Figure 6 Experimental schematic

CHAPTER V
RESULTS

5.1 Preliminary formulation trial

5.1.1 Process adjustment and selection of the control formula

During the first stage of the study, attempts were made to determine the basic fresh wheat noodle and instant noodle formulas and process condition for using as the control formula.

5.1.1.1 Selection of fresh wheat noodle control formula

Control fresh wheat noodle formulas were chosen from basic formula of Loahavaleesant’s study and modified (no egg) Taeteang's basic formula. Both noodles were compared for noodle quality and sensory evaluation.

a. Quality evaluation of fresh wheat noodle

Table 8 Noodle quality from preliminary trial of control fresh wheat noodles

Formula	Cooking time (min)	Appearance	Eating quality	Ease of processing
Loahavaleesant’s basic formula	4	Yellow color	Elastic and moderately soft texture	Easy to process
Modified Taeteang's basic formula (no egg)	4	Yellow color	Elastic and moderately soft texture	Easy to process

From **Table 8**, the appearance of the two fresh wheat noodle formulas were the same. Cooking time, eating quality and ease of processing were also similar.

b. Sensory evaluation

Table 9 Sensory screening test [1,2] for control fresh wheat noodles

Formula	Before tasting		After tasting		
	General[3] appearance	Color[4] suitability	Overall[3] acceptability	Elasticity[3] suitability	Softness[4] suitability
Loahavaleesant's formula	6.86 (1.28)	2.92[a] (0.44)	6.16 (1.88)	2.82 (0.66)	2.98 (0.59)
Modified Taeteang's formula (no egg)	6.87 (0.64)	3.4[b] (0.51)	6.13 (0.74)	2.80 (0.68)	3.00 (0.53)

[1] Mean and standard deviation from CRB design, n=20
[2] Means with the same or without superscripts in a column are not significantly different (p>0.05)
[3] Rated on 9-point hedonic scales (1=dislike extremely, 5=neither like nor dislike, 9=like extremely)
[4] Rated on 5-point just-about-right scales (1 = much too light/brittle/soft, 3 = just-about-right, 5 = much too dark/elastic/hard)

From the results in **Table 9**, the acceptability scores of general appearance, overall acceptability, elasticity and softness suitability showed no significant difference (p> 0.05) among all samples. The color score of Laohavaleesant's formula were closer to 3 (just-about-right) than modified Taeteang's formula. However according to the good appearance, ease handling and processing as well as acceptable sensory scores of both formulas, it was difficult to choose which formula should be use for further development. Thus, 10% RS was substitute for wheat flour in both formulas in order to compare the effect of RS on noodle quality evaluation among the two formulas.

c. Quality evaluation of fresh wheat noodle with 10% RS substitution

Table 10 Noodle quality of control fresh wheat noodles with 10% RS substitution

Formula	Cooking time (min)	Appearance	Eating quality	Ease of processing
Loahavaleesant's formula with 10%RS	4	Yellow color	Elastic and moderately soft texture	Easy to process
Modified Taeteang's formula (no egg) with 10%RS	4	Yellow color	Soft and fragile texture	Too moist dough, hard to manage in sheeting and cutting steps

From **Table 10**, the appearance of fresh wheat noodles of Loahavareesant' formula with 10%RS substitution were better in eating quality and ease of processing compared to modified Taeteang's basic formula. Therefore, this formula was selected as the control fresh wheat noodle formula in further study.

5.1.1.2 Selection of instant noodle control formula

Control instant noodle formulas were chosen from basic formula of Kounhawej's basic formula and Prairahong's (no egg) basic formula. The both of noodles were compared for noodle quality and sensory evaluation.

a. Quality evaluation of instant noodle

Table 11 Noodle quality from preliminary trial of control instant noodles

Formula	Cooking time (min)	Appearance	Eating quality	Ease of processing
Kounhawej's basic formula	2	Yellow color	Elastic and moderately soft texture	Easy to process
Prairahong's basic formula (no egg)	2	Yellow color	Elastic and moderately soft texture	Easy to process

From **Table 11**, the appearance of the two instant noodle formulas were the same. Cooking time, eating quality and ease of processing were also similar.

b. Sensory evaluation

Table 12 Sensory screening test [1,2] for control instant noodles

Formula	Before tasting		After tasting		
	General[3] appearance	Color[4] suitability	Overall[3] acceptability	Elasticity[3] suitability	Softness[4] suitability
Kounhawej's formula	6.26 (1.56)	2.52 (0.54)	5.98 (1.47)	2.96 (0.67)	3.40 (0.61)
Prairahong's formula (no egg)	6.25 (1.52)	2.25 (0.44)	5.65 (1.79)	3.00 (1.03)	3.50 (0.76)

[1] Mean and standard deviation from CRB design, n=20
[2] Means with the same or without superscripts in a column are not significantly different (p>0.05)
[3] Rated on 9-point hedonic scales (1=dislike extremely, 5=neither like nor dislike, 9=like extremely)
[4] Rated on 5-point just-about-right scales (1 = much too light/brittle/soft, 3 = just-about-right, 5 = much too dark/elastic/hard)

From the results in **Table 12**, the acceptability scores of general appearance, overall acceptability as well as color, elasticity and softness suitability showed no significant difference ($p > 0.05$) among all samples. According to the good appearance, ease of handling, and acceptable sensory scores of both formulas, it was difficult to choose which formula should be used for further development. Thus, 10% RS was substituted for wheat flour in both formulas in order to compare the effect of RS on noodle quality evaluation.

c. Quality evaluation of instant noodle with 10% RS substitution

Table 13 Noodle quality of control instant noodles with 10% RS substitution

Formula	Cooking time (min)	Appearance	Eating quality	Ease of processing
Kounhawej's formula with 10% RS	2	Yellow color	Elastic and moderately soft texture	Easy to process
Prairahong's formula (no egg) with 10% RS	2	Yellow color	Soft and fragile texture	Too moist dough, hard to manage in sheeting and cutting steps

From **Table 13**, the appearance of instant noodles of Kounhawej's formula with 10%RS substitution were better in eating quality and ease of processing than the other. Hence, this formula was selected as the control instant noodle formula in further study.

5.1.2 RS-enriched noodle products

As the amount of RS increased, the dough became too dry and could not be made into both noodle products (fresh and instant noodles). Consequently, but with most ingredients and method of preparation were kept the same as a control formula, the substitution of RS for wheat flour in the formula, an additional amount of water was required. However, when more water was added, the dough became too sticky, too fragile and still could not be managed. Therefore, gluten was added in the RS-enriched noodle formulas to improve their quality.

5.2 Formulation adjustment of RS-enriched noodle products.

5.2.1 Effect of gluten

Both fresh noodles and instant noodles with 30, 35, 40% RS substitution were chosen to add gluten in order to obtain the highest possible RS substitution level. Different amount of gluten was added to RS-enriched noodle formulas to determine the optimum amount of gluten for each formula. The suitable amount of gluten for 30, 35, 40% RS substitution (data show in **Appendix E** and **F**) in fresh noodles were 10, 12, 14% by weight, respectively and for 30, 35, 40% RS substitution in instant noodles were 21, 23, 25% by weight, respectively. Gluten level in excess of these amount presented difficulties in dough sheeting process.

In addition, instant noodles with higher level than 30% RS substitution had moist interior (noticed from fried noodle still stuck with a drilled stainless steel noodle block mold) and required longer frying time which extended from 45 sec to 60 sec.

5.2.2 Noodle quality evaluation

The noodle quality data of RS-enriched fresh noodles and instant noodles with added gluten are shown in **Table 14** and **Table 15**, respectively.

Table 14 and **Table 15** represent the noodle quality evaluation of 30, 35, 40% RS-enriched fresh noodles and instant noodles with added gluten, respectively. The results reveal that the cooking time of all formulas of RS-enriched fresh noodles was 6 min and for all of RS-enriched instant noodles was 3 min. Good eating quality was found in all RS enriched formulas. They were also similar to control noodles in terms of cooking time, color, shape and appearance as well as ease of processing except at 40% RS substitution where they were formed slightly hard to process.

Table 14 Noodle quality evaluation of 30, 35, 40% RS-enriched fresh wheat noodles with added gluten.

Formula	Added gluten (% flour weight)	Cooking time (min)	Appearance	Eating quality	Ease of Processing
Fresh noodles with 30% RS substitution	10	6	Light yellow color Symmetrical shape	No gritty mouthfeel Soft and elastic texture	Easy to process
Fresh noodles with 35% RS substitution	12	6	Light yellow color Symmetrical shape	Very slightly gritty mouthfeel Soft and elastic texture	Easy to process
Fresh noodles with 40% RS substitution	14	6	Light yellow color Symmetrical shape	Slightly gritty mouthfeel Soft and elastic texture	Slightly hard to process

Table 15 Noodle quality evaluation of 30, 35, 40% RS-enriched instant noodles with added gluten.

Formula	Added gluten (% flour weight)	Cooking time (min)	Appearance	Eating quality	Ease of Processing
Instant noodles with 30% RS substitution	21	3	Light yellow color Symmetrical shape	No gritty mouthfeel Soft and elastic texture	Easy to process
Instant noodles with 35% RS substitution	23	3	Light yellow color Symmetrical shape	No gritty mouthfeel Soft and elastic texture	Easy to process
Instant noodles with 40% RS substitution	25	3	Light yellow color Symmetrical shape	Very slightly gritty mouthfeel Soft and elastic texture	Slightly hard to process

5.2.3 Sensory evaluation

Part I. Sensory screening test

RS-enriched noodle products adjustment indicated that 30, 35, 40% RS substitution with added gluten yielded noodle products with satisfactory characteristics. Therefore, sensory screening test was carried out for these noodles. All sensory screening tests were performed by 25 panelists according to the procedure described in Section 4.3.2 (Part I). The sensory acceptability scores of the RS-enriched fresh noodles and instant noodles are shown in **Table 16** and **Table 17**, respectively.

Table 16 Sensory screening test[1, 2] of 30, 35, 40% RS-enriched fresh noodles with added gluten.

Formula	Before tasting		After tasting		
	General[3] appearance	Color[4] suitability	Overall[3] acceptability	Elasticity[4] suitability	Softness[4] suitability
Fresh noodle with 30% RS substitution	6.40 (1.37)	2.75 (0.97)	6.50 (1.58)	2.95 (0.68)	2.90 (0.55)
Fresh noodle with 35% RS substitution	6.25 (1.25)	2.75 (0.85)	6.50 (1.19)	2.90 (0.55)	2.85 (0.48)
Fresh noodle with 40% RS substitution	6.00 (1.31)	2.70 (0.80)	6.15 (1.75)	2.90 (0.78)	2.85 (0.48)

[1] Mean and standard deviation from CRB design, n=20
[2] Means with the same or without superscripts in a column are not significantly different (p>0.05)
[3] Rated on 9-point hedonic scales (1=dislike extremely, 5=neither like nor dislike, 9=like extremely)
[4] Rated on 5-point just-about-right scales (1 = much too light/brittle/soft, 3 = just-about-right, 5 = much too dark/elastic/hard)

All formulas presented no significant differences (p>0.05) in terms of general appearance, color suitability, overall acceptability, elasticity suitability and softness suitability. Overall acceptability ranged from 6.15 to 6.50 of 9-point hedonic scales which mean that these formulas were liked slightly.

However, when considering the trend of the highest score in all of sensory test along together with other noodle quality particularly ease of processing, fresh noodle with 30% RS substitution formula was then chosen for an in-house consumer test, comparing with the control formula. The results are shown in **Table 18**.

Table 17 Sensory screening test[1, 2] of 30, 35, 40% RS-enriched instant noodles with added gluten.

Formula	Before tasting		After tasting		
	General[3] appearance	**Color[4] suitability**	**Over all[3] acceptability**	**Elasticity[4] suitability**	**Softness[4] suitability**
Instant noodles with 30% RS substitution	6.75 (1.21)	2.85 (0.81)	6.85 (1.53)	2.95 (0.69)	2.90 (0.55)
Instant noodles with 35% RS substitution	6.50 (1.15)	2.70 (0.87)	6.55 (1.32)	2.90 (0.45)	2.95 (0.51)
Instant noodles with 40% RS substitution	6.30 (1.53)	2.65 (0.81)	6.85 (1.14)	2.85 (0.88)	2.85 (0.49)

[1] Mean and standard deviation from CRB design, n=20
[2] Means with the same or without superscripts in a column are not significantly different (p>0.05)
[3] Rated on 9-point hedonic scales (1=dislike extremely, 5=neither like nor dislike, 9=like extremely)
[4] Rated on 5-point just-about-right scales (1 = much too light/brittle/soft, 3 = just-about-right, 5 = much too dark/elastic/hard)

All formulas presented no significant differences ($p>0.05$) in terms of general appearance, color suitability, overall acceptability, elasticity suitability and softness suitability. Overall acceptability ranged from 6.55 to 6.85 of 9-point hedonic scale which means that these formulas were liked slightly.

However, when considering the trend of the highest score in all of sensory test along together with other noodle quality particularly ease of processing, instant noodle with 30% RS substitution formula was then chosen for an in-house consumer test, comparing with the control formula. The results are shown in **Table 19**.

Part II. In-house consumer test

After sensory screening test, fresh and instant noodles with the suitable level of RS substitution were evaluated by 50 panelists in an in-house consumer test according to the method described in Section 4.3.2 (Part II).

Table 18 In-house consumer test of 30% RS-enriched fresh noodles compared to control.[1, 2]

Formula	Before tasting		After tasting		
	General[3] appearance	Color[4] suitability	Overall[3] acceptability	Elasticity[4] suitability	Softness[4] suitability
Control fresh noodles	6.86[a] (1.27)	2.92 (0.44)	6.16 (1.87)	2.92[a] (0.66)	2.98[a] (0.58)
Fresh noodle with 30% RS substitution	6.16[b] (1.63)	2.76 (0.93)	6.32 (1.85)	3.18[b] (0.80)	3.26[b] (0.52)

[1] Results are the means (SD) of 50 panelists.
[2] Means with the same or without superscripts in a column are not significantly different (p>0.05)
[3] Rated on 9-point hedonic scales (1=dislike extremely, 5=neither like nor dislike, 9=like extremely)
[4] Rated on 5-point just-about-right scales (1 = much too light/brittle/soft, 3 = just-about-right, 5 = much too dark/elastic/hard)

From the result in **Table 18**, no significant differences (p>0.05) were found for the color suitability and overall acceptability between control and 30% RS-enriched fresh wheat noodles. The general appearance score of control fresh wheat noodles was significantly higher (p<0.05) than that 30% RS-enriched noodles whereas the control fresh wheat noodles gave significantly more suitable elasticity and softness than that 30% RS-enriched noodles. Nevertheless, fresh wheat noodle with 30% RS substitution was judged by the panelists as like slightly (overall acceptability score above 6.00).

Table 19 In-house consumer test of 30% RS-enriched instant noodles compared with control.[1, 2]

Formula	Before tasting		After tasting		
	General[3] appearance	Color[4] suitability	Overall[3] acceptability	Elasticity[4] suitability	Softness[4] suitability
Control instant noodles	6.06 (1.63)	2.74 (0.52)	6.42 (1.14)	2.26[a] (0.66)	2.36[a] (0.63)
Instant noodle with 30% RS substitution	6.30 (1.54)	2.90 (0.58)	6.68 (1.37)	3.00[b] (0.53)	2.96[b] (0.28)

[1] Results are the means (SD) of 50 panelists.
[2] Means with the same or without superscripts in a column are not significantly different (p>0.05)
[3] Rated on 9-point hedonic scales (1=dislike extremely, 5=neither like nor dislike, 9=like extremely)
[4] Rated on 5-point just-about-right scales (1 = much too light/brittle/soft, 3 = just-about-right, 5 = much too dark/elastic/hard)

From the results in **Table 19**, there were significant differences ($p<0.05$) among control and 30% RS-enriched instant noodles in terms of elasticity and softness suitability whereas no significant differences ($p>0.05$) were found for general appearance as well as color suitability and overall acceptability. Furthermore, instant noodle with 30% RS substitution was judged by the panelists as like slightly (overall acceptability score above 6.00).

5.3 Physical quality determination

5.3.1 Color measurement

Table 20 Noodle dough sheet color of RS-enriched fresh noodle compared with control formula[1, 2]

Formula	Color		
	L*	**a***	**b***
Control	71.40[a] (0.32)	-3.08[a] (0.09)	19.04[a] (0.71)
30% RS	74.00[b] (0.91)	-1.28[b] (0.07)	18.88[b] (0.01)

[1] Results are means (SD) of triplicate analysis
[2] Means with the same superscripts in a column are not significantly different ($p>0.05$)

Table 21 Noodle dough sheet color of RS-enriched instant noodle compared with control formula[1, 2]

Formula	Color		
	L*	**a***	**b***
Control	71.68[a] (0.20)	3.15[a] (0.04)	24.01[a] (0.04)
30% RS	75.23[b] (0.39)	2.41[b] (0.05)	21.58[b] (0.07)

[1] Results are means (SD) of triplicate analysis
[2] Means with the same superscripts in a column are not significantly different ($p>0.05$)

From **Table 20** and **Table 21**, the flour replacement by RS in both fresh noodle and instant noodle formulas had an effect on L* values (lightness) of noodle dough sheet. L* values of the RS-enriched noodles significantly increased ($p<0.05$) from that of control noodles. Whereas a* and b* value, as the percentage of RS increased, a* and b* value decreased significantly ($p<0.05$). Overall, RS-enriched fresh noodles and instant noodles were lighter and lower in yellow color than control formula and the color weakened correspondingly with the level of RS used.

5.3.2 Texture analysis

Table 22 Tensile strength and break length of RS-enriched fresh noodles compared with control formula

Formula	Tensile strength (g)	Break length (mm)
Control	13.92[a] (1.68)	9.33[a] (2.58)
30% RS	14.00[a] (0.69)	15.8[b] (2.48)

[1] Results are means (SD) of
[2] Means with the same superscripts in a column are not significantly different ($p>0.05$)

From **Table 22**, there was significant difference ($p<0.05$) among control and 30% RS-enriched fresh wheat noodles in terms of break length whereas no significant difference ($p>0.05$) was found for tensile strength.

Table 23 Tensile strength and break length of cooked RS-enriched instant noodles compared with control formula

Formula	Tensile strength (g)	Break length (mm)
Control	15.40[a] (1.77)	5.10[a] (1.21)
30% RS	16.58[a] (2.10)	13.29[b] (1.20)

[1] Results are means (SD) of
[2] Means with the same superscripts in a column are not significantly different ($p>0.05$)

From **Table 23**, there was significant difference ($p<0.05$) among control and 30% RS-enriched instant noodles in terms of break length whereas no significant difference ($p>0.05$) was found for tensile strength.

5.4 Chemical analysis

Proximate analysis

Proximate composition and DF content of control and RS-enriched noodles (dry basis), are shown in **Table 24** and **Table 25**. The RS-enriched fresh noodles contained slightly lower ash content than the control, whereas the ash content of control and RS-enriched instant noodles were the same.

The protein content of RS-enriched fresh noodles was slightly higher than the control (about 18%), whereas the protein content of RS-enriched instant noodles was about 1.5 times higher than that of the control (about 45%).

Control and RS-enriched fresh noodles contained similar fat content but the fat content of RS-enriched instant noodles were higher than that of the control.

RS-enriched fresh noodles contained slightly higher moisture content than the control. On the other hand, the moisture content of RS-enriched instant noodles were slightly lower than that of the control.

As expected, both RS-enriched fresh noodles and instant noodles contained more TDF content with less carbohydrate and energy content when compared with that of the control.

Table 26 and **Table 27** show percentage of RS in RS-enriched fresh noodle and instant noodle compared to control in uncooked and cooked sample, respectively. As expected, the percentage of RS in RS-enriched fresh noodles and instant noodles were higher than that of the control. Furthermore, the cooked noodles showed a higher percentage of RS (moisture-free basis) than uncooked noodles.

Table 28 shows the amount of TDF per serving (50g) and % Thai RDI. One serving of RS-enriched fresh noodles and instant noodles contained 8.4 g and 8.35 g of TDF, respectively. These amounts were equivalent to about 33% Thai RDI. Thus these products could be labeled as “high fiber product” according to the Ministry of Public Health Notification no, 182 (B.E. 2541) on nutrition labeling.

Table 24 Nutrient composition of a composite RS-enriched fresh noodles (2 batches of samples) compared to control (per 100 g fresh weight)

Nutrient composition	Control formula	30% RS-enriched fresh noodles
Energy (kcal)	258	192
Moisture (g)	33.3	35.1
Protein (g)	9.2	10.9
Fat (g)	1.6	1.6
Carbohydrate (g) (excluding TDF)	51.6	33.5
Dietary fiber(g) [1] (by calculation)	1.8	16.8
Ash (g)	2.5	2.1

[1] Result obtained from calculation method; based on 52% TDF of Hi-maize® and 2.7% TDF of wheat flour (Data from: www.nal.usda.gov/)

Table 25 Nutrient composition of a composite RS-enriched instant noodles (2 batches of samples) compared to control (per 100 g fresh weight)

Nutrient composition	Control formula	30% RS-enriched fresh noodles
Energy (kcal)	483.4	446.2
Moisture (g)	1.6	1.2
Protein (g)	10.6	15.4
Fat (g)	21.2	25.4
Carbohydrate (g) (excluding TDF)	62.5	39.1
Dietary fiber(g) [1] (by calculation)	1.9	16.7
Ash (g)	2.2	2.2

[1] Result obtained from calculation method; based on 52% TDF of Hi-maize® and 2.7% TDF of wheat flour (Data from: www.nal.usda.gov/)

Table 26 RS content of a composite RS-enriched fresh noodles (2 batches of samples) compared to control (moisture-free basis)

Formula	**% RS**	
	Uncooked	**Cooked**
Control fresh wheat noodles	0.47	3.90
30% RS-enriched fresh wheat noodles	15.70	42.90

Table 27 RS content of a composite RS-enriched instant noodles (2 batches of samples) compared to control (moisture-free basis)

Formula	**% RS**	
	Uncooked	**Cooked**
Control instant noodles	0.45	0.80
30% RS-enriched instant noodles	7.69	31.20

Table 28 TDF content (with percent Thai RDI) of RS-enriched fresh noodles and instant noodles compared with control (per serving) [1].

Formula	**TDF (g)**	**%RDI [2]**
Control fresh wheat noodles	0.90	3.60
RS-enriched fresh wheat noodles	8.40	33.60
Control instant noodles	0.95	3.80
RS-enriched instant noodles	8.35	33.40

[1] Amount one serving of noodles = 50 g.
[2] Percent RDI, based on 2,000 kcal diet.

5.5 Cost of raw materials of RS-enriched noodle products

The cost of RS-enriched fresh noodles and instant noodles (based on raw materials) were compared with the cost of control noodles and are shown in **Table 29**. Raw materials include commercial resistant starch (Hi-maize®), wheat gluten and commercial wheat flours (based on average 30 baht/ kg.) The cost of Hi-maize® 1043 purchased from National starch Co., Ltd. is 200 baht/ kg and wheat gluten purchased from Nutrition Ltd., Part is 60 baht/ kg. All other ingredients were used at the same amount for control and RS-enriched fresh noodles. For fresh noodles and instant noodles, the total cost of raw materials for RS-enriched noodles was about 3 baht and 1.50 baht more than that of the control for 100 g and for one serving of noodles, respectively.

Table 29 Cost estimation of RS-enriched fresh wheat noodles and instant noodles compared to that of control formula (raw material only).

Product	**Cost (Baht/ 100 g product)**	**Cost (Baht/ serving)**
Control fresh noodles	X	X
RS-enriched fresh noodles	X+3.10	X+1.55
Control instant noodles	Y	Y
RE-enriched instant noodles	Y+3.20	Y+1.60

CHAPTER VI
DISCUSSION

6.1 Preliminary formulation trial

Process adjustment and selection of the control formula

During the first stage of the study, attempts were made to determine the basic fresh wheat noodle and instant noodle formulas and process condition for using as the control formula. In addition, the control formula served to ensure that the declined acceptability score from sensory evaluation of RS-enriched noodle products did not result from inferior cooking skill and technique used by the researcher (178).

6.1.1 Selection of fresh noodle control formula

The control fresh noodles were selected between Laohavaleesant's and modified Taeteang's formulas. Both formulas were reported to be well-accepted in terms of noodles quality and sensory evaluation. Both fresh noodle formulas were prepared in order to compare for noodles quality and sensory evaluation. According to the good appearance, ease of handling as well as good sensory acceptability score of noodles made from the two formulas, it was difficult to choose which one to be control fresh noodles. Thus 10% RS was substituted in both of those two formulas and then the noodles were compared again for quality.

Laohavaleesant's formula was better accepted due to better eating quality, which included more elasticity and not too fragile texture along with not too moist dough and easier to manage in sheeting and cutting step. The reason may be because of egg incorporation in Laohavaleesant's formulas. Adding eggs to noodles gives a firmer texture and also helps improving the texture, increases dough elasticity and extensibility in noodles (163, 166).

6.1.2 Selection of instant noodle control formula

The control instant noodles were selected between, Kounhawej's and Prairahong's formulas, based on the same procedure as the selection of fresh noodle control formula.

Similarly, both instant noodle formulas differed only slightly. Then 10%RS substitution was performed. Kounhawej's basic formula with 10%RS substitution gave better eating quality and was easier to process.

6.2 Formulation RS-enriched fresh noodles and instant noodles

The starting level of RS substitution was determined by reviewing the previous research (181) which could substitute wheat flour noodles with 30% (w/w) soybean hulls. Therefore substitution level close to 30% of RS should be investigated. For this study, the flour was replaced with RS varying from 30, 35 to 40%. The substitution of flour with RS in the noodles caused some negative effects on their appearance and texture of the noodle strands. This result agreed very well with earlier studies, which reported that replacement of wheat flour by traditional fiber (such as soy bean hull, garbanzo bean flour, green and yellow pea flour) resulted in a decrease in texture profile analysis (TPA) parameters of cooked noodles (18, 157, 179). Consequently, it necessitated formula adjustment in the amount of water and gluten. The addition of gluten to formation in pasta was shown to increase dough elasticity (180).

6.3 Formula adjustment

Replacement with RS caused weak and fragile noodles strands. They resulted from the decrease of wheat gluten in mixed flour that affected the amount of disulfide bond available in dough forming. Laohavaleesant and Prairahong (18, 171) studied the effect of gluten on noodle texture and reported that addition of gluten could improve the noodle texture. Thus in this study, gluten was added in order to obtain dough which was similar to the control. In addition, this study found that fresh noodles and instant noodles could increase RS substitution level to 45-50% of wheat flour with increasing gluten and water at suitable amounts. However, RS-substitution level was stopped at 40% because of the difficulty in dough producing. The resulting noodles also had a rough texture and had a little gritty mouthfeel when used more than 40% substitution in RS-enriched noodles. The formulated RS-enriched noodles were further tested for noodle quality, sensory and physical quality.

Pre-test

The sensory results showed that 30, 35, 40% RS substitution in both fresh noodles and instant noodles gave no significant difference in sensory scores ($p>0.05$). However, when considering the trend of the scores in all of the sensory characteristics evaluated, as the amount of RS substitution increased, the trend of sensory scores gradually decreased. This result agreed with the result from Gomolmanee's study (155). Besides, noodles with 40% RS was remarked by some panelists as having a slightly dry mouthfeel. The increase in RS level directly affected the appearance and texture of the products and therefore, causing a decrease in overall acceptability (155)

In the case of RS-enriched instant noodles, frying time was extended from 45 sec to 60 sec because the fried noodles still stuck to the drilled stainless steel noodle block mold. Furthermore for RS-enriched fresh noodles and instant noodles, the cooking time was extended from 4 min to 6 min and 2 min to 3 min, respectively. At the beginning, cooking time was kept the same as control but the cooked noodles were not acceptable in eating quality, having grittier mouthfeel and hard texture. They also appeared like uncooked noodles. Therefore, cooking time was increased to improve the quality of RS-enriched noodle products. The RS used in this study was a high-amylose maize starch which was reported to have a high gelatinization temperatures, requiring temperatures that are often not reached in conventional cooking practices (154 °C to 171 °C) before the granules are completely disrupted (36). Thus it is possible that the RS-enriched noodles required longer cooking time and also frying time in RS-enriched instant noodles than that of control. This result agrees with Jangchaimonta that waffle and cake enriched with RS required longer baking time than the control (192). On the contrary, in Gomolmanee's study, alkali noodles contained 15% RS with xanthan gum still required the same cooking time as control noodles. The reason may be because the RS content in those alkali noodles was low so longer cooking time was not necessary (155).

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In-house

The results from the in-house consumer test confirmed that there was significant impact ($p<0.05$) on sensory acceptability scores in terms of elasticity and softness suitability in both RS-enriched fresh noodles and instant noodles. The results showed that the panelists preferred the elasticity and softness of control fresh noodles than RS-enriched fresh noodles. Nevertheless, the panelists preferred the elasticity and softness of RS-enriched instant noodles to that of the control. These results agree with texture analysis results and are explained in next part.

In terms of general appearance, control fresh noodles received significantly higher score ($p<0.05$) than RS-enriched fresh noodles. However, the overall acceptability and color suitability scores were not significantly different ($p>0.05$) between the control and RS-enriched noodles. In addition the scores of both general appearance and overall acceptability were above 6. This means RS-enriched fresh noodles was judged by the panelists as liked slightly. Therefore the results indicate that wheat flour mixed with 30% (by weight) Hi-maize® could be utilized in fresh noodles and instant noodles production with minimal defects in eating quality.

In case of color measurement, according to the results, as the level of RS substitution increased, L* value or lightness increased whereas a* value or redness and b* value or yellowness decreased. It could be indicated that incorporation of RS made the noodle dough brightened and appeared light yellow in color. Generally, consumers prefer the brightness in noodles (182) Therefore, the color suitability score of RS-enriched noodles in sensory evaluation was not significantly different ($p<0.05$) when compared to control for both fresh noodles and instant noodles. The reason for color lightening in noodle dough may be due to the water holding characteristic of RS which led to decrease activity of browning reaction (155). These results were similar to earlier researches. Gomolmanee reported that L* value of alkali noodles slightly increased with 15% RS substitution and 0.5% xanthan gum added (155). Similarly, Yue & Waring reported that pasta with 15% RS substitution gave lighter color compared to the control that had no added fiber (183).

For texture measurement, tensile strength gives an indication of how the sample holds together during cooking (184). High values of tensile strength and breaking distance in noodles are associated with noodle-eating texture (185). The tendency to have higher tensile strength (no significant difference, $p>0.05$) and the higher break length (significant difference, $p<0.05$) of RS-enriched fresh noodles and instance noodles compared to the control indicated that RS-enriched noodles possessed a more strengthened network. This may be explained by the effect of added gluten. These results were similar to earlier researches. Prairahong (18) reported that substitutions of flour with soybean hull in the noodles provided more tensile strength than the control. Similarly, Seib stated that protein in cooked noodles would be expected to increase tensile strength, depending mostly, however, on the level present (184). This reason agreed with sensory result in terms of elasticity and softness that panelists preferred softer and did not like too much elasticity of RS-enriched fresh noodles. On the other hand, the decrease in tensile strength with increasing RS-content could be explained by the weaker gel strength of starch since the RS particles may interfere with the starch gel network (155)

6.4 Chemical analysis of RS-enriched noodles and control

The results showed that TDF content of RS-enriched noodles was higher than that of the control noodles. The RS-enriched fresh noodles, control fresh noodles, RS-enriched instant noodles and control instant noodles had TDF values of 16.8, 1.8, 16.7, 1.9, g/100g, respectively. These TDF values in this study were only the approximate values because they were obtained from the calculation method based on the original TDF of wheat flour and the TDF content of Hi-maize® added. Chemical analysis could give more reliable values. On a serving basis, the product (50g) provided about 8 g DF which equals to about 33% Thai RDI. In addition, when considering the nutrition labeling option, current regulation states that nutrition-related terms of nutrient content claims can be used on label as specified in the Ministry of Public Health Notification No.182/2541(186). For term such as “High fiber”, a product must have not less than 5g DF per reference amount (serving). Thus, the term “High fiber” could be used for the RS-enriched fresh noodles and instant noodles formulated in this study.

The protein content of RS-enriched fresh noodles and instant noodles were higher than the control, especially RS-enriched instant noodles with about 5g/100g more protein than the control. RS-enriched fresh noodles had about 1.7g/100g more protein than the control. This result could be explained by addition of gluten in these formulas. Similarly, Prairahong and Laohavaleesant added gluten in to their DF-enriched noodles and found that the protein content subsequently increased (18, 171).

The ash content of RS-enriched fresh noodles was slightly lower than the control while that of RS-enriched instant noodles was similar to the control.

Regarding the fat content, RS-enriched fresh noodles contained similar amount of fat compared to the control. In contrast RS-enriched instant noodles contained more fat than the control. This result was unexpected because earlier researches reported the benefit of RS and most DF sources in decreasing oil uptake of DF-enriched products (187). When considered the instant noodles processing applied for RS-enriched instant noodles in this study, it may be possible that a longer frying time required for RS-enriched instant noodles caused the higher uptake of oil. Kim and Lee reported that a long frying time increased uptake of oil (188). A high uptake of oil during frying not only increased the cost of the finished product, but also could adversely affect its shelf life. Thus, to obtain an optimum RS-enriched instant noodles product, frying time should be improved by either increasing frying temperature or using a fryer with a better efficiency. Moreover, frying in a closed system may also help to attain better heat transfer (18).

The amount of carbohydrate in RS-enriched products was lower than that of the control because of the partial substitution of RS for wheat flour. This also led to a slight reduction in energy content of RS-enriched noodles.

Regarding the effect of cooking on RS content, this study found that RS content in both of RS-enriched and control noodles increased after cooking. This evident could be explained by the study of Yue & Waring (1998) that RS can be inherently present in food or can be increased due to processing conditions involving heat and high temperature (183). The RS content was particularly high in RS-enriched noodles from the direct addition of RS in the product formulation. Therefore, these RS-enriched noodle products could be presented as an alternative food source of dietary fiber for the consumers.

6.5 Cost of the products

The results of cost estimation (raw materials only) indicated that the cost of RS-enriched fresh noodles and instant noodles were about 3 baht higher than that of the control when comparing on a per 100g basis. The more expensive cost resulted from the cost of RS and gluten added in RS-enriched products. Nevertheless, the cost estimation of RS-enriched fresh noodles and instant noodles were based on a pilot scale study. Therefore, their cost could be lower in a large-scale production with bulk purchase of ingredients.

CHAPTER VII
CONCLUSION

The study indicated that RS-enriched fresh wheat noodles and instant noodles could be formulated to contain a commercial RS (Hi-maize®). RS was applied by partially substituting wheat flour in the noodles formulation. High level of RS incorporation in noodle products caused adverse effects to the rheological property of noodle dough as well as the quality of finished product. To improve the quality of RS-enriched noodle products, it was necessary to add gluten and additional amount of water. Results from product application testing and sensory evaluation indicated that RS could partially substitute wheat flour up to 30%.

RS-enriched fresh noodles and instant noodles in this study had TDF values of 16.8 and 16.7 g/100g, respectively. This is markedly higher than the control noodles (about 9 times). The TDF content of RS-enriched fresh noodles and instant noodles was about 8 g per reference amount which equals to 33% Thai RDI. Therefore, these products can be classified as "high fiber" products.

The cost of Hi-maize® was relatively higher than the commercial wheat flour. Considering the higher cost and the final noodle product quality, the recommended substitution level of RS that could be used without adverse effects on eating quality was 30% wheat flour substitution. Due to the high TDF content of RS-enriched noodles, they could be recommended as an alternative food choice for health conscious consumers. This, in turn, will enhance their dietary fiber intake towards the recommended level. Moreover, the results of this study could be used as a guideline for developing high-dietary fiber products containing RS.

Recommendations for future study

The RS-enriched noodles developed in this study had been shown to provide better nutritive values, particularly TDF, than regular noodles. Nevertheless, the fat content in RS-enriched instant noodles is still high. Therefore, the formula or processing procedure of RS-enriched instant noodles should be modified in order to reduce oil absorption. Furthermore, the beneficial effects of RS supplementation in noodle products on various physiological effects and health; such as prebiotic effects, reduction of glycemic index and weight management, should be determined.

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APPENDIX

APPENDIX A

PENFORD
AUSTRALIA LIMITED

TYPICAL NUTRITIONAL INFORMATION
HI-MAIZE 1043

	Per 100g of product (as is basis)
Weight	100g
Water	14.0g
Energy Value	986kJ
Protein	0.5g
Total carbohydrates*	33g
Dietary Fibre*	52g
Fat-total	NA
Fat-saturated	NA
Fat-mono	NA
Fat-poly	NA
Cholesterol	None
Calcium (48ppm)	4.8mg
Chloride (44ppm)	4.4 mg
Iron (5ppm)	0.5mg
Magnesium (41ppm)	4.1mg
Manganese (41ppm)	4.1mg
Phosphorus (110ppm)	11.0mg
Potassium (20ppm)	2.0mg
Sodium (95ppm)	9.5mg
Zinc (3ppm)	0.3mg
Vitamins	None

* as defined by ANZFA food regulations

The above information is considered to be typical and not part of the product specification.

Date of Issue: January 2002

APPENDIX B

National Starch
Carbohydrate Nutrition

PRODUCT SPECIFICATION

HI-MAIZE® 1043

Natural Hi-maize

PRODUCT DESCRIPTION

Hi-maize®1043 is a natural, unmodified, food grade, high amylose maize starch that is an enriched source of total dietary fibre and resistant starch, and is not organoleptically detected in most product applications.

CHARACTERISTICS

A fine, white, free-flowing powder, with no off odours or flavours.

APPLICATIONS

Hi-maize®1043 may be used as a fibre source in bakery products, bread, breakfast cereals, pasta and noodles, snack foods, soups, cereal drinks, yoghurt and select dairy products.

Note: Hi-maize®1043 is not soluble.

LABELLING

Recommended labelling is MAIZE STARCH or STARCH however please check local food labelling requirements to ensure compliance.

PACKAGING

Available in 25 kg and 50lb moisture barrier, multiwall paper bags.

TYPICAL ANALYSIS

Moisture	: 10.0 – 13.0%
Dietary Fibre (AOAC)	: 60% dsb min
Protein (N x 6.25)	: 1.0% max
Particle Size	
Retained 212 micron	: 1.0% max
Ash dsb	: 0.5% max
pH	: 4.0 – 6.0
Sulphur Dioxide	: <10mg/kg

Nutritional Information
Typical "as is" 13.0% moisture

Total Carbohydrate*	: 34%
Sugars	: Nil
Fat	: <0.1%
Sodium	: 65 mg/kg
Dietary Fibre*	: 52% min

* as defined by FSANZ food regulations

STORAGE

We recommend that Hi-maize 1043 be stored in a clean, dry area at ambient temperature and away from heavily aromatic materials. The shelf life for Hi-maize 1043 is 24 months from the date of manufacture.

GENERAL

Hi-maize®1043 is both Kosher & Halal certified and is Non-GM Identity Preserved.

Issued: August 2003

APPENDIX C

แบบสอบถามการประเมินผลผลิตภัณฑ์บะหมี่สด

ตัวอย่างหมายเลข.....................

ผู้ประเมิน อายุ..........................ปี เพศ (........)ชาย (........)หญิง

วันที่...เวลา........................

กรุณาชิมตัวอย่างอาหารที่เสนอและขีดเครื่องหมาย ✓ หน้าคำอธิบายความชอบที่มีต่อผลิตภัณฑ์

I. กรุณาดูลักษณะอาหารก่อนชิม (คนและดมได้)

ลักษณะโดยทั่วไป (พิจารณาเฉพาะตัวเส้นบะหมี่)	ลักษณะสีของเส้นบะหมี่
____ชอบมากที่สุด	____เข้มมากไป
____ชอบมาก	____เข้มไป
____ชอบปานกลาง	____กำลังดี
____ชอบเล็กน้อย	____จางไป
____เฉยๆ *	____จางมากไป
____ไม่ชอบเล็กน้อย	
____ไม่ชอบปานกลาง	
____ไม่ชอบมาก	
____ไม่ชอบมากที่สุด	

II. หลังจากชิมแล้ว

ความชอบโดยรวม (พิจารณาเฉพาะตัวเส้นบะหมี่)	ความเหนียวของเส้น	ความนุ่มของเส้น
____ชอบมากที่สุด	____เหนียวมาก	____แข็งมาก
____ชอบมาก	____เหนียว	____แข็ง
____ชอบปานกลาง	____กำลังดี	____นุ่มกำลังดี
____ชอบเล็กน้อย	____เปราะ	____เละ
____เฉยๆ *	____เปราะมาก	____เละมาก
____ไม่ชอบเล็กน้อย		
____ไม่ชอบปานกลาง		
____ไม่ชอบมาก		
____ไม่ชอบมากที่สุด		

ข้อเสนอแนะเกี่ยวกับเส้นและลักษณะทั่วไปของบะหมี่

....

หมายเหตุ * หมายถึงความรู้สึกระหว่างชอบและไม่ชอบ

APPENDIX D

แบบสอบถามการประเมินผลผลิตภัณฑ์บะหมี่กึ่งสำเร็จรูป

ตัวอย่างหมายเลข......................

ผู้ประเมิน อายุ..........................ปี เพศ (........)ชาย (........)หญิง

วันที่..เวลา........................

กรุณาชิมตัวอย่างอาหารที่เสนอและขีดเครื่องหมาย ✓ หน้าคำอธิบายความชอบที่มีต่อผลิตภัณฑ์

I. <u>กรุณาดูลักษณะอาหารก่อนชิม (คนและดมได้)</u>

ลักษณะโดยทั่วไป (พิจารณาเฉพาะตัวเส้นบะหมี่)	ลักษณะสีของเส้นบะหมี่
____ชอบมากที่สุด	____เข้มมากไป
____ชอบมาก	____เข้มไป
____ชอบปานกลาง	____กำลังดี
____ชอบเล็กน้อย	____จางไป
____เฉยๆ *	____จางมากไป
____ไม่ชอบเล็กน้อย	
____ไม่ชอบปานกลาง	
____ไม่ชอบมาก	
____ไม่ชอบมากที่สุด	

II. <u>หลังจากชิมแล้ว</u>

ความชอบโดยรวม (พิจารณาเฉพาะตัวเส้นบะหมี่)	ความเหนียวของเส้น	ความนุ่มของเส้น
____ชอบมากที่สุด	____เหนียวมาก	____แข็งมาก
____ชอบมาก	____เหนียว	____แข็ง
____ชอบปานกลาง	____กำลังดี	____นุ่มกำลังดี
____ชอบเล็กน้อย	____เปราะ	____เละ
____เฉยๆ *	____เปราะมาก	____เละมาก
____ไม่ชอบเล็กน้อย		
____ไม่ชอบปานกลาง		
____ไม่ชอบมาก		
____ไม่ชอบมากที่สุด		

ข้อเสนอแนะเกี่ยวกับเส้นและลักษณะทั่วไปของบะหมี่

....

หมายเหตุ * หมายถึงความรู้สึกระหว่างชอบและไม่ชอบ

APPENDIX E

Fresh wheat noodle

Fresh wheat noodle formation with three levels of RS incorporation

Ingredient (g)	Control	30% RS	35% RS	40% RS
Wheat flour	100	70	65	60
Resistant starch	0	30	35	40
Gluten	0	10	12	14
Water	30	37	39	41
Sodium chloride (NaCl)	1.5			
Di-sodium carbonate (Na_2CO_3)	1.5			
Di-potassium carbonate (K_2CO_3)	0.17			
Beaten whole egg	15			

APPENDIX F

Instant noodle

Instant noodle formation with three levels of RS incorporation

Ingredient (g)	Control	30%RS	35%RS	40%RS
Wheat flour	88	58	53	48
Modified potato starch	12	12	12	12
RS	0	30	35	40
Gluten	0	21	23	25
Water	35	50	53	55
Di-potassium carbonate (K_2CO_3)	0.02			
Di-sodium carbonate (Na_2CO_3)	0.02			
Sodium chloride (NaCl)	0.5			
Gaur gum	0.4			
Sodium acid Pyrophosphate (SAPP)	0.04			
Sodium tri-polyphosphate (STPP)	0.04			
Beaten whole egg	5.5			

APPENDIX G
RESISTANT STARCH ANALYSIS

Principle

Sample is incubated with pancreatic α-amylase and amyloglucosidase (AMG) for 16 hours at 37° C in shaking water bath. Non-resistant starch is solubilised and hydrolyzed to glucose by the combined action of the two enzymes. The reaction is terminated by the addition of an equal volume of ethanol. Resistant starch (RS) is recovered as a pellet on centrifugation. This is then washed twice by suspension in ethanol (50%v/v), followed by centrifugation.

RS in the pellet is dissolved in 2M KOH by viscously stirring in an ice-water bath over a magnetic stirrer. This solution is neutralized with acetate buffer and the starch is quantitatively hydrolyzed to glucose with AMG. Glucose is measured with glucose oxidase/peroxidase reagent (GOPOD), and this is a measure of the RS content of the sample.

Calculations

Resistant starch is calculated according to the following:

Resistant Starch (g/100g sample) (samples containing > 10 % RS):

$= \Delta E \times F \times 100/0.1 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 90.$

Resistant Starch (g/100g sample) (samples containing < 10 % RS):

$= \Delta E \times F \times 10.3/0.1 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 9.27.$

where:

ΔE = absorbance (reaction) read against the reagent blank.

F = 100 divided by absorbance of glucose standard

W = dry weight of sample analyzed

= "as is" weight x (100-moisture content)/100].

Measurement of Resistant starch

(1). Hydrolysis and solubilisation of non-resistant starch.

Weigh 100 ± 5 mg sample

↓

Screw cap tube (Corning culture tube; 16 x 125 mm)

↓ *Gently tap tube: ensure sample falls to bottom*

Add 4.0 ml of pancreatic α-amylase (10 mg/ml) containing AMG (3 U/ml)

↓

Mix by a vortex mixer

↓

Attach them horizontally in a shaking water bath

↓

Incubate at 37ºC with continuous shaking

(200 strokes/min) **for exactly 16 hr.**

↓

Remove tubes from water bath

↓ *Remove excess surface water with paper*

Remove the tube caps and treat with 4.0 ml of ethanol (99 %)

↓ *Vigorous stirring by vortex mixer*

Centrifuge at 1,500 g (approx. 3,000 rpm) for 10 min (non-capped)

↓

Re-suspend the pellets in 2 ml 50 % ethanol

↓ *Vigorous stirring by vortex mixer*

Add 6 ml of 50 % ethanol

↓

Mix the tubes and centrifuge at 1,500 g for 10 min

↓

Decant the supernatants

Repeat suspension and centrifugation once more

↓

Carefully decant the supernatants

↓

Invert the tubes on absorbent paper to drain excess liquid

(2). Measurement of Resistant Starch

Add magnetic stirrer bar (5 x 15 mm) + 2 mL of 2 M KOH to each tube

↓

Dissolve RS by stirring for ~ 20 min
(in an ice/water bath over a magnetic stirrer)

↓

Add 8 mL of 1.2 M sodium acetate buffer (pH 3.8) to each tube
with stirring on the magnetic stirrer.

↓

Immediately add 0.1 mL of AMG (3300 U/ml) (f]

↓

Mix well and place the tubes in a water bath at 50º C

↓

Incubate the tubes for 30 min with intermittent mixing on a vortex mixer

For samples containing > 10 % RS content;
Transfer the contents of the tube to a 100 mL volumetric flask

↓

Adjust to 100 ml with distilled water and mix well

↓

Centrifuge aliquot of the solution at 1,500 g , 10 min

For samples containing < 10 % RS content;
Directly centrifuge the tubes at 1,500 g for 10 min (no dilution)

↓

Final volume in the tube is approximately 10.3 ml

Transfer 0.1 mL aliquots (in duplicate) of either the diluted or undiluted supernatants
into glass test tubes (16 x 100 mm)

↓

Add with 3.0 mL of GOPOD reagent

↓

Incubate at 50º C, 20 min

↓

Measure the absorbance at 510 nm against a reagent blank

Prepare reagent blank solutions by mixing 0.1 mL of 0.1 M sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD reagent.

Prepare glucose standards (in quadruplicate) by mixing 0.1 mL of glucose (1 mg/ml) and 3.0 mL of GOPOD reagent.

BIOGRAPHY

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