DEVELOPMENT OF READY-TO-COOK CAMBODIAN KORKO SOUP BASE

Miss Meng Sreang Loem

จุฬาลงกรณ์มหาวิทยาลัย
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การพัฒนาเครื่องปรุงแกงกอโอร์ของกัมพูชาพร้อมปรุง

น.ส.เมง สรีแอง เลียม

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งานวิจัยนี้มีวัตถุประสงค์ในการพัฒนาเครื่องปรุงแกงกอโอร์ของกัมพูชาพร้อมปรุง โดยเริ่มจากการศึกษาทัศนคติของผู้บริโภคทั่วไปจำนวน 400 คน ต่อผลิตภัณฑ์เครื่องแกงสำเร็จรูป พบว่าผู้บริโภคให้คะแนนความชอบของรูปแบบเครื่องปรุงแกงกอโอร์สำเร็จรูปแบบผงมากที่สุดถึงร้อยละ 44.5 จากนั้นศึกษาผลของอุณหภูมิที่ต่างกันในการทำแห้งสมุนไพร เครื่องเทศ และปลาร้า ในการพัฒนาแกงกอโอร์ของกัมพูชา (Cambodian Korko soup) ต่อสมบัติทางด้านเคมีกายภาพ จุลชีววิทยา และ สารต้านอนุมูลอิสระของสมุนไพร เครื่องเทศ และปลาร้าที่ใช้ โดยการทำแห้งที่อุณหภูมิ 80 องศาเซลเซียส ให้ค่ากิจกรรมของน้ำและความชื้นน้อยกว่าอุณหภูมิอื่นๆ ในทุกตัวอย่าง รวมถึงค่าการต้านอนุมูลอิสระด้วยวิธี DPPH และ FRAP มีค่าลดลง เมื่อเปรียบเทียบกับการทำแห้งที่อุณภูมิ 60 และ 70 องศาเซลเซียส ในทางตรงกันข้ามพบว่าปริมาณฮิสตามีนของปลาร้ากลับเพิ่มขึ้นที่อุณหภูมิและค่าบริกซ์ที่สูงขึ้น หลังจากคัดเลือกสภาวะที่เหมาะสมในการทำแห้ง จึงพัฒนาต้นแบบผลิตภัณฑ์ออกเป็นสี่ประเภท ได้แก่ 1) ผงแห้งผสม 2) เพสท์ที่ 60 องศาบริกซ์ 3) เพสท์ที่ 45 องศาบริกซ์ และ 4) เพสท์ที่ 30 องศาบริกซ์ พบว่าค่ากิจกรรมของน้ำ ความชื้น ปริมาณฮีสตามีน ปริมาณจุลินทรีย์ทั้งหมด รวมทั้งราและยีสต์ของตัวอย่างที่เป็นเพสท์มีค่าสูงกว่าเกณฑ์ความปลอดภัย ต้นแบบที่เป็นรูปแบบผงแห้งผสมซึ่งมีค่ากิจกรรมของน้ำที่ 0.38±0.56 จึงถูกเลือกไปทำการศึกษาต่อไป ค่าไอโซเทอมความชื้นของแกงผงสำเร็จรูปที่อุณหภูมิ 30 องศาเซลเซียสมีค่าอยู่ในช่วง 0.113-0.970 โดยใช้วิธี static gravimetric มีลักษณะเป็นไอโซเทอมประเภทที่สาม โดยค่าสมการการดูดซับความชื้นนั้นเข้ากับแบบจำลองของ GAB ระดับความชื้นชั้นบาง (monolayer moisture content) ที่ปลอดภัยในผลิตภัณฑ์ต้นแบบคือ 0.068 กรัมน้ำต่อกรัมตัวอย่างแห้ง จากนั้นนำแกงผงสำเร็จรูปมาตรวจสอบการเปลี่ยนแปลงในระหว่างการเก็บรักษา 6 สัปดาห์ที่อุณหภูมิ 30, 40 และ 50 องศาเซลเซียส โดยพิจารณาในเกณฑ์ของความชื้น ค่ากิจกรรมของน้ำ สี ความสามารถในการละลาย การจับตัวเป็นก้อน ความเหม็นหืน และสมบัติทางจุลชีววิทยา ผลการทดลองพบว่ามีการเปลี่ยนแปลงคุณสมบัติทางกายภาพและเคมีของผลิตภัณฑ์ต้นแบบระหว่างการเก็บรักษา โดยค่ากิจกรรมของน้ำ ความสามารถในการละลาย และเนื้อสัมผัส มีค่าเพิ่มขึ้นเล็กน้อย ในขณะที่ปริมาณความชื้นและพิกัดสีมีค่าลดลง นอกจากนั้นจำนวนจุลินทรีย์ระหว่างการเก็บรักษายังคงอยู่ในเกณฑ์ความปลอดภัย ผลการประเมินทางประสาทสัมผัสของแกงสำเร็จรูปโดยมีผู้ทดสอบ 32 คน พบว่าให้คะแนนความชอบที่ 4.1±1.32 จากการใช้เกณฑ์ความพอดี (JAR) บ่งชี้ว่ามีคะแนนในด้านกลิ่นและรสต่ำ จากนั้นได้ปรับต้นแบบและให้ผู้บริโภคประเมินรสสัมผัสครั้งที่สอง (ผู้ทดสอบ 30 คน) พบว่าผลิตภัณฑ์ที่ปรับปรุงแล้วได้รับคะแนนความชอบ 4.67±1.01 ซึ่งเพิ่มขึ้นจากเดิม ในส่วนของค่าความชื้น ค่ากิจกรรมของน้ำ เถ้า โปรตีน เส้นใย ไขมัน คาร์โบไฮเดรต ความสามารถในการละลาย ปริมาณฮิสตามีน, $L*$, $a*$ และ $b*$ มีค่าอยู่ที่ 2.79, 0.375, 22.39, 10.22, 4.27 , 2.63, 62.97, 60.82, 32.56, 62.07, 2.37 และ 20.55 ตามลำดับ และปริมาณจุลินทรีย์ยังอยู่ในเกณฑ์ความปลอดภัย

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สาขาวิชา	วิทยาศาสตร์และเทคโนโลยีทางอาหาร	ลายมือชื่อนิสิต ....
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6278016023 : MAJOR FOOD SCIENCE AND TECHNOLOGY
KEYWORD: ready-to-cook quality antioxidant product development

Meng Sreang Loem : DEVELOPMENT OF READY-TO-COOK CAMBODIAN KORKO SOUP BASE. Advisor: Associate Professor CHALEEDA BOROMPICHAICHARTKUL, Ph.D.

This study develops a prototype of a ready-to-cook Cambodian Korko soup base by surveying the perception of 400 Cambodian consumers and found that 44.5% of replies prefer ready-to-cook Cambodian Korko soup base in powder form. After that, the effect of different drying temperatures on the properties of Cambodian Korko soup base ingredients (herb, spice, and fermented fish) was investigated on physicochemical, antioxidants, and microbiological properties. At higher temperature 80 ºC, water activity, moisture content was decreased faster and DPPH and FRAP were decreased compared to lower temperatures 70 ºC and 60 ºC. On the other hand, histamine in fermented fish was increased at higher temperatures and Brix. After drying, there were four product prototypes developed including (1) dried form and (2) paste form in the ºBrix of 60, 45, and 30. Water activity, moisture content, histamine content, total plate count, yeast, and mold were found over the safety limitation on paste product prototypes such as ºBrix of 60, 45, and 30. Therefore, the powder form at 0.38±0.56 water activity was selected for further study. Moisture sorption isotherm of the ready-to-cook Korko soup powder at 30 ºC and water activity in the range of 0.113-0.970 were determined by a static gravimetric method. The isotherm exhibited Type III behavior. The moisture sorption data were fitted to the GAB model. The monolayer, taken as the safe minimum moisture level in the product prototype found at 0.068-gram water per gram dry matter. In addition, the ready-to-cook Korko soup powder was monitored during 6 weeks of storage at different temperatures 30 ºC, 40 ºC, and 50 ºC in terms of moisture content, water activity, color, solubility, caking, rancidity, and microbiological properties. The results showed that the changes in physical and chemical properties of the product prototype during storage slightly increased in water activity, moisture content, TPA, and decrease in solubility and color coordinates. In addition, at 30-40 °C and 40-50 °C, the Q10 values were 1.11 and 1.10, respectively. As a result, the instant Korko powder had predicted expiry dates of 3.96, 3.56, and 3.24 years when kept at 30, 40, and 50 °C, respectively. The microorganisms during the storage were within the safety limit under the regulation. Then, the ready-to-cook Korko soup base was evaluated by 32 panelists to score the product prototype preference for further development. The prototype got an overall liking score of 4.1±1.32. From the JAR rating, the prototype had a lower aroma and flavor. The second sensory evaluation (n=30) of the improved prototype showed that the overall liking was 4.67±1.01. Moisture Content, water activity, ash, protein, crude fiber, fat, carbohydrate, solubility, histamine, $L*$, $a*$, $b*$ and microorganisms were analyzed on the final product prototype and found at 2.79, 0.375, 22.39, 10.22, 4.27, 2.63, 62.97, 60.82, 32.56, 62.07, 2.37, 20.55, respectively. The number of microorganisms was at the safe level to consume. Hence, the consumer survey (n=400) on the product preferred form was found 44.5% of the powder form.

Field of Study:	Food Science and Technology	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....

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Meng Sreang Loem

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CHAPTER 1
INTRODUCTION

1.1 Background

Korko is one of the great fusions of fresh ingredients fused into a soup usually served with lots of meals all week long. It is a delicious and healthy soup originating from Cambodia, has been widely known as one of the most popular and traditional dishes among Cambodians. It is increasingly consumed in other regions of the world, particularly those with significant Cambodian populations (Asian Society, 2008). Korko soup is consumed over the country because of its health benefits, taste, and appearance. It is unique, delicious, and low in fat and calories, full of multivitamin and other nutrition due to the combination of various vegetables such as pumpkin, taro, papaya, green jack fruit, green banana, moringa, edible amaranth, ivy gourd, and pea eggplant (Ryan, 2018).

Currently, Korko soup has usually been prepared by hand from fresh ingredients in a small batch at home and restaurants over the country. It takes such a long time to find and prepare lots of ingredients such as fermented fish, roasted rice, lemongrass, kaffir lime leaves, garlic, turmeric, galangal, salt, monosodium glutamate, and sugar. Those ingredients have to be prepared in different ways such as finely grinding, thinly slicing, and also roasting. This is resulting in a long period of time to make this dish. Yet, cooking procedure is quite complicated as well. Today, most people prefer something fast and instant, especially food. Besides being more practical and faster, instant seasonings have the advantages of being more durable and overcoming the unavailability of some herbs or spices. Hence, in the market, various instant seasoning products are found, however, until now there has not been found instant Korko seasoning products yet. Therefore, a ready-to-cook Korko soup base would be an option to improve convenience and decrease the cooking time of Korko soup.

In this study, dehydration will be used to develop this ready-to-cook Korko soup base. However, the compounds in this developing product are sensitive to drying conditions especially temperature which can cause quality deterioration of the products through a color change, shrinkage, or loss of texture, nutritional, and functional properties (Vega-Gálvez et al, 2008). The ready-to-cook Korko soup base is also a potential functional food source since many ingredients such as galangal, turmeric, lemongrass, garlic, and kaffir-lime leaves have health-giving benefits such as antitumor and anti-cancer in the digestive tract antimicrobial, antifungal, antiparasitic, and antiviral activities, as well as antidiabetic. However, those essential compounds can be destroyed by drying temperature (Nishimura et al, 2000). Therefore, to develop the ready-to-cook Korko soup base product with quality and safety, drying temperature will be investigated through the determination of physical and chemical properties that would be an alternative of a healthy seasoning to meet consumers' desires. Hence, the sensory evaluation will either be conducted to ensure the consumer product preference as well as physicochemical, microbiological, and stability of the ready-to-cook Korko soup base product.

1.2 Research objectives

This research was aimed to develop a ready-to-cook Korko soup base by study effect of drying temperatures as well as the determination of physiochemical, microbiological, shelf life, and sensory evaluation of the product.

1.3 Expected outcomes

The ready-to-cook Korko soup base is developed successfully with an accepted quality, safety as well as consumer acceptability.

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CHAPTER II
LITERATURE REVIEW

2.1 Seasonings powder

Seasonings powder are the mixtures of ground spices, herbs, seeds, or other flavorings. Seasoning includes a large or small amount of salt, sugar, and other ingredients being added to preparation but retain their physical and chemical properties (Underriner et al., 1994). Generally, the seasonings are ground to powder for convenience. Seasonings powder has a low moisture content which reduces the rate of quality degradation. As a result, seasonings powder can be stored longer than other forms of food products. Based on previous studies, there are two categories of seasonings powder. The first category is commonly produced from meat or poultry to enhance various soups' flavor. In various cultures, meat may be existing as a seasoning technique too (Poste et al., 1993). The second category is known as ready-to-cook seasonings powder made entirely from total ingredients for making the specific dishes, for instance, Korko soup. A well-designed dish may combine seasonings that complement each other. Seasonings powder is very important in daily life, especially in food, such as improving the taste of food. It is therefore also intended to enhance the natural taste of the food to be more intense or noticeable. Balancing tastes, seasonings help to overcome very strong tastes, especially sour, sweet or bitter tastes. Both synthetic and natural seasonings powder are needed to enhance sensory characteristics. In processed foods, seasonings powder may be more economical to use than fresh seasonings. For example, seasonings powder do not require the cutting or grinding of fresh forms.

2.1.1 Korko soup base and origin

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Korko soup base is an instant seasoning powder, one package full-flavored for cooking only Korko soup. Korko soup is one of the most popular traditional healthy dishes in Cambodia. It is one of the great fusions of fresh ingredients fused into a soup usually served with lots of meals all week long. It has usually been prepared by hand from fresh ingredients in a small batch at home and restaurants over the country. It is a delicious and healthy soup originating from Cambodia, has been widely known as one of the most popular and traditional foods among Cambodians. It is increasingly consumed in other regions of the world, particularly those with significant Cambodian populations (Asian Society, 2008). Korko soup is consumed over the country because of its health benefits, tastes, and appearance. It is unique, delicious, and low in fat and calories, full of multivitamins and other nutrition. According to a local legend, the dish has been the king's favorite. It is a popular and delicate flavor normally prepared by mixing several herbs and spices, including fermented fish, roasted rice, lemongrass, kaffir lime leaves, garlic, turmeric, galangal, salt, MSG, and sugar. This soup can be cooked by various meat such as fishes, chicken, or pork with many kinds of vegetables such as pumpkin, taro, papaya, green banana, green jack fruit, pea eggplant as well as green leafy vegetables such as Yardlong bean, edible amaranth, Coccinia Grandis, moringa. Korko soup is a potential functional food since many ingredients such as galangal, turmeric, lemongrass, and kaffir-lime leaves have health-giving benefits such as anti-tumor and anti-cancer in the digestive tract (Mackeen et al., 1997). Moreover, some ingredients such as garlic also have antifungal, antiparasitic, and antiviral activities, as well as antidiabetic, hypocholesterolemic, and cancer preventive agents (Nishimura et al., 2000).

2.2 Seasonings powder and analogue

Seasonings are often used in powder form because they are not subject to seasonal availability, are easier to process, have a longer shelf life, are convenient, and have lower costs. These powder forms are most commonly used for processed products especially for ready-to-cook seasonings or for wholesale use. Dry seasonings come whole, finely or coarsely ground, cracked, and as diverse particles. Seasonings are ground by milling them into various sizes of particulate matter (Underriner, 1994). This grinding also generates rapid air movement and heat that dissipates some of the volatile oils and even changes some of the natural flavorings through oxidation. Depending on its shape, the same powder spice will produce different flavor perceptions in the finished product. Ground seasonings are better available in food products than fresh whole seasonings. Some volatile oils are released by grinding, which partially breaks down the cellular matrix of the spice (Bhesh et al., 2013). In some seasonings, the taste is increased by drying due to the elimination of most moisture. This leaves a higher concentration of low volatile compounds that give a stronger flavor but less aroma due to the loss of volatile components. Seasonings powder can withstand higher temperatures and processing conditions than fresh seasonings. To develop their taste, many ground seasonings need to be "rehydrated." This addition of water triggers an enzyme reaction that releases the aroma of the spice. The sensory, physical, and chemical characteristics of seasonings powder are determined by the environment, climate, soil conditions, harvesting time, and post-harvest handling. The same type of spice can have different sensory characteristics depending on where it has been grown and how it has been harvested, stored, and processed. For example, Indian powder ginger has a subtle lemon-like flavor. Spice flavor can be easily oxidized and losses occur during milling and storage of seasonings. When the seasonings are ground, the oils tend to volatilize, causing a loss of aroma. To better retain color, flavor, and aroma, seasonings are sometimes milled or dried at lower temperatures. While seasonings lose more aroma as they are ground more finely, the advantage is that finely ground seasonings blend better in finished products requiring a smooth texture. Seasonings powder may have certain disadvantages, such as poor flavor intensity, may cause discoloration in the finished product, and may therefore create an undesirable appearance in the product (Bhesh, 2013).

2.2.1 Advantages of seasonings in powder form

Seasonings are ground by milling them into varying particulate matter sizes. The low moisture content of seasonings powder can reduce the rate of quality degradation and long shelf life than other forms. Hence, low bulk weight, diverse applications, conveniences, and also easy to transport. The same powder spice can create different taste perceptions in the finished product, based on its form. In food products, ground seasonings are better available than fresh whole spices. Spices powder can tolerate higher temperatures and processing conditions than fresh spices. Spices powder can be easier to use than fresh seasonings. For instance, it does not require the chopping, cutting or grinding of new forms. Hence, the sensory, physical, and chemical properties of seasonings powder are determined by the environment, the climate, and the conditions of the soil, the time of harvest, and the handling after harvest (Vega et al, 2009).

2.2.2 Seasonings Powder Nutrition

Since seasonings powder tend to have strong flavors and are used in small quantities, they also tend to add few calories to food, even though many seasonings contain large amounts of fat, protein and carbohydrate by mass. When used in greater quantities, however, seasonings can either add substantial amounts of minerals and other micronutrients to the diet, including iron, magnesium, calcium, and many others. Most herbal and seasonings powder has significant antioxidant activity, primarily due to phenolic compounds, in particular, flavonoids, which affect nutrition through many pathways, including other nutrient absorption (Underriner, 1994). Such antioxidants can also serve as natural preservatives, preventing or slowing food spoilage, resulting in an increased nutritional content is stored food.

2.2.3 Seasonings powder applications

Seasonings powder has various roles in foods. Their primary functions include flavoring food and providing scent, texture, and color. Seasonings can have side effects such as preservative, dietary, and functional protection. Seasonings consist of starch, fat, sugar, protein, gum, ash, and other non-volatile materials. Both of these components impart the different taste, color, nutritional, environmental, or preservative effects of each spice. Within a matrix of carbohydrate, protein, fiber, and other cell components, the flavor components (volatile and non-volatile) are covered.

2.2.3.1 Seasonings powder primary functions

Seasonings powder is anything we apply in cooking to increase the taste of foods. In a recipe or formula, the overall flavor, taste, aroma, texture, or color that spices add to the food determines its efficacy. There are prevailing chemical components in each spice or flavoring that generate these sensual qualities. The chemical compounds of spice can have moderate to intense flavors. Spices have flavors and aromas that are distinctive. Coloring, some spices provide color as well as flavor to foods such as turmeric and so on (Susheela Raghavan, 2007). In spices, the ingredients responsible for coloring are soluble in oil or soluble in water. Often the general coloring offered by a spice is a combined result of two or more of its coloring elements.

2.2.3.2 Seasonings powder secondary functions

Traditionally, spices powder has been used to enhance appetite, improve digestion, reduce tension and increase energy. Spices can also promote nutrition as they are used in prepared foods to improve flavor instead of salt, fat or sugar. Spices may be used as preservatives in food products. Spices as preservatives, antimicrobials, and antioxidants have long been recognized for their preservative properties. Many ancient cultures have used them to fumigate cities, embalm royalty, preserve food, and prevent diseases and infections. For both bactericidal and nutritional purposes, spices have also been used. Spices such as cinnamon, garlic, and oregano were used to treat cholera and other infectious diseases during the middle ages (Susheela, 2007). As preservatives, a mixture of spices may be more effective than a single spice powder. To be effective antimicrobials, spices must be used at high volumes, although this would, in food products, taste problems exist. Generally, the amount of spices usually applied to western-style foods is not adequate to fully inhibit microorganisms, but can to some degree inhibit spoilage. Today seasonings powder can be used commercially as natural antioxidants in foods, with a market preference for natural products. (Bhesh et al., 2013).

2.2.3.3 Seasonings powder emerging applications

Spices as medicines, the increasing emphasis on healthy eating brings attention not only to spices as critical ingredients for the production of delicious low-fat or low-salt foods, but also as a natural way to improve health and promote well-being. Consumers prefer to take medicine or drugs to eat a natural food product. Spices are becoming more attractive to consumers with greater research into their medical benefits. In traditional medicine, in many western cultures, spices are used to improve energy, relieve stress, improve the nervous system, help digestion, reduce cold symptoms and headaches, and treat many diseases. In modern medicine, Spices are becoming more attractive to consumers with greater study into their medicinal benefits. Many Asian and western researchers have detected active compounds in spices that have medicinal effects using modern testing techniques.

Sulfides, thiols, terpenes, and their derivatives include the active components of spices (or phytochemicals), phenols, glycosides, alcohols, aldehydes, and esters (Susheela Raghavan, 2007).

2.3 Seasonings powder stability

Minimizing their exposure to air, heat, light, and moisture is key to minimise deterioration and maximise shelf life of seasonings powder (Ansley, 2020). During storage and handling, seasonings powder can become sticky, and cake. Moisture is sometimes defined as the main factor, or trigger when caking occurs. Knowing the water sensitivity and hygroscopicity of the major powder components for predicting the caking kinetics is therefore very important. The fact that they contain several different ingredients complicates many food powders and food ingredient blends, making it difficult to predict their susceptibility to cakes. Furthermore, during handling, storage, processing, and delivery to the end-user, the powders can change temperature and atmospheric humidity that can alter the handling behavior and appearance such as color and flavor of the powders. This is particularly important as powders are transported to hotter, more humid conditions where a mixture is trapped or solidly liquefied from water absorption.

2.3.1 Water activity

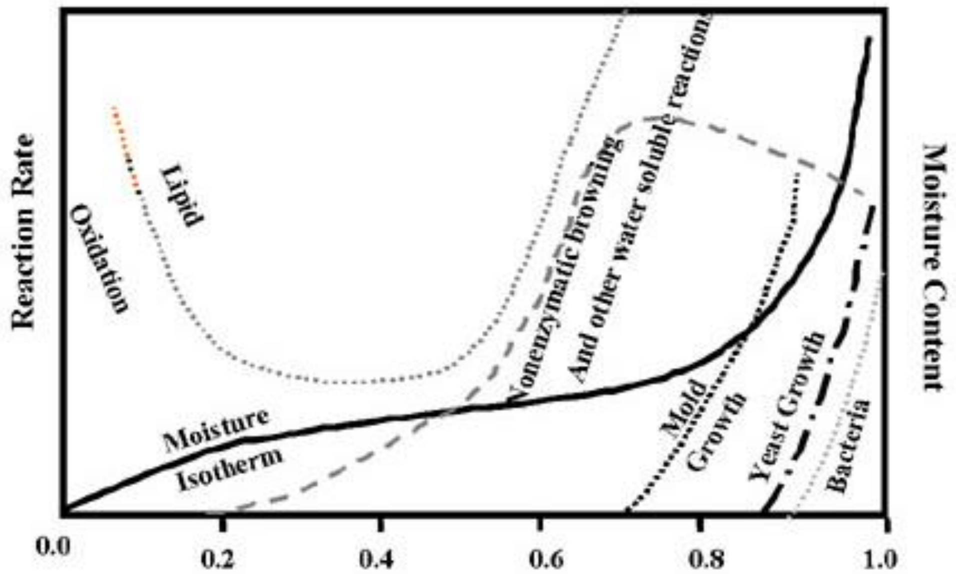

Figure 2.1 Relationship of water activity to food quality and safety (Bell et al., 2000)

Low moisture food ingredients are utilized by most food manufacturers because of their easy handling, longer shelf life, and other advantages. All the common modes of failure for seasonings powder are related to and can potentially be controlled by water activity. When considering the stability of spices powder, the most common mode of failure is likely caking or clumping because it makes them difficult to handle and impacts production efficiency (Aguilera et al., 1995). However, an additional concern for seasonings powder which has more recently gained attention due to several products recalls originating from low moisture ingredients is microbial safety (Cavallaro et al., 2011). While it may seem strange to discuss the microbial safety of ingredients whose water activities fall well below the lower limit for microbial growth, it is still possible for low moisture ingredients to be carriers of microorganisms. Spices powder are also particularly susceptible to changes in their organoleptic profile due to chemical reactions that produce off colors, flavors, and odors. As with other low moisture ingredients, spices have been increasingly targeted as potential carries of microorganisms (Muggeridge et al., 2001). There may be a tendency to consider water activity testing of seasonings powder unnecessary because they are low moisture. Identifying and maintaining an ideal water activity range for the stability of seasonings powder may be the easiest and most effective way to maximize their utility and shelf life. The objective of this whitepaper is to discuss the theory of water activity and describe how to identify the critical water activity that will limit seasonings powder modes of failure including glass transition, caking and clumping, chemical degradation, and microbial safety.

2.3.2 Critical water activity and caking

Caking or clumping of seasonings powder during handling, packaging, and storage is a ubiquitous problem. Problems can happen during both the processing and storage of powders and spices. Caking is the formation of permanent clumps due to the stickiness of particles which eventually can result in a loss of functionality and lowered quality (Bell et al., 2000). Caking can reduce product recovery during drying, slow processing time by clogging up hoppers and pipes, and reduce product shelf life. Caking is water activity, time, and temperature-dependent. Factors known to affect caking kinetics may be divided into intrinsic properties of the powder itself (water activity, particle size distribution, presence of impurities, and glass transition temperature) and external factors such as temperature, relative humidity, and mechanical stress applied to the substance. Moisture is characterized as the primary factor or trigger that causes caking. Caking may occur due to attractions between particles of van der Waals and may also occur due to the creation of liquid bridges where the capillary forces present are sufficiently strong to capture the material (Susheela, 2000). The strength of the cake can also be affected by compaction stress, where greater stress contributes to stronger cakes, such as in the case of tableting a free-flowing powder where compaction triggers van der Waals attractions. To understand the caking mechanisms, it is useful to research the intermolecular structure within the powder particles, as the caking mechanisms for crystalline and amorphous particles are distinct. At lower relative humidity, crystalline materials tend to have a poor ability to absorb water, but they can readily adsorb water above critical relative humidity. This will result in liquid bridges being produced and a partial breakdown of soluble materials on the crystal surfaces. The strength of these liquid bridges containing low dissolved molecular compounds relies primarily on capillary powers. A large rise in cohesive forces that contributes to the solidification of the remaining solids in the bridge can only be accomplished by drying. This also refers to the dissolved solids being crystallized in the liquid to create stable bridges between the particles. Above the glass transition temperature, the powder particles become sticky, and this is often expressed by the temperature of the sticky point. For carbohydrates with low molecular weight, the stopping point is typically about 10-20 °C above the glass transition temperature. A highly viscous flow between particle surfaces is this stickiness, causing the surfaces to bond and connect, making the powder much more compact. The stickiness first increases to a limit when passing through the sticky zone with increasing water content or temperature, after which it then decreases because of the continuous reduction in viscosity. The collection of powder particles consisting mainly of amorphous substances depends heavily on the relative humidity and temperature of the air around them. When exposing amorphous materials to a moist atmosphere, the mechanism for bridging between single particles differs from that of crystalline materials, since the amorphous particles drain together. Sintering is a method in which molecules move into the gap between two adjacent particles, and this is allowed above the glass transition so that the molecules flow. The mechanism is driven by surface friction and/or external forces. When reducing the distance between the particles, the device's free specific surface energy is decreased. The rate of production of the sinter bridge would depend on the molecular mobility associated with the viscosity of the material, which in turn is linked to the temperature difference (T-Tg) between the temperatures of the material. Humidity cycling, where powders are exposed to high relative humidity followed by low relative humidity, can lead to the formation of strong bridges between the powder particles. When the powder particles contain water-soluble constituents, this can occur. During high humidity exposure, water bridges containing dissolved constituents will form and these will be transformed into solid bridges when the water is vaporized during low humidity exposure.

2.3.3 Critical water activity and chemical stability

Unwanted chemical reactions in seasonings powder can lead to the development of off colors, odors, and flavors. Chemical reaction rates will be at a minimum and caking remains the most likely mode of failure, but reactions can still occur and potentially reduce the shelf life of the product. For these seasonings powder, the chemical breakdown is their primary mode of failure. The primary chemical reactions responsible for the quality loss in seasonings powder are Maillard browning, lipid oxidation, enzymatic, and hydrolysis reactions. The products of these reactions, when the reaction progresses sufficiently, affect the taste, appearance, and nutritional quality. Water activity influences reaction rates by reducing activation energy, increasing mobility, and increasing the rate constant. Consequently, reaction rates are better correlated to water activity than moisture content. Most reactions will reach a maximum in the range of 0.70-0.80 a_w due to dilution at high water activities, but lipid oxidation also increases at low water activity. These reactions are often complex with multiple possible pathways and require the presence of specific reactants or enzymes to occur. While lower water activity often reduces the reaction rate to provide sufficient stability, it may also be necessary to restrict the reactants, such as oxygen for rancidity or reducing sugars for Maillard Browning. Seasonings powder are susceptible to both browning and rancidity and a product that has experienced chemical degradation will likely be rejected on inspection due to unexpected color changes or odor (Brady, 2019).

2.3.4 Microbial contamination

The microorganism is contaminated from many sources on seasonings powder. Microbiological seasoning criteria is included counts of total bacteria, yeast, mold, *coliforms,* and food pathogens such as *E. coli*, with *Salmonella.* During the growing and post-harvesting handling high microbial numbers are caused by contamination. During the drying process of the seasoning powder, spore-forming bacteria such as the *Bacillus* species or *Aerobacter aerogenes* contained in the soil may be transferred to the spice, in particular with seasonings such as turmeric, ginger, galangal, and garlic, under the ground. The type and amount of molds and bacteria on a spice powder depends on the type of spice and the conditions in which it is processed. Seasonings that show strong antimicrobial properties tend to have low microbial counts (Shamsudeen, 2009). In the drying process and during storage molds continue to multiply. Good storage conditions, monitoring, and specifications are critical for retaining seasonings powder quality attributes. Microbial growth and infestation of insects can occur to varying degrees during storage, depending on the level of contamination during harvesting, transporting, and processing conditions. The growth of insects and molds will change the color and, to some degree, the spice flavor. Seasonings powder does not normally spoil but over a period of time lose their strength in fragrance, taste, and color. Low humidity levels in the soil or entire seasonings are potential for mold and microbial growth (Susheela, 2007). Insect and microbial control are important when obtaining a quality spice. To that the initial bacteria or mold content in processed foods, seasonings powder must be free of microbes.

2.3.5 Effect of drying process on seasonings powder

In the ready-to-cook seasoning product preparation, herbs will primarily be used. These herbs contain many phytochemicals, including antioxidants, which play a significant role in protecting humans from infection and degenerative diseases

(Ebrahimzadeh et al., 2008). Drying will also be involved in the preparation of this process repetitively. The benefits of dehydration are well known, as the decrease in moisture in the product significantly delays microbial and chemical deterioration and results in a significant decrease in volume (Doymaz and Pala, 2003). Many herbs are susceptible to drying conditions that can cause its quality deterioration through oxidation, color change, shrinkage or loss of texture, and especially nutritional and functional properties, there are both advantages and disadvantages of drying methods (Vega et al., 2009). Under this technique, however the rise in temperature can contribute to a decrease in antioxidant compounds as well as in antioxidant activity, depending on the air velocity and duration of heat exposure as well (Miranda et al., 2010). Many researchers have stated that the overall antioxidant activity will influence the drying temperature because most antioxidant compounds are temperature-influencing phenolic compounds. Some studies indicate that the quality of certain antioxidant compounds is impaired by heating due to the shift in extractability due to the rupture of the cell wall and isomerization of certain compounds (Peleg et al., 1991 and Wang, 2000). A decrease in antioxidant capacity was promoted by drying at higher temperatures (80 ºC and 90 ºC), drying at 70°C resulted in reasonably acceptable in physical, chemical, and sensory properties it was observed that an increase in drying temperature caused degradation of antioxidant compounds and sensory properties of onion powder such as color, and flavor in a fresh sample based on research by Muhaba Seifu (2018).

2.3.6 Moisture sorption isotherm

Moisture sorption isotherms of seasonings powder provide critical information that can be used in predicting stability by theoretical calculation. Moisture sorption isotherms can also be used to investigate structural features such as specific surface area, pore-volume, pore size distribution, and crystallinity of food products (Rizvi et al., 1995). During the processing and storage of agricultural products, physical, chemical, and microbiological changes occur. The changes are influenced particularly by the moisture content and water activity of food material. The equilibrium moisture content of a food material is defined as its moisture content attained when the water vapor present in the food material has reached an equilibrium with its surroundings. It is a thermodynamic property and has practical significance in both the drying and storage of foods. It is affected by relative humidity and temperature. Water activity on the other hand is defined as the ratio of the vapor pressure of water over the foodstuff to that of pure water at the same temperature. The relationship between moisture content and water activity in food at constant temperature and pressure is often expressed as a moisture sorption isotherm. It can give information on the sorption mechanism and the interaction of food with water. The typical shape of the sorption isotherm may change depending on the type of product and reflects how water binds to the system (Bell, 2000). The drying method can also significantly affect the sorption properties of some dry products such as model fruit powders (Tsami et al., 1999) and dried locust bean gum-pectin-starch composite gels (Sundaram et al., 2008). Several equations have been used to describe the sorption isotherms of many food materials (Muhtaseb et al., 2002). Some of these models are based on theories on the sorption mechanism, others are purely empirical or semi-empirical. However, none of these equations accurately describes the sorption isotherm over the whole range of water activity or for different food materials. According to Muhtaseb et al., 2002, no sorption isotherm model could fit data over the entire range of relative humidity because water is associated with the food matrix by different mechanisms in different water activity regions. The information on the moisture sorption behavior of seasoning powder is essential for determining the interaction of water with the product substances. It is also useful for food processing operations such as drying, mixing, packing, and storage since it can be used to calculate drying time and predict the behavior of ingredients upon mixing. It can also help make packaging selections, model moisture changes that occur during storage, and estimate shelf life stability (Martinelli, 2007). Furthermore, the monolayer moisture content or the minimum moisture level is of importance to the physical and chemical stability of dehydrated materials concerning lipid oxidation, enzyme activity, nonenzymatic browning, and structural characteristics (Bell, 2000).

2.3.7 Shelf life

The shelf life of seasonings powder is defined by the time during a commodity processed under certain temperature conditions. It is subjected to changes that are deemed acceptable to a certain extent by the manufacturer, consumer, and current food legislation. Shelf life depends on extrinsic factors such as production, packaging properties, ambient air temperature, and relative humidity, luminosity, and conditions of headspace, as well as intrinsic food factors such as acidity, usable oxygen, chemicals, microbial contamination levels, redox capacity, and water activity. Mixed spices powder and seasonings last around one or two years (Susheela, 2007). During preparation, seasonings powder will appear homogeneous and retain free-flowing properties while minimizing chemical reactions. As determined by both physical modifications and chemical reactions, the availability of water, processing, and storage conditions control the shelf life of food powders. Crystallization and non-enzymatic browning and oxidative degradation are avoided for food powders for glassy states, and water movement and temperature toleration together determine critical storage conditions. During the storage time, the consistency and protection of seasonings powder should be closely monitored to obtain the appropriate properties of seasonings powder which will have a long shelf life. The shelf life of seasonings powder is regulated mainly by the availability of water, and also by conditions of processing and storage to stabilize the product. The shelf life of seasonings powder was stated to be regulated mainly by 8% to 10% water availability, storage conditions in between 27°C and 38°C with a relative humidity of 65% to 92% (Gaiani et al., 2013). In short, the consistency and health of seasonings powder should be closely checked during storage time to achieve the appropriate properties of seasonings powder which will have a long shelf life.

2.3.8 Packaging

Packaging is really important for seasonings power. The packaging materials to be used must be chosen with attention, taking into account the practical and marketing specifications, to ensure the consistency of the seasonings powder during handling, transport, storage, and delivery. In general, the packaging specifications for this product are listed as, to protect the product from spillage and spoilage, to protect from conditions such as light, sun, humidity, and oxygen in the atmosphere, to be high water vapor and oxygen barriers for the chosen packaging materials. To the above functional demands, it should be easily accessible and disposable and have good machinability, printability and to avoid aroma/flavor losses and the introduction of additional odor. The packaging content should have a high barrier property. Ground herbs are sensitive and are prone to different forms of changes such as volatile oil degradation, property of free flow, microbial spoilage, and infestation of insects. Light and oxygen give way to discoloration. The lack of essential oils can also lead to a degradation of the quality of herbal powders. The glass bottles and pouches of Al foil laminate are the most suitable packages (Susheela, 2007).

2.4 Product development

Product development has evolved into an essential approach for product invention and enhancement. Product development also helps to stabilize and sustain a business. It is a synthesis of many predictive information, such as business model, product marketing, product design, cost analysis, and consumer research. This procedure is the most important for product and market management during the product's saturation and decline stages. As a result, product enhancement and new product creation can decrease and diverse business risks, allowing it to stable or expand in the future. Furthermore, this study combines relevant knowledge for ready-to-cook Korko soup base manufacturing in order to fulfill customer demand. This information was used to simplify and alter these product development goals.

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2.4.1 Consumer survey

The consumer survey is a prominent method for gathering and expressing consumer information as well as customer acceptability and attitude. The consumer survey may help the researcher to quickly understand customer behavior and develop an acceptable product. As a result, several studies have been conducted on the consumer survey of food products. In addition, the Taro Yamane equation is a well-known approach for selecting sample size in different product development studies (Yamane, 1973). Taro Yamane determination is appropriate for finite population research and dichotomous equations (Moathong et al., 2017). Hence, many studies choose a method to perform their research. However, consumer survey research must consider a variety of aspects, including cost, population type, and size, and level of confidence (Moathong et al., 2017 and Yothongyos et al., 2014).

2.4.2 Sensory evaluation

Sensory evaluation is defined as the study of human reaction to physicochemical qualities of a product through a sensory quality and can provide information about the affectability of the human sense on the product (Pimentel et al., 2016). Sensory perception has several aspects, including quantitative, qualitative, sensory features, and hedonic factors. Furthermore, sensory assessment tests are commonly used in product development, quality control, and shelf life studies to measure the quality of food samples, particularly product development guidelines and customer acceptability (Anprung, 2008 and Chompreeda, 2013). Product developers have a variety of tools at their disposal to assist them in developing or revising products that satisfy customers. Generally, hedonic reactions are acquired by product acceptability rating scales or through choice behavior (preference). However, merely achieving acceptability (in the form of overall like or preference ratings) does not automatically imply that additional product development or refinement is possible. This is due to the fact that acceptability scores or preference ratings only transmit a product's hedonic or choice status. While these scores can be compared across products or over time and supplemented with liking or preference ratings for specific attributes, liking scales and preference ratings by themselves do not help the developer understand how product attributes should be changed to improve product acceptability or relative preference. The product developer has a number of options for identifying attributes that need to be adjusted. One such strategy is the use of just-about-right (JAR) scales in customer testing. JAR scales are bipolar labeled attribute scales with a 'just about right' or 'just right' center point. The end-points are anchored by labels that signify degrees of the attribute that deviate in opposite directions from a respondent's theoretical ideal point. The goal of employing JAR scales in consumer testing is to find out whether the attribute levels under discussion should be increased or decreased.

The use of JAR scales necessitates the creation of bipolar scales for the traits of interest, which can be challenging, especially when looking for semantic opposites. While 'too thick' and 'too thin' can be used as semantic opposites (in many, but not all circumstances), 'too sweet' does not have a semantic opposite. One solution is to identify the scale with the attribute name ('sweetness,') and the end-points simply with 'much too weak' or 'not nearly enough,' and 'much too strong' or 'much too many,' respectively. Another issue comes when one of the scale's end points is difficult to link to food.

When evaluating particular component additions or flavor qualities, the usage of JAR scales may be challenging. Toppings and inclusions are common examples of such ingredients. On the pizza, chocolate chips in the cookie, and almonds in the ice cream, there may never be enough sausage. Even if the quantity of these substances is always deemed to be insufficient, future additions may not boost liking, and may even help to diminish liking. Certain flavor or texture traits have such a pleasant halo that customers will always say they want them to be improved. There may never be enough chocolate flavor in the cake, and there may never be enough creaminess in the Alfredo sauce. In other words, it's likely that the theoretical "ideal" level of that feature will never be reached. More probable, future rises will eventually result in products that are less widely appreciated.

The JAR scale data by itself do not indicate the researcher how much the attribute(s) should be increased or decreased. While it's easy to believe that a higher percentage of ratings on the "too much" side of the scale equates to a larger required attribute reduction than a lower percentage, this isn't necessarily the case. The ingredients that convey those attributes may react differently to variations in intensity within the food matrix; likewise, the sensitivity of the JAR scale to formulation modifications may vary by attribute. Finally, it's likely that modifications in formulation resulting from data in the not-quite-right' sections of the scales will alienate respondents who now think the qualities are "just right."

Many sensory evaluation studies have been undertaken to determine the quality of food products, notably seasoning products. Previously, the Hendonic scale was used in studies such as the production of ready-to-cook Tom Yam seasoning (Somkiat, 2008). The product was evaluated for overall acceptability by customers aged 15-45 using the hedonic rank test, which evaluated color, texture, and flavor after altering the amounts of a modified Tom-Yam formula. A prior study on the development of a ready-to-cook curry used the Hedonic scale and just-about-right to determine the preference score, as well as features such as appearance, color, texture, and flavor, and to select the best curry (Tanu et al., 2008). It was either used to evaluate the preference score and attributes such as appearance, aroma, texture, taste, and general acceptability of spice-mix seasonings made from functional leaves of Utazi (*Gongronema latifolium*), Uda (*Xylopia aethiopica*), Nchanwu (*Ocimum gratissimum*), and Uziza (*Piper guineense*).

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw materials

Fresh herbs such as lemongrass, kefir lime leaves, galangal, turmeric, and fermented fish were purchased from King Phet fresh market, Ratchathewi, Bankok. Seasoning such as salt, sugar and monosodium glutamate were bought from Tesco Lotus Express, Chamchuri square, Thailand.

3.1.2 Chemicals and instruments

Table 3.1 and 3.2 listed ready-to-cook Korko soup base raw materials as well as instruments and apparatus that were used in this research.

Table 3.1 Raw materials and Chemicals

No.	Raw materials and chemicals	Company, Origin
1.	Lemongrass, turmeric, garlic, galangal and kefir lime leaves, fermented fish	No brand
2.	Salt	Prung Thip, Thailand
3.	Sugar	Mitr Phol, Thailand
4.	Monosodium glutamate	Ajinomoto, Thailand
5.	Ethanol AR-grade	Sigma-Aldrich, Singapore
6.	2,2-diphenyl-1-picrylhydrazyl (DPPH)	Sigma-Aldrich, Singapore
7.	6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox)	Sigma-Aldrich, Singapore
8.	2,4,6-tripyridyl-s-triaze (TPTZ)	Sigma-Aldrich, Singapore
9.	Hydrochloric acid 37%	Sigma-Aldrich, Singapore
10.	Sodium Hydroxide Pellet (NaOH)	Sigma-Aldrich, Singapore
11.	Lithium chloride (LiCl)	Sigma-Aldrich, Singapore
12.	Potassium acetate (CH3CO2K)	Sigma-Aldrich, Singapore
13.	Magnesium chloride (MgCl2)	Sigma-Aldrich, Singapore
14.	Potassium carbonate (K2CO3)	Sigma-Aldrich, Singapore
15.	Sodium bromide (NaBr)	Sigma-Aldrich, Singapore
16.	Sodium chloride (Nacl)	Sigma-Aldrich, Singapore
17.	Potassium chloride (KCL)	Sigma-Aldrich, Singapore
18.	Potassium nitrate (KNO3)	Sigma-Aldrich, Singapore
19.	Histamine Test kit	Megazyme, Ireland
20.	Tetrasodium Salt (EDTA)	Sigma-Aldrich, Singapore

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Table 3.2 Instruments and apparatus

Name	Model	Company	Origin
1. Water activity meter	Series3 TE	AquaLab	United State
2. Chroma meter	CR-400	Konica Minolta	Japan
3. 2-Digit balance	PB1502-S	Mettler Toledo	Switzerland
4. 4-Digit balance	MS304S/01	Mettler Toledo	Switzerland
5. Hot plate stirrer	IKA C-MAG HS7 digital	Staufen	German
6. PE zip lock	-	No brand	Thailand
7. Spectrophotometer UV- Vis	Spectronic 20® Genesys™, Thermo Electron Corporation	Waltham	USA
8. Centrifuge	Hettich-Zentrifugen	Universal 32 R	Germany
9. Oven	-	-	-
10. Fume hood	-	Mastap	Thailand
11. Vortex	-	Vel Scientifica	China
12. Micropipette	-	Mettler Toledo Rainin	Thailand
13. Moisture analyzer	-	Mettle Toledo	USA

3.2 Consumer survey for preference form of product

The objective of this consumer survey was to identify directions for developing ready-to-cook Cambodian Korko soup base product especially the product form by Cambodian consumers people who in Phnom Penh, Cambodia. Also, their behaviors and attitudes toward the ready-to-cook Korko soup base product could be studied. The consumers answered a prepared questionnaire (Google form). The consumers were people from Phnom Penh, Cambodia, who are busy with working and have no enough time to cook Korko soup with fresh ingredients. The information from the consumer was used to direct the general development of the ready-to-cook Korko soup product. The selected population was Phnom Penh where there were 2,078,000 people (National Institute of Statistics, 2021). The sample size was calculated by using the simplified sample size formula, Taro Yamane's formula, there were 400 people (Dachruangsri, 2014 and Yamane, 1973). The Taro Yamane's formula was aimed at determining the sample population size that accepted, as follows:

$$n = \frac{N}{1 + Ne^2}$$

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Where n is the sample size
N is the population size
e is the acceptable sampling error
* p = 0.05 are assumed at 95% confidence level
Solve for n

$$n = \frac{2,078,000}{1+(2,078,000)(0.05)^2}$$

n=399.87 ≈ 400 people

The demographic information, consumer behavior, and attitudes about the ready-to-cook Korko soup base product were collected and analyzed using descriptive statistics. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) Version 22 (SPSS lnc., Chicago, IL, USA).

3.3 Selection of suitable drying condition (temperature and time) for drying herbs

The drying conditions may have some effect on the quality of herbs. Therefore, the experiments on drying conditions of herbs had been conducted. Herbs that were used as ingredients to develop a ready-to-cook Korko soup base include lemongrass, kefir lime leaves, galangal, garlic, and turmeric. Those mentioned herbs were washed, peeled, thinly sliced to 2 mm, and dried separately at different temperatures at 60 ºC, 70 ºC, and 80 ºC by a tray dryer to a stable weight. Then, all the dried herbs were finely ground and mixed based on a standard formula. Parameters such as drying time, moisture content, water activity, antioxidant activity in terms of DPPH, and FRAP were determined on this dried mixed herb (procedure in section 3.3.1-3.3.3). Antioxidant activity was the selection criteria to choose the best temperature for drying herbs that can maintain the product quality.

3.3.1 Moisture content

The dried mixed herbs of 2 g were weighed into an aluminum plate of moisture analyzer % (w.b.). After the sample was already placed into the instrument, its cover was closed. The result showed automatically in the final moisture content number after waiting for 2 minutes. Three replicate measurements were conducted within each sample (AOAC, 1995).

3.3.2 Water activity

A dried mixed herbs of 2 g was weighed into a plastic plate of water activity analyzer, Series3 TE, AquaLab, United State. After the sample was already placed into the instrument, it started to measure. The result showed automatically in the final water activity number after waiting for 2-5 minutes. Three replicate measurements were conducted within each sample (AOAC, 1995).

3.3.3 Antioxidant activity

The DPPH radical scavenging activity of extracts and standards was evaluated using a modified method (Shimada et al, 1992). 0.5ml of extracted samples, standard Trolox, and ethanol (blank) in the test tube was mixed with 5 mL of 0.1 mM DPPH in 100% ethanol. Left the mixed substances in the dark at room temperature for 30 min and Measured absorbance at 515 nm. The blank must be measured.

$$\text{DPPH (mg Trolox/g)} = \left[\frac{\text{AB} - \text{AS)}}{\text{AS}}\right] \times 100 \quad (1)$$

Where
AB: the absorbance of blank,
AS: the absorbance of the sample

The FRAP of extracts and standards was determined using a modified method (Benzie et al, 1996). 0.5ml of extracted samples, standard Trolox, and distilled water in the test tube were mixed with 7.5 mL of FRAP reagent. Left the mixed substances in the dark at room temperature for 30 min then measured absorbance at 593 nm. Results were reported as mg of Trolox equivalents per ml of solution (mg TE/g).

3.4 Selection of suitable total soluble solid (TSS) of fermented fish and drying condition (temperature and time) to use for developing dried mixed prototype

Fermented fish was used as an important ingredient to prepare a dried mixed prototype. However, different TSS of fermented fishes have different quality and safety, therefore, selecting a suitable TSS of fermented fish was conducted between 30 (original TSS), 45, and 60 ^{o}Brix. The different TSS of fermented fishes were made differently. (1) 30 ^{o}Brix fermented fish was prepared by blending the ordinary wet fermented fish. The total soluble solid was measured by a refractometer. (2) 45 ^{o}Brix fermented fish, the ordinary wet fermented fish was blended to become a mixture then stirred at 40 °C for 2 h to evaporate the moisture out until reaching 45 °Brix. (3) 60 ^{o}Brix fermented fish, similar to 45 ^{o}Brix, blending was conducted on the ordinary wet fermented fish to become a mixture then stirred at 40 °C for 3 h to evaporate the moisture out until reaching 60 ^{o}Brix. Then, 30, 45, and 60 ^{o}Brix fermented fishes were died separately at 70 ^{o}C, 75 ^{o}C, and 80 ^{o}C to a constant weight. Drying time, moisture content, water activity, and histamine content were determined. Moisture content, and water activity were measured according to AOAC, 1995 method as stated on 3.3.1 and 3.3.2. The suitable TSS of fermented fish and drying temperature were selected by using histamine content (section 3.4.1) as the selection criteria. The selected fermented fish was used for making a dried mixed product prototype.

3.4.1 Histamine measurement

2g of sample was added 25ml EDTA (37.2g of EDTA+750ml distilled water, adjusted the pH to 8 with sodium hydroxide solution, added water to 1000ml). The solution was boiled at 100 ºC for 20 minutes then cooled down. The mixture was centrifuged at 10,000 ×g for 5 minutes and the sample was ready to analyze. Colorimetric Enzyme of Megazyme Company, Ireland, was used for detecting histamine in samples. A1 blank (distilled water 0.2ml, EDTA 1ml, buffer 0 (distilled water 0.2ml, sample solution 1ml, buffer 0.15ml, and INT/mPMS 0.1ml,). Both blank and standard were kept for 5 minutes at dark room temperature then measured the absorbance at 492 nm. A1 blank and standard were added 0.05ml HDH then kept for 20 minutes at dark room temperature and measured the absorbance at 492 nm. Histamine concentration will be calculated by:

$$\text{Histamine(ppm)} = \left[\frac{\text{M x25 x1}}{\text{1.5 x2}}\right] \text{xF x } \Delta\text{Ahist (2)}$$

Where
M: value of histamine standard,
25: original sample extract volume,
1: sample volume in the assay,
1.5: total assay volume,
2: the weight of original sample material, a
F: dilution factor,
ΔA: absorbance change of sample (Bakke and Sato, 2005).

3.5 Prototype fabrication – powder, and different TSS fermented fish paste (60, 45, and 30)

The different forms of the ready-to-cook Korko soup base prototypes were prepared. The prototypes were a mixture of the powder of the herb (such as lemongrass, kefir lime leaves, garlic, galangal, and turmeric) drying at a selected temperature with roasted rice flour, salt, sugar, MSG, and fermented fish. Each form of the prototype was mixed with the same ingredients and formula except fermented fish. There were 4 types of fermented fishes were used to prepare 4 prototypes namely, a selected fermented fish powder, and the fermented fish paste at 60, 45, and

30 °Brix. The 4 product prototypes were mixed by blending base on the following formula:

Table 3.3 The ingredients and standard formulas of the 4 product prototypes at different forms

Prototype I ingredients (Powder form)	Standard formula (%)
Lemongrass	12.73
Kefir lime leaves	1.37
Garlic	2.89
Galangal	1.96
Turmeric	0.27
Selected fermented fish powder	21.3
Roasted rice flour	18.76
Salt	10.71
Sugar	20.79
Monosodium glutamate	2.55
Prototype II ingredients (Paste form)	**Standard formula (%)**
Lemongrass	12.73
Kefir lime leaves	1.37
Garlic	2.89
Galangal	1.96
Turmeric	0.27
60 °Brix fermented fish	25.8
Roasted rice flour	18.76
Salt	10.71
Sugar	20.79
Monosodium glutamate	2.55
Prototype III ingredients (Paste form)	**Standard formula (%)**
Lemongrass	12.73
Kefir lime leaves	1.37
Garlic	2.89
Galangal	1.96
Turmeric	0.27
45 °Brix fermented fish	31.6
Roasted rice flour	18.76
Salt	10.71
Sugar	20.79
Monosodium glutamate	2.55

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Prototype IV ingredients (Paste form)	Standard formula (%)
Lemongrass	12.73
Kefir lime leaves	1.37
Garlic	2.89
Galangal	1.96
Turmeric	0.27
30 ^{o}Brix fermented fish	47.4
Roasted rice flour	18.76
Salt	10.71
Sugar	20.79
Monosodium glutamate	2.55

Parameters such as moisture content, water activity, histamine, and microbial properties were determined on the 4 different prototypes. Moisture content, and water activity were measured according to AOAC, 1995 method as stated in 3.3.1 and 3.3.2. Histamine was measured as stated in section 3.4.1. Microbial properties were determined as the following methods. The selection criteria on those prototypes were moisture content, water activity, microbial and histamine. Besides, only one product prototype was selected for further study.

3.5.1 Total plate count

To determine total plate count, 1 ml of each dilution sample, the ready-to-cook Korko soup base, was placed in a sterile petri dish. Then, add 15-20 mL of liquid PCA media into the petri dish. Until the sample is uniformly combined, petri dishes are rotated and pushed horizontally or parallel (or in an eight shape). A blank check is performed at the same time by mixing the buffer into the media. Allow the mixture in the next petri dish to freeze. The final step is to incubate the petri dishes by placing them in the incubator upside down. For 24-48 hours, the plates were incubated at 36 ± 1 ^{o}C. Colony growth is calculated and reported in colony units forming units per gram or ml of sample (cfu / gr or ml) (Hazan et al., 2012).

3.5.2 Yeast and mold

Aseptically made 1:10 dilution of ready-to-cook Korko soup base with dilution H_2o, then blended for 2 minutes before plating. On the plate surface, yeast and mold count plates were inserted. Lift top film and carefully inoculate 1 ml test suspension onto center of film base, holding pipet perpendicular or plate. Placed the top film on top of the inoculum. Lift plastic spreader with approximate center of plate. Distributed suspension evenly using gentle downward pressure on center of spreader. Removed spreader and left plate undisturbed 1 minute to let gel solidify. The plates were placed in incubator in horizontal position, clear side up, in stacks not exceeding 20 units. At 20-25 ^{o}C, the plates were incubated for 5 days. After the incubation period, they were counted right away. Yeast had a blue-green appearance and produced tiny, well-defined colonies. Mold colonies are normally blue, although they can also take on their original color (e.g., black, yellow, green). Multiply the total number of yeast and mold colonies/plate by the appropriate dilution ratio to get the yeast and mold count. Estimated counts can be performed on plates with more than 150 colonies and should be reported as such. Doing such counts, take the average count per 1 cm^2 and multiply by 30 (Circular growth area is ca 30 cm^2). The presence of a large number of yeast colonies may cause the entire growing region to become blue. The presence of a large number of mold colonies may cause the growing region to become blue, black, yellow, or other colors (AOAC, 2000).

3.6 Proximate analysis, solubility, color and microbial property of the final product prototype

The selected prototype was determined on physical, chemical, and also microbiological properties to examine the compounds of interest related to product quality and safety. Some parameters such as moisture content, water activity, histamine, total plate count, yeast, and mold were determined based on the methods as mentioned in 3.3.1, 3.3.3, 3.4.2, 3.5.1, and 3.5.2 respectively. Ash, protein, crude fiber, fat, carbohydrate, solubility and color were determined based on the following methods:

3.6.1 Determination of Ash Content

In the porcelain crucible, 1 g of sample was accurately weighted. Over a hot plate, the crucible and sample were carefully ignited and heated until the sample was totally charred. The residue was then deposited in the muffle furnace for 5 hours at 550 °C until it was carbon-free. The crucible and ash were then weighed after cooling in the desiccator. Weighing, heating in the furnace, and chilling were all repeated until the weight remained consistent. The sample's ash content was determined as follows: Stability of the cream (AOAC, 2000).

$$\text{Ash (\%)} = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

3.6.2 Determination of Protein Content

2 g of sample was transferred to a digestion flask followed by the addition of 3 g of catalyst mixture (K2SO4:CuSO4: SeO2 in 100:20:2.5) and 20 ml of concentrated sulfuric acid. The content was then digested till transparent, liquid was

obtained. The volume of digested material was made up to 100 ml with distilled water. Carry out blank digestion without the sample and make the digested to 100 ml. Measured a liquor of digested, material was distilled with an excess of 40% NaOH solution and the liberated ammonia were collected in 20 ml of 2% boric acid solution containing 2-3 drops of mixed indicator (10 ml of 0.1 percent bromocresol green + 2 ml of 0.1 percent methyl red indicator in 95 percent alcohol). The entrapped ammonia was titrated against 0.01 N of hydrochloric acid. A reagent blank was similarly digested and distilled. Nitrogen content in the sample was calculated as follows and a factor of 6.25 was used to convert nitrogen to protein.

$$N2(\%) = \frac{Sample\ titre - blank\ titre \times HCL\ normality \times 14 vol.made\ of\ digest \times 100}{Aliquot\ of\ the\ digest\ taken \times weight\ of\ sample \times 1000}$$

Protein content= % Nitrogen× 6.25 (AOAC, 2000).

3.6.3 Determination of Crude Fiber Content

2 g of sample was weighed into 500 ml of the beaker and 200 ml of boiling 0.255 N of sulfuric acid (1.25 percent w/v) was added. The mixture was boiled for 30 min keeping the volume constant by the addition of hot water at frequent intervals (a glass rod stirred in the beaker helps smooth boiling). At the end of this period, the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from acid. The material was then transferred to the same beaker and 200 ml of boiling 0.313 N of NaOH (1.25 percent w/v) was added. After boiling for 30 min, the mixture was filtered to a crucible, dried overnight at 80-100 °C and weighed (W2). The crucible was kept in a muffle furnace at 550°C for 3 hours. Then it was cooled in desiccators and weighed again (W3). The difference in residue weights and ash represents the weight of crude fiber.

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$$\text{Crude fiber content (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where

W1= Weight of the sample (g)

W2= Weight of insoluble matter (g)

W3= Ash weight (g) (AOAC, 2000).

3.6.4 Determination of Fat Content

Accurately weighed 5 g of sample was introduced inside the thimble and a piece of cotton was placed at the open and of the thimble. The thimble containing the sample was kept inside a soxhlet apparatus fixed with a round bottom flask (500 ml) containing petroleum ether (B. P 40-60°C) 250 ml. The extraction flask was heated on the heating mantle for 14 hours at the boiling point of petroleum ether. After the extraction was completed, the ether dissolving oil was transferred into the beaker. Then, the ether was removed by evaporation. Fat content was calculated as follows: (AOAC, 2000)

$$\text{Fat (\%)} = \frac{\text{Fat weight}}{\text{sample weight}} \times 100$$

3.6.5 Determination of Carbohydrate Content

Carbohydrate value of the sample will be determined by using the following formula: (AOAC, 2000)

$$\text{Carbohydrate (\%)} = 100(\text{moisture} + \text{ash} + \text{protein} + \text{fiber} + \text{fat})$$

3.6.6 Solubility

The sample was prepared in 100 ml centrifuge tubes with 2.5 g of sample, 30 ml of distilled water, and 30 minutes of mixing with occasional stirring. The supernatant was gently poured into the petri dish after centrifugation at 3000 rpm for 10 minutes. The supernatant liquid was placed in a hot air oven to dry out. The petri dishes were cooled and weighed after drying. The solubility was calculated as weight of solids to the initial weight of the sample (g/g) (Machado et al., 1998).

3.6.7 Color

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The color of the ready-to-cook Korko soup base was measured by using Minolta® chroma meter and calibrated with a white standard plate. The measurement was obtained in the CIE system (L^*, a^*, and b^*). The results of color measurement were shown as the L^*, lightness (L^*=0 is darkness, and L^*=100 is lightest), a^* is red (+a^*) to green (-a^*), b^* is yellow (+b^*) to blue (-b^*).

3.7 Moisture sorption isotherm

Moisture sorption isotherm was conducted on the selected product prototype by a static-gravimetric method. To determine the sorption, about 0.5 (±0.001) gram of a sample of the selected ready-to-cook Korko soup base was accurately weighed into a previously weighed aluminum pan. The pan was then placed on a plastic receptacle inside the jar over different saturated salt: LiCl, CH3C00K, MgCl2, K2CO3, NaBr, NaCl, KCL, and KNO3, giving aw of 0.1130, 0.2260, 0.3280, 0.4330, 0.64, 0.7510, 0.8360, and 0.9230, respectively. The jar was then tightly closed and placed in an electric oven at 30 ^{o}C. All sample was weighed every week until a difference of less than 0.001 gram in two consecutive weighing was achieved when the moisture in the sample was assumed to be at equilibrium. After the equilibrium was reached, the moisture content was determined using the oven method by heating at 105 ºC to constant weight. All determinations were performed in triplicate. (Lewicki, 1997, and Rahman, 2006). The experimental data of all samples were fitted to sorption equations i.e. GAB model. The coefficient of determination (R^2), reduced chi-square (X^2), and root mean square error (RMSE) was used to assess the goodness of fit of the mathematical models to the experimental data. The better the fit, the higher the R^2 value and lower the X^2 and RMSE values. It is possible to calculate the X^2 and RMSE in the following way:

$$Me = \frac{X_m . C . K . a_w}{(1 - K . a_w)(1 - K . a_w + C . K . a_w)}$$

$$\chi^2 = \frac{\sum_{i=1}^{N} (W_{e,exp,i} - W_{e,pre,i})^2}{N - z}$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (W_{e,exp,i} - W_{e,pre,i})^2}$$

Where a_w= water activity; Me= moisture content at equilibrium (grams water per gram dry matter); Xm= monolayer moisture content (grams water per gram dry matter); C, and K= moisture sorption constants, We,exp,i is the ith experimental moisture content at equilibrium, and We,pre,i is the ith predicted moisture content at equilibrium. In the sorption model, N is the number of observations, and z is the number of constants.

3.8 Changes of properties during storage and shelf-life determination

3.8.1 Packaging and temperature

Laminated Aluminum bag was used to pack 300 g of the selected prototype and stored in the temperature at 30 ^{o}C, 40 ^{o}C and 50 ^{o}C for 45 days. The physical, chemical, and microbiological analysis such as moisture content, water activity, color, solubility, caking, rancidity, total plate count, yeast and mold were determined every week.

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3.8.1.1 Quality characterization

The properties and variations among the product samples were determined by quality characterization. Furthermore, the findings will be used by the researcher to better understand the causes for product potential and to address any necessary product problems. Moisture content, water activity, total plate count, yeast, and mold, solubility, and color were determined based on the methods which have stated in 3.3.1, 3.3.2, 3.5.1, 3.52, 3.6.6, and 3.6.7 respectively. Other parameters such as caking and rancidity were measured based on the following methods:

3.8.1.2 Caking

The powder caking of the ready-to-cook Korko soup base was assessed visually after exposure to various temperatures, including room temperature, 30 °C, 40 °C, and 50 °C for 45 days. The oven device was used to maintain the desired temperature. The powder was poured into the tray and spread out into a thin, 1 cm thick layer. Once the sample was spread out on the plate, it was visually examined for caking activity by running a spatula through the powder and determining if it had caked or not, as well as whether the cake was soft, solid, or hard. Triplicate samples were conducted (Fitzpatrick et al., 2007).

3.8.1.3 Rancidity

The TBA assay was carried out according to Buege and Aust, (1978). A stock solution containing 0.375 % TBA, 15 % TCA, and 0.25-N HCl was combined to 2.5 mL with 0.5 g of triplicate samples. The mixture was heated in a boiling water bath (100 °C) for 10 minutes to produce a pink color, cooled in tap water, and centrifuged for 10 minutes at 6000 rpm. The supernatant's absorbance at 532 nm was measured spectrophotometrically against a blank. The following equations were used to convert the absorbance values to ppm MDA:

TBA (ppm) = Sample A532 × 2.77 (where MDA = malonaldehyde).

3.8.2 Determination of shelf-life

The shelf-life of ready-to-cook Korko soup base was determined by monitoring changes at different temperatures. Regression equations were established using the water activity and TPA characteristics. The Q10 values and predicted shelf life were obtained at 30-40 °C and 40-50 °C, respectively.

$$Q_{10} = \frac{K_{T+10}}{K_T}$$

$$t = \frac{(A - Ao)}{K}$$

$$t = \frac{(R - Ro)}{K}$$

Where Q_{10} is the Q_{10}-value, K is the rate constant, and T is the absolute reaction temperature, A is the maximum water activity, Ao is the initial water activity, R is the maximum TPA, Ro is the initial TPA.

3.9 Sensory evaluation

The selected prototype of the ready-to-cook Korko soup base was used to cook Korko soup with chicken, and fresh vegetables such as pumpkin, green papaya, green jack fruit, pea eggplant as well as green leafy vegetables such as Yardlong bean, edible amaranth, Coccinia Grandis, and moringa by using 90 g of the sample cooking with 1.5 L of freshwater. The sample was served within 10 minutes after cooking to control the serving temperature. The panelists were all above the age of 18, lived in Phnom Penh, and had no allergies to fermented fish or herbs. Each member of the panel was given a tiny plate of the sample (20 mm x 20 mm x 3 mm). The experiment was carried out in the sensory evaluation laboratory at the Royal University of Agriculture, Department of agro-industry, under daytime fluorescent lights in separate individual sensory booths with no other odors. The sensory evaluation was conducted two times. The first sensory test was evaluated by 32 untrained panelists. The samples were evaluated for overall liking and sensory characteristics using a 6-point Hedonic scale (1= extremely dislike, and 6= extremely like) and for a just-about-right level of the characteristics using a 5-point Just-About-Right (JAR) scale before, during, and after eating (Lim, 2011; Rothman, 2007). The attributes that were evaluated for a developing direction were appearances, color, flavors, and textures. The weak attributes of the prototype were improved. The second sensory evaluation was tested by 30 untrained panelists. The process was the same as the initial sensory evaluation.

Net score

The net score, also known as graphical scaling, is calculated by subtracting the ‘Too weak' percentage from the ‘Too strong' percentage, then applying the formula below.

Net score = %Too strong - %Too weak

The attribute is identified and concerned when the net score value exceeds ±2%, however if the difference percentage is less than ±2%, the attribute is marked as non-significant difference for attributed adjustments (Anprung, 2008; Chompreeda, 2013).

The descriptive data (i.e., percentage, mean, and frequency), net score was used to examine the sensory assessment findings. After significant differences in one-way Analysis of Variance, Duncan's Multiple Range Test (p=0.05) was performed to compare the significant differences across the samples (ANOVA). These statistical analyses were performed by using SPSS program version 22.0 for windows (SPSS lnc., Chicago, IL, USA).

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CHAPTER IV
RESULTS AND DISCUSSIONS

4.1 Consumer survey for preference form of product

The results of the consumer survey (n=400) indicated a potential for developing the ready-to-cook Cambodian Korko soup base by the consumer who lived in Phnom Penh, Cambodia. The information identifies the needs and directions to develop the product prototypes. The respondents' data on the ready-to-cook Korko soup base product was obtained on the product form, packaging, and serving sizes.

Table 4.1 shows that the ready-to-cook Korko soup base should be produced in the powder form due to the easiness of the product consumption.

Table 4.1 Consumer survey of the ready-to cook Korko soup base on the product form

Form	Frequency	Percentage
Powder	178	44.5%
Cube	162	40.6%
Paste	60	14.9%

Table 4.2 shows that the ready-to-cook Korko soup base should be produced in the serving size of cooking for 4 people followed by cooking for more than 6 people. The average family members in Cambodia is 4 people (National Institute of Statistics, 2021) that is why cooking for 4 people was the most selected serving size.

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Table 4.2 Consumer survey of the ready-to-cook Korko soup base on the product serving size

Serving size	Frequency	Percentage
Cooking for 2 people	71	17.7%
Cooking for 4 people	167	41.8%
Cooking for 6 people	76	18.9%
Cooking for more than 6 people	86	21.6%

Table 4.3 shows that the ready-to-cook Korko soup base should be packed in laminated aluminum bag. Laminated aluminum bag is a good choice for the seasoning products due to it is a good barrier of oxygen and moisture. Furthermore, this kind of

packaging is convenience to consume as it is light, flexible and also printable (Amudha et al., 2011).

Table 4.3 Consumer survey of the ready-to-cook Korko soup base on the product packaging

Packaging	Frequency	Percentage
Laminated aluminum bag	122	30.5%
Paper	93	23.3%
Glass	89	22.3%
Plastic	96	23.9%

In short, the consumer survey results found that the ready-to-cook Korko soup base should be produced in powder form due to it is easy to consume, packed in an aluminum bag sealed because it is good oxygen and moisture barrier which can protect the product and also light, flexible, printable, besides, cooking for 4 people was the most favorite serving size due to it is the average family members in Cambodia.

4.2 Selection of suitable drying condition (temperature and time) for drying herbs

4.2.1. Drying duration at different drying temperatures

The initial moisture content of fresh lemongrass, kaffir lime leaves, galangal, garlic, and turmeric samples were found to be 73, 66, 71.2, 69, and 72.3 % (w.b.) respectively. It could be observed from Table 4.4 that when all herb was dried until its weight is constant, the final moisture content of mixed herbs was between 1.7-1.8 % (w.b.) and water activity is around 0.37-0.38. The drying time is varied according to the different drying temperatures.

Table 4.4 Drying time of dried mixed herbs at different drying temperatures

Drying temperature (°C)	**Drying hour (h)**	**Final moisture content % (w.b.)**	**Water activity (a_w)**
60	10	$1.80^{a}\pm0.00$	$0.38^{a}\pm0.01$
70	7.5	$1.79^{a}\pm0.00$	$0.37^{a}\pm0.00$
80	5	$1.78^{a}\pm0.01$	$0.37^{a}\pm0.05$

[a] Mean values±SD (n=3) were indicated significant difference within the same column ($p\leq0.05$)

The dried mixed herbs from 80 °C showed shorter drying time than 70 °C, and 60 °C. This indicated that the higher temperature can lead to faster decrease in moisture content as the previous research also reported that. An increase in drying temperature can increase the rate of heat transfer and, consequently, reduced the total drying time. The moisture loss occurs differently because the water transfer mechanism occurs by mass diffusion. The shelf stability of a food product depends on the moisture content, therefore, the higher the moisture content, the lower the shelf stability (Siripongvutikorn et al., 2008). The moisture contents of dried mixed herbs were within the recommended moisture content (<12 % (w.b.) (Barbosa et al., 2008). This is an indication that all the dried samples are likely to stay longer before further use or processing due to their low moisture content and water activity since the low moisture content and water activity of the herbs coupled with drying could hinder the growth of microorganisms, hence storage life would be longer (Awogbemi et al., 2009 and Shelef, 1983). The water activity at the equilibrium of dried mixed herbs powder and fermented fishes from all drying temperatures was lower than 0.6 which suggested that at this level those samples contained lower risk where deteriorative chemical and biochemical reaction rates are reduced to a minimum (Vera et al., 2007). Those reducing water activity below 0.6 also indicated the prevention of microbiological spoilage (Shelef, 1983).

4.2.2 Antioxidant activity via DPPH radical-scavenging activity and FRAP

DPPH free radical-scavenging activity and FRAP of the dried mixed herbs powder were investigated to determine their antioxidant activities. The findings are expressed as milligram Trolox per gram sample in Table 4.5.

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Table 4.5 Antioxidant properties of dried mixed herbs powder using different drying temperatures

Drying temperature (^{o}C)	**DPPH (mg Trolox/g)**	**FRAP (mg Trolox/g)**
60	288.73^{a}±0.05	67.4^{a}±0.02
70	278.48^{ab}±0.02	64.8^{a}±0.05
80	238.00^{b}±0.05	60.37^{a}±0.04

^{a,b} Mean values±SD (n=3) were indicated significant difference within the same column (p≤0.05)

The DPPH radical scavenging activity and FRAP were investigated based on different drying temperatures ($p<0.05$). The current study found that the variation of drying temperature affects the antioxidant activity of dried mixed herbs powder, as shown in table 4.5. The antioxidant activity of the dried mixed herb powder reduced as the drying temperature increased.

DPPH radical-scavenging activity determined during the drying process was 238.00 ±0.05 mg Trolox/g at the temperature of 80 ^{o}C. The results of this present investigation showed that the variation of drying temperatures affect the antioxidant activity of the dried mixed herbs powder as presented in table 4.5. The antioxidant activity of the dried mixed herbs powder decreased with increasing drying temperature. Higher drying temperature can be affected on the total antioxidant activity because most antioxidant compounds are phenolic compounds that can converse or release glucosides or bound phenolics into free phenolic derivatives that influence by temperature (Pokorny and Schmidt, 2003). As reported by some authors, long drying times associated with low process temperature may also promote a decrease in antioxidant activity due to heat-sensitive and easily oxidized (Garau, 2007 and Khoddami et al., 2013).

The experimental results showed that FRAP decreased with increasing temperature as observed in table 4.5. At 80 ^{o}C, the highest drying temperature, the degradation of FRAP is the least compared to other temperatures, 70 ^{o}C, and 60 ^{o}C. It was observed that an increase in drying temperature caused degradation of FRAP concerning corresponding content in the fresh samples ($p<0.05$). Prolonged drying time did not necessarily produce the severe degradation, a temperature rise led to greater cause degradation of antioxidant substances that contributed in FRAP (Ponciano et al., 1996). This is probably due to high convective heat transfer acting at the air–solid interface retarding heat diffusion into the solid herbs which can breakdown the antioxidant activity. Decreasing correlation between antioxidant activities has been reported during food dehydration. However, data on the effects of drying on antioxidant activities of different samples are rather conflicting due to several factors, such as the drying method, the type of extraction solvent, the antioxidant assays, and the interactions of several antioxidant reactions (Réblová, 2012). As mentioned above, decreasing antioxidant activity with increasing temperature is generally explained by the acceleration of initiation reactions associated with faster utilization of antioxidants (Lara et al., 2000). This study implies that a decrease in antioxidant activity with increasing temperature (faster for antioxidants with higher oxidation potential) is caused by a decrease in the ability of antioxidants to react with free radicals. Consequently, it is logical that the easily oxidizable antioxidants lose their antioxidant activity sooner than the less oxidizable ones.

In short, according to the finding, DPPH and FRAP were significantly decreased when increasing temperature, then, we can assume that the higher temperature, the lower the antioxidant activity. Therefore, all the fresh herbs should be dried at 60 ^{o}C to maintain the quality of the final product.

4.3 Selection of suitable total soluble solid (TSS) of fermented fish and drying condition (temperature and time) to use for developing dried mixed prototype

4.3.1 Duration of drying and physical property of fermented fish at different TSS

Table 4.6 Drying time and physical property of 30 ^{o}Brix fermented fish at different drying temperatures

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Drying temperature	**Drying hour (h)**	**Final moisture content % (w.b.)**	**Water activity (a_w)**
70	22	$9.77^{a}\pm0.05$	$0.38^{a}\pm0.02$
75	19	$9.72^{a}\pm0.02$	$0.375^{a}\pm0.02$
80	14	$9.69^{b}\pm0.01$	$0.355^{b}\pm0.01$

^{a,b} Mean values±SD (n=3) were indicated significant difference within the same column (p≤0.05)

Table 4.7 Drying time and physical property of 45 ^{o}Brix fermented fish at different drying temperatures

Drying temperature	**Drying hour (h)**	**Final moisture content % (w.b.)**	**Water activity (a_w)**
70	18	$9.73^{a}\pm0.02$	$0.384^{a}\pm0.05$
75	16	$9.79^{a}\pm0.01$	$0.367^{b}\pm0.02$
80	12	$9.65^{b}\pm0.07$	$0.349^{b}\pm0.05$

^{a,b} Mean values±SD (n=3) were indicated significant difference within the same column (p≤0.05)

Table 4.8 Drying time and physical property of 60 ^{o}Brix fermented fish at different drying temperatures

Drying temperature	**Drying hour (h)**	**Final moisture content % (w.b.)**	**Water activity (a_w)**
70	16	$9.7^{a}\pm0.01$	$0.381^{a}\pm0.05$
75	14	$9.65^{a}\pm0.05$	$0.362^{a}\pm0.01$
80	10	$9.61^{b}\pm0.05$	$0.331^{b}\pm0.07$

^{a,b} Mean values±SD (n=3) were indicated significant difference within the same column (p≤0.05)

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Final moisture content of the fermented fishes was significantly decreased while the temperature was increased. At 70 ^{o}C, the moisture content of the 30 ^{o}Brix fermented fishes was 9.77 ±0.05% (w.b.) higher than drying at 75 ^{o}C, and 80 ^{o}C, 9.69 ±0.01 % (w.b.). For 45 and 60 ^{o}Brix fermented fishes moisture contents were also decreased when the temperatures were increased. Because of the decrease in water in the external resistance during the first drying phase, as well as increased heat transfer at higher drying temperatures, moisture content decreases as drying temperature rises (Femenia et al., 1998). The dried mixed herbs from 80 °C took less time to dry than those from 70 °C and 60 °C. The moisture content of fermented fishes was within the recommended range (12 % (w.b)) (Barbosa et al., 2008). This indicates that due to their low moisture content and water activity, all dried samples are likely to last longer before being used or processed. Because the fermented fishes' low moisture content and water activity, combined with drying, could inhibit the growth of microorganisms, storage life would be extended (Awogbemi et al., 2009 and Shelef, 1983). The water activity (a_w) of all the fermented fishes at 70 °C of drying temperature showed higher water activity than the temperatures of 75 °C and 80 °C. Furthermore, the water activity of dried mixed herbs powder and fermented fishes at equilibrium from all drying temperatures was less than 0.6, implying that at this level, those samples included lower risk, with deteriorative chemical and biochemical reaction rates lowered to a minimum (Vera et al., 2007).

4.3.2 Histamine measurement

Histamine was investigated in this study to see the effect of drying temperatures and °Brix on histamine level in fermented fish. The result showed significantly different at different drying temperatures as 70°C, 75 °C, 80 °C, and °Brix as 30 °Brix, 45 °Brix, and 60 °Brix (p< 0.05) as observed in Table 4.9.

Table 4.9 Histamine contents of different TSS of fermented fish at different drying temperatures

Drying temperature (°C)	**Histamine (ppm)**		
	30 °Brix	**45 °Brix**	**60 °Brix**
70	$4.2^{a}\pm0.02$	$16.5^{a}\pm0.05$	$17.2^{a}\pm0.05$
75	$39^{b}\pm0.04$	$40.3^{b}\pm0.06$	$139.8^{b}\pm0.06$
80	$45^{c}\pm0.09$	$49.7^{c}\pm0.05$	$148.9^{c}\pm0.05$

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^{a,b,c} Mean values±SD (n=3) were indicated significant difference within the same column (p≤0.05)

At 30 °Brix, the histamine level of fermented fish drying at 70 °C (4.2±0.02ppm) was significantly lower than 75 °C (39±0.04ppm) and 80 °C (45±0.09ppm). Fermented fish at 45 °Brix showed the lowest histamine level at 70 °C (16.5±0.05ppm) significantly lower than 75 °C (40.3±0.06ppm) and 80 °C (49.7±0.05ppm). Drying temperature at 80 °C (148.9±0.05ppm) was significantly higher than 75 °C (139.8±0.06ppm) and 70 °C (17.2±0.05ppm), at 60 °Brix. The histamine level of fermented fish was increased while increasing drying temperature and °Brix. The histamine level of most fishery products and processed marine products was increased by temperature, and it seems that most of them increased greatly. Pre-requisites for the formation of histamine are the availability of free amino acids, such as histidine, presence of decarboxylase active microorganisms, and favorable conditions for decarboxylation of amino acids. The increase of histamine by the dehydrated process has usually been reported (Bo Young et al., 2017). The possible reason for these changes can be explained by the moisture loss by evaporation during drying could cause the histamine concentration to increase faster due to the condensation. In addition, another possible reason for the differences is that the histamine formation is affected by histidine decarboxylase activity. It is known that there are several factors (e.g., pH, temperature, and NaCl concentration) that affect histidine decarboxylase activity. This enzyme activity increased with increasing temperature. Consequently, during the dehydration process, the histamine in fermented fish would accumulate continuously until the enzyme was inactive (Kanki et al., 2007). This may explain why the histamine level was increased by temperature and °Brix in this study.

Based on the above investigation, at a lower temperature, the moisture evaporation is slower than at a higher temperature that can slower the histamine concentration. Therefore, 30 °Brix fermented fish drying at 70 °C was selected for further study due to its histamine content was significantly lower compared to fermented fishes at higher concentrations and temperature. Choosing a suitable concentration and drying temperature for fermented fish is really important to avoid food allergies to the consumer, which cause by histamine, due to concentration of fermented fish and drying temperature can change the histamine content in the product. Likewise, controlling these two parameters can help to maintain product safety while making the further product prototypes.

4.4 Prototype fabrication – powder, and different TSS fermented fish paste (60, 45, and 30)

4.4.1 Physical, chemical, and microbiological properties of the four different prototypes

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Four final product prototypes exhibited different water activities and forms based on the properties of the prepared fermented fishes. Water activity, moisture content, histamine, total plate count, yeast, and mold were measured on those four final product prototypes.

Figure 4.1 Ready-to-cook Cambodian Korko soup base in dried form

Figure 4.2 Ready-to-cook Cambodian Korko soup base at 60 °Brix form

Figure 4.3 Ready-to-cook Cambodian Korko soup base at 45 °Brix form

Figure 4.4 Ready-to-cook Cambodian Korko soup base at 30 °Brix form

Table 4.10 Physical, chemical, and microbiological properties of the four prototypes

Physical and microbial properties	Dried form	60 °Brix paste	45 °Brix paste	30 °Brix paste
Moisture content % (w.b.)	$2.67^a \pm 0.34$	$16.5^b \pm 1.04$	$31.32^c \pm 0.43$	$45.26^d \pm 0.23$
Water activity (a_w)	$0.38^a \pm 0.56$	$0.69^b \pm 0.76$	$0.74^c \pm 0.76$	$0.81^d \pm 0.76$

Histamine (ppm)	48.2^{a}±0.02	102.32^{b}±1.12	88.32^{c}±1.48	43.17^{d}±2.28
Total plate count < 250 CFU/g	< 250 CFU/g	>250 CFU/g	>250 CFU/g	>250 CFU/g
Yeast and mold < 100 CFU/g	< 100 CFU/g	> 250 CFU/g	> 250 CFU/g	> 250 CFU/g

^{a,b,c,d} Mean values±SD (n=3) were indicated significant difference within the same raw ($p\leq0.05$)

The dried form was the prototype which was composed of all dried powder materials such as lemongrass, turmeric, garlic, kefir lime leaves, galangal, sugar, salt, MSG, selected fermented fish, and roasted rice. Hence, 60, 45, and 30 ^{o}Brix pastes were the product prototypes composed of the same powder materials as dried form excepted fermented fish. The fermented fish was the only main ingredient to define the concentration and form of the prototype 2,3 and 4.

All the dried flour raw materials such as lemongrass, turmeric, garlic, kefir lime leaves, galangal, sugar, salt, MSG, and roasted rice were ready to mix with different forms and concentrations fermented fishes to become final product prototypes. They were in dried, and paste form (°Brix 60, 45, and 30) based on the standard formula. Table 4.10 shows that the lowest moisture content of dried form seasoning was 2.67±0.34 % (w.b.) and 45.26±0.23 % (w.b.) was the highest moisture content of 30 °Brix ready-to-cook Korko soup base. The moisture content % (w.b.) of the 60, 45, and 30 °Brix samples were more than criterion 12 % and they may not stable during storage and prone to microbiological deterioration. The water activity of dried form samples was found 0.38±0.56 and this was the lowest number compared to the highest level 0.81±0.76 for 30 °Brix samples. Besides, the number of water activities found on the 60, 45 and 30 °Brix ready-to-cook Korko soup base were higher than 0.6 which is over the criterion and is not good for the product stability. Above 0.6 the chemical reaction rates are increased (Vera et al., 2007). Hence, microbiology was also increased among those that had water activity above 0.6 (Shelef, 1983). Besides, histamine content in the dried form was also significantly the lower compared to the other product prototype excepted 30 °Brix. Then, total plate count, yeast, and mold on the four different water activities product prototypes showed that only the lowest water activity sample was found under the limitation, however, 60, 45, and 30 °Brix were found over the limitation which is unsafe to the consumer. The over limitation of total plate count, yeast, and mold maybe because of lacking hygiene practice while making fermented fish and sometimes also caused by the source of fishes for making fermented fish as it lived under unclean water and cause contamination to the final product due to high °Brix fermented fishes did not involve with any thermal process to kill the microbial before making the prototypes. Based on the results on moisture content, water activity, histamine, and microbial analysis on the four different concentrations, and forms product prototypes, there was only dried form ready-to-cook Korko seasoning was selected for further study.

4.5 Proximate analysis, solubility, color and microbial property of the final product prototype

The final product was investigated for proximate, physical, chemical, and microbiological, shows in Table 4.11. The final product prototype found 2.79±0.67 % (w.b.) of moisture content under Codex Alimentarius criteria, (<12 % (w.b.) and the water activity was found 0.375±0.04 lower than 0.6, which suggested that at this level those samples contained lower risk where deteriorative chemical and biochemical reaction rates are reduced to a minimum (Vira et al., 2007). Those reducing water activity below 0.6 also indicated the prevention of microbiological spoilage (Shelef, 1983). The proximate parameters such as ash, fat, protein, crude fiber, and carbohydrate were found 22.39, 2.63, 10.22, 4.27, 62.97 g/100 g, respectively. Hence, the product solubility found 60.82±1.56 which is not exhibited excellent solubility in water (>90%). The product was involved with fermented fish which also can cause risk to the consumer. However, the product prototype resulted from 32.56±0.97 ppm of histamine which is under 200 ppm (FDA, 2001) and this is safe for the customer to consume the product.

In Table 4.11, the mean values and standard derivations of color coordinates *L**, *a**, *b** of the product prototypes shows 62.07±0.56, 2.37±0.25, and 20.55±0.03 respectively. The evolution of the microorganisms in the ready-to-cook Korko soup powder is also presented in Table 4.11. The total plate count (TPC), yeasts, and molds determined less than 25 cfu/g. Because the ready-to-cook Korko soup base is a dehydrated product that contained low water activity, below 0.6, that not suitable for microorganism growth (Shelef, 1983).

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Table 4.11 Physical, chemical and microbiological analysis on the final product prototype (dried form)

Physical, chemical and microbiological properties	Value
1. Moisture content % (w.b.)	2.79±0.67
2. Water activity (a_w)	0.375±0.04
3. Ash content (g/100 g)	22.39
4. Protein content (g/100 g)	10.22
5. Crude fiber (g/100 g)	4.27

6. Fat content (g/100 g)	2.63
7. Carbohydrate (g/100 g)	62.97
10. Solubility %	60.82±1.56
8. Histamine (ppm)	32.56±0.97
9. Color	*L**=62.07±0.56
	*a**=2.37±0.25
	*b**=20.55±0.03
10. Total plate count	15 CFU/g
11. Yeast and mold	< 100 CFU/g

4.6 Moisture sorption isotherm

The dried powder form of the instant Korko soup base was selected to do moisture sorption isotherm. During the processing and storage of the ready-to-cook Korko soup base, physical, chemical, and microbiological changes occur. The changes are influenced particularly by the moisture content and water activity of food material. The equilibrium moisture content of a food material is defined as its moisture content attained when the vapor pressure of water present in the food has reached an equilibrium with its surroundings. It is affected by relative humidity and temperature. Water activity on the other hand is defined as the ratio of the vapor pressure of water material over the foodstuff to that of pure water at the same temperature. The relationship between moisture content and water activity in food at constant temperature and pressure is often expressed as a moisture sorption isotherm. It can give information on the sorption mechanism and the interaction of instant Korko seasoning with water. The typical shape of the sorption isotherm may change depending on the type of product and reflects how water binds to the system (Bell and Labuza, 2000). The moisture sorption data obtained could give useful information to guide the storage of instant Korko powder.

The experimental equilibrated moisture content data of ready-to-cook Korko soup powder was fitted against the water activity on multiple sorption models to fit sorption models to equilibrium moisture data. To choose the best fitting equation, statistical test methods such as the coefficient of determination (R^2), the reduced chi-square (χ^2) and the root mean square error (RMSE) were considered. The GAB model for the ready-to-cook Korko soup powder had the highest chance of fitting the experimental data with the highest values for R^2 and the lowest values for (χ^2) and

RMSE, according to the results. R^2 values of 0.87, (χ^2) values of 0.11, and RMSE values of 0.17 are calculated using the experimental and predicted findings. In comparison to the GAB equation, the model was found to have a higher chance of excellent fit for the experimental data.

Normally, the GAB equation's parameters have physical meaning: Xm is the monolayer content, C is the total heat of sorption of the first layer, and K is a factor that corrects multilayer molecules' properties in relation to the bulk liquid. However, based on the mathematical study of the model, Lewicki, 1997 proposed several alternate limit values for parameters C and K. According to the above author, the parameters should have values in the ranges $C \geq 5.67$ and $0.24 \leq K \leq 1$. The calculated C results in the present study are not in accordance with the limit values, and hence are not regarded to meet the theoretical requirements. Despite this, no physical significance could be attributed to the equation's parameters. For apple cellular fiber, carrot, coffee, mushroom, wheat bran flour, and yeast isotherms, Lewicki 1997 reported similar results.

The monolayer of ready-to-cook Cambodian Korko soup powder was found at 0.068 gram water per gram dry matter while the total heat of sorption of the first layer (C) is 1.08 and the factor correcting the properties of multilayer molecules (K) is 0.83. The moisture sorption profile and fitted with GAB model of ready-to-cook Korko soup base are shown in Figure 5.1. As the initial moisture content of the ready-to-cook Korko soup base was low, adsorption was dominant. The equilibrated moisture content (Me) of the ready-to-cook Korko soup base increased with a water activity (a_w). This might be since the vapor pressure of water present in samples increased with that of the surroundings. The moisture content increased very slowly with an increase in water activity up to 0.432. From this point on there was a gradual increase in moisture content with an increase in water activity up to 0.851, beyond which there was a steep rise in moisture in the sample. The sorption isotherm allows having a general idea of the characteristic of the hygroscopicity of the powder. Hence it can also predict the shelf stability of this product under specific process and environmental conditions.

Table 4.12 Statistical results and estimated values of GAB model parameters

Model	**Parameters**					
GAB	X_m	C	K	R^2	χ^2	RMSE
	0.068	1.08	0.83	0.87	0.11	0.17

$$Me = \frac{0.06.a_w}{(1-0.83.a_w)(1-0.83.a_w + 0.89.a_w)}$$

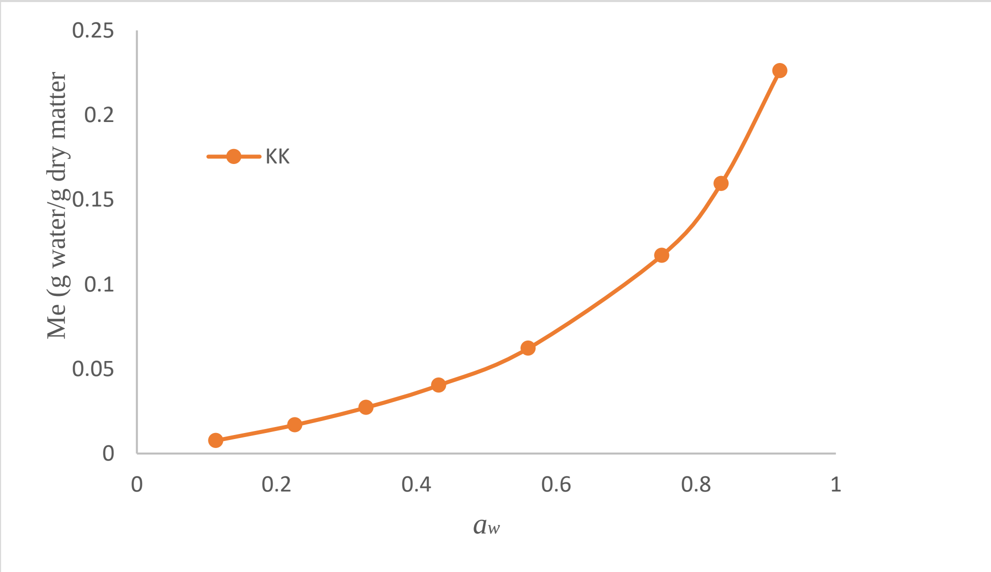

Figure 5 Moisture sorption isotherm of ready-to-cook Korko soup base powder (KK) at 30 ^{o}C and fitted with the GAB model.

Figure 4.5 shows KK was the sorption isotherm curve obtained from the ready-to-cook Korko soup at equilibrium moisture content and fitted with the GAB model.

According to the classification of Al-Muhtaseb et al, 2002, sorption isotherms obtained exhibited type III behavior in which a small amount of water is adsorbed at low water activity and a larger amount is adsorbed at higher water activity, and once the bulk moisture point has been reached, the powder rapidly adsorbs large amounts of water vapor, causing it to deliquesce and leading to a steep rise in the third part of the curve, corresponding to the formation of hydrate (Sundaram et al, 2008). The linear shape at the first part of the isotherms is caused by water adsorption on to the biopolymers and the sharp increase in water content at high water activity is due to the gradual dissolution of solutes such as salts and sugars (Tsami et al, 1999).

Al-Muhtaseb et al, 2002 reported that foods rich in soluble components show isotherms with type III behavior owing to the solubility of the components in water. Similar isotherm behavior has been found in crushed chilies (Arslan et al, 2005), pistachio nut paste (Maskan et al, 1997), model fruit powder (Drouzas et al, 1999), pineapple pulp powder, fruits rich in sugar such as grape, apricot and apple (Gabas et al, 2007) and salted alligator meat (Filho et al, 2002). It is known that the shape and position of an isotherm for food is influenced by sample composition, physical structure (crystalline or amorphous), pretreatment, and method of processing (Gabas et al, 2007). According to the finding, the ready-to-cook Korko powder inhibited type III behavior.

The value of the monolayer moisture content (Xm) is of particular interest since it indicates the amount of water that is strongly adsorbed to specific sites at the food surface. It is considered as the optimum value to assure food stability. For most dry foods, the rate of quality loss due to chemical reactions is negligible at the monolayer value. Therefore, this value is important for the storage of Korko instant powder, since at this moisture level water does not act as a solvent, being biologically inert. The monolayer value in this study was determined by using the GAB equation and was found at 0.068 gram water per gram dry matter, the values at which the Korko instant powder keeps very well on storage for a long period of time. Tsami et al, 1999 following adsorption at 25°C found for model fruit powder the Xm values between 0.060-0.097, which is the same in the order of magnitude as Xm estimated in the present work. The monolayer values for pineapple powder, however, were between 0.146-0.166 gram water per gram dry matter following adsorption at 20-50°C (Martinelli, 2007). Kaymak-Ertekin and Gedik, 2004 found for grapes, apricots and apples Xm values between 0.095-0.220 gram water per gram dry matter, and those for potatoes between 0.067-0.073 gram water per gram dry matter following adsorption and desorption at 30-60°C. The value of Xm for Korko instant powder seems to be lower than those high-sugar fruits and fruit powder but higher than those for starchy foods. Due to the monolayer value was found 0.068-gram water per gram dry matter, the values at which the instant Korko seasoning powder keeps very well on storage for a long period of time, the instant Korko seasoning powder should make at this value.

4.7 Changes of properties during storage and shelf-life determination

4.7.1 Packaging and temperature

The shelf life of spice mixed powder is relatively long compared with other type of foods. Accelerated shelf life testing (ASLT) is a method that has been used to predict shelf life of many food products. Exposing the food to extreme conditions by accelerating the degradation mechanism will lead to a shorter time to reach the endpoint of shelf life (Lloyd et al., 2009). Accelerating conditions can be any factors that increase the rate of degradation. The most common example is using temperature (Lilliebjelke, and Torbjörn, 2010). The objective of the presented master thesis work was to examine the possibility to use ASLT to determine the quality changes, and shelf life of the ready-to-cook Korko soup seasoning powder. The accelerated storage process was done using temperature as the critical factor.

Table 4.13 Changes in moisture content (% (w.b.) of ready-to-cook Korko soup base keeping for 6 weeks at different temperatures

Storage duration (Weeks)	Moisture content % (w.b.)		
	30 ºC	40 ºC	50 ºC
0	2.96^{a}±0.00	2.96^{a}±0.00	2.96^{a}±0.09
1	2.96^{a}±0.007	3.02^{b}±0.01	3.18^{b}±0.02
2	2.96^{a}±0.007	3.12^{b}±0.05	3.27^{b}±0.04
3	2.97^{a}±0.007	3.19^{b}±0.02	3.32^{c}±0.03
4	2.98^{a}±0.00	3.38^{b}±0.07	3.46^{c}±0.02
5	3.11^{a}±0.05	3.44^{b}±0.09	3.52^{c}±0.07
6	3.16^{a}±0.5	3.49^{b}±0.05	3.62^{c}±0.04

^{a,b,c} Mean values±SD (n=3) were indicated significant difference within the same raw ($p \leq 0.05$)

The numbers indicated that there was moisture content % (w.b.) changes of ready-to-cook Korko soup base which had been kept in 3 different levels of temperature. When the storage duration of 6 weeks, the moisture content in ready-to-cook Korko soup base powder had increased each week. But the moisture content % (w.b.) of the samples was less than the criterion which was under the percentage of 12 which is the safe level for the product.

Table 4.14 Changes in water activity of ready-to-cook Korko soup base keeping for 6 weeks at different temperatures

Storage duration (Weeks)	Water activity (a_w)		
	30 ºC	40 ºC	50 ºC
0	0.34^{a}±0.00	0.34^{a}±0.00	0.34^{a}±0.00
1	0.35^{a}±0.007	0.37^{a}±0.04	0.37^{b}±0.13
2	0.35^{a}±0.007	0.39^{a}±0.2	0.38^{b}±0.27

3	0.36^{a}±0.01	0.40^{a}±0.12	0.39^{b}±0.17
4	0.36^{a}±0.00	0.42^{a}±0.007	0.43^{b}±0.02
5	0.37^{a}±0.00	0.44^{b}±0.005	0.44^{b}±0.05
6	0.37^{a}±0.00	0.46^{a}±0.009	0.47^{b}±0.09

^{a,b} Mean values±SD (n=3) were indicated significant difference within the same raw ($p \leq 0.05$)

The numbers indicated that when keeping the ready-to-cook Korko soup base in 3 different levels of temperature for 6 weeks, the water activity (a_w) was increased each frequency. Hence, the ready-to-cook Korko soup base had a lower water activity than the criterion of 0.6 which is good for the product stability where deteriorative chemical and biochemical reaction rates are reduced to a minimum (Vera et al., 2007). Microbiological deterioration was also avoided among those that reduced water activity below 0.6 (Shelef, 1983).

Table 4.15 Changes in water solubility (%) of ready-to-cook Korko soup base keeping for 6 weeks at different temperatures

Storage duration (Weeks)	**Water solubility (%)**		
	30 ºC	**40 ºC**	**50 ºC**
0	60.00^{a}±0.00	60.00^{a}±0.00	60.00^{a}±0.00
1	60.05^{a}±0.49	59.89^{a}±0.43	59.21^{b}±0.23
2	60.00^{a}±0.14	59.52^{b}±1.27	59.01^{c}±0.12
3	59.8^{a}±0.14	59.03^{b}±1.87	58.87^{c}±0.09
4	60.03^{a}±0.14	58.85^{b}±0.98	58.32^{c}±1.28
5	60.01^{a}±0.00	58.27^{b}±0.54	57.38^{c}±0.43
6	60.0^{a}±0.00	57.75^{b}±1.12	56.72^{c}±0.36

^{a,b,c} Mean values±SD (n=3) were indicated significant difference within the same raw ($p \leq 0.05$)

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Table 4.15 shows that there was the amount of water-soluble (%) changed of the ready-to-cook Korko soup base which had been kept in 3 different levels of temperature. The amount of water-soluble was significantly decreased in each frequency. This may be due to the moisture of powder and formation of insoluble compounds. This can be also explained by the formation of a thin layer of wet particles on the phase's border which makes the penetration of water into the particles of powder difficult.

Table 4.16 Changes in rancidity of ready-to-cook Korko soup base keeping for 6 weeks at different temperatures

Storage duration (Weeks)	**TPA value (ppm)**		
	30 ^{o}C	**40 ^{o}C**	**50 ^{o}C**
0	$0.8^{a}\pm0.00$	$0.8^{a}\pm0.00$	$0.8^{a}\pm0.00$
1	$0.80^{a}\pm0.007$	$0.81^{a}\pm0.04$	$0.83^{c}\pm0.007$
2	$0.81^{a}\pm0.01$	$0.83^{b}\pm0.02$	$0.85^{c}\pm0.05$
3	$0.79^{a}\pm0.007$	$0.88^{a}\pm0.22$	$0.89^{b}\pm0.21$
4	$0.79^{a}\pm0.01$	$0.92^{b}\pm0.21$	$0.99^{c}\pm0.007$
5	$0.8^{a}\pm0.00$	$1.07^{b}\pm0.12$	$1.25^{c}\pm0.07$
6	$0.81^{a}\pm0.00$	$1.19^{b}\pm0.00$	$1.28^{c}\pm0.72$

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[a,b,c] Mean values±SD (n=3) were indicated significant difference within the same raw ($p\leq0.05$)

The TBA values in the ready-to-cook Korko soup base after 6 weeks storage was as high as 2.43 for 50 ^{o}C, and as low as 0.8 for 30 ^{o}C, respectively (Table 4.16). Thus, the ready-to-cook Korko seasoning at 30 ^{o}C was the most potent sample where TBA values at 1-10 (ppm) are possibly associated with the perception of rancid flavor. Lipid oxidation is one of the major reasons that foods deteriorate and is caused by the reaction of fats and oils with molecular oxygen leading to off-flavors that are generally called rancidity. Exposure to light, pro-oxidants and elevated temperature will accelerate the reaction. Rancidity is associated with characteristic off-flavor and odor of the food. There are two major causes of rancidity. One occurs when oil reacts with oxygen and is called oxidative rancidity. The other cause of rancidity is by a combination of enzymes and moisture. Enzymes such as lipases liberates fatty acids from the triglyceride to monoglycerides and free fatty acids and such liberation of free fatty acids is called hydrolysis (Hamilton, 1994). According to Hamilton (1994) hydrolysis is also caused by chemical action that is prompted by factors such as heat or presence of water. (Hamilton, 1994). Rancidity caused by hydrolysis is called hydrolytic rancidity. Oxidation is concerned mainly with the unsaturated fatty acids. Oxidative rancidity is off special interest as it leads to the development of unfavorable off-flavors that can be detected early on the development of rancidity.

Table 4.17 Changes in color of ready-to-cook Korko soup base keeping for 6 weeks at different temperatures

Temperature	**Time week**	**Color coordinates (nd)**		
		L*	***a****	***b****
30 ^{o}C	0	64.67±0.0	2.47±0.0	20.35±0.0
	1	64.62±007	2.43±001	20.08±0.04
	2	64.4±67	2.26±004	19.88±0.12
	3	64.34±0.12	2.16±0.07	19.45±0.01
	4	64.12±072	1.97±0.02	19.07±0.07
	5	63.97±042	1.94±0.03	18.95±0.02
	6	63.87±004	1.92±0.01	18.46±0.02
40 ^{o}C	0	64.67±0.0	2.47±0.0	20.35±0.0
	1	64.62±0.35	2.43±001	20.07±0.07
	2	64.42±0.63	2.26±004	19.59±0.35
	3	64.38±0.15	2.16±0.07	19.44±0.01
	4	64.15±0.73	1.97±0.02	18.48±0.23
	5	63.87±098	1.94±0.03	18.06±0.09
	6	62.85±0.25	1.92±0.01	17.22±0.02
50 ^{o}C	0	64.67±0.0	2.47±0.0	20.35±0.0
	1	64.72±0.21	2.45±0.02	20.09±0.09
	2	64.17±0.7	2.28±0.1	19.58±0.38
	3	63.42±0.35	1.99±0.02	19.44±0.05
	4	62.65±0.02	1.52±0.57	18.51±0.23
	5	61.37±028	1.34±0.24	16.62±2.19
	6	60.78±0.16	1.32±0.13	15.25±0.03

^{a,b,c} Mean values±SD (n=3) were indicated significant difference within the same raw ($p \leq 0.05$)
nd means not significantly different

The color coordination in the CIE system of the ready-to-cook Korko soup base for six weeks storage (Table 4.17) showed that color coordinates were slightly different from the initial values. The *L**, *a**, and *b** values were slightly

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decreased with a significant difference ($p \leq 0.05$) when increasing temperature and storage time. The decreasing color coordinates made the ready-to-cook Korko soup base color slightly darker. The color change during storage was due to chemical reactions, such as a browning reaction and oxidative reaction. The previous research also found that the coordination values and other color functions represent color changes of red pepper powder as influenced by temperature and water activity.

Table 4.18 Changes in caking of ready-to-cook Korko soup base keeping for 6 weeks at different temperatures

Storage duration (Weeks)	Caking		
	30 ^oC	40 ^oC	50 ^oC
0	No caking	No caking	No caking
1	No caking	No caking	No caking
2	No caking	No caking	No caking
3	No caking	No caking	No caking
4	No caking	No caking	No caking
5	No caking	No caking	No caking
6	No caking	No caking	No caking

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Experimentation was carried out to also investigate how caking behavior of the ready-to-cook Korko soup base powder. The sample was exposed to 30 ^oC had no caking behavior along 6 weeks storage process. Hence, there weren't any caking behavior too for the sample that kept in higher temperature 40 ^oC and 50 ^oC. There is also previous research that mentioned no caking characteristics on skim milk powder exposed to 20 to 60 ^oC keeping for 8 weeks (Fitzpatrick et al., 2007). Several studies have shown that the main factors affecting stickiness and caking in amorphous foods are water plasticization on particle surfaces and temperature (Boonyai et al., 2004). Being part of the atmosphere, an increase in relative humidity could lead to an increased amount of water being absorbed on the particle surface or into the bulk (Adhikari et al., 2001 and Bhusari et al., 2014). Water absorption may occur if there is a temperature gradient in the environment in which the powder is stored, since moisture will migrate from warmer regions towards colder regions (Mehos and Clement, 2008). Hence, Caking also can occur due to van der Waals attractions between particles and may also occur due to liquid bridge formation where the capillary forces present are strong enough to cake the powder (Bhesh et al., 2013).

Table 4.19 Changes in microbial properties of ready-to-cook Korko soup base keeping for 6 weeks at different temperatures

Storage duration (Weeks)	Total plate count, yeast, and mold		
	30 °C	**40 °C**	**50 °C**
0	<100 CFU/g	<100 CFU/g	<100 CFU/g
6	<100 CFU/g	<100 CFU/g	<100 CFU/g

The study of microbiological quality factors indicated that the length of shelf-life duration, more of yeast amount would increase from the 12 weeks of shelf life. The estimated quantity was less than 100 CFU/g which was not over the standard of the community dried seasoning product. Hence, the total plate count was stated within the standard limitation. Synopsis, the developed Instant Korko soup base that had been kept for 6 weeks was safe and harmless to consumers.

4.7.2 Determination of shelf-life

The results of the present study indicated that the shelf-life of the ready-to-cook Korko soup seasoning powder can be determined by monitoring changes at different temperatures. Based on the results of this study, regression equations were established using the water activity and TPA characteristics. For safety acceptance, the Q10 values were 1.11 and 1.10 at 30-40 °C and 40–50 °C, respectively. Therefore, the expected expired dates of the ready-to-cook Korko soup powder was almost 4 years at 30 °C, 3 years and a half at 40 °C, and almost 3 years and a half at 50 °C. On the other hand, at 30-40 °C and 40–50 °C, the Q10 values were 1.98, 166 and 1.35, respectively. When stored at 30, 40, and 50 °C, the ready-to-cook Korko soup powder had projected expiration dates of almost 5 years and a half, almost 3 years and around 2 years, respectively.

Table 4.20 Q10 and shelf life of ready-to-cook Korko powder at different temperatures (a_w based)

Safety acceptance	Temperature (°C)	Q10	Shelf life (year)
Water activity (a_w)	30	1.11	3.96
	40	1.11	3.56
	50	1.10	3.24

Table 4.21 Q10 and shelf life of ready-to-cook Korko powder at different temperatures (TPA based)

Quality acceptance	Temperature (°C)	Q10	Shelf life (year)
TPA	30	1.98	5.48
	40	1.66	2.77
	50	1.35	2.05

In short, based on the result mentioned in 4.7.1, packaging and temperature, the product properties were significantly changed at 40 °C and 50 °C, especially TPA. TPA is a critical quality acceptance of the ready-to-cook Korko soup base. It results in rancidity and will be increased when the temperature is increased due to the oxidation of fatty acids and essential oils. The estimated shelf-life of the ready-to-cook Korko soup base is based on quality acceptance (TPA) to maintain both safety and quality of the product during on the shelve because the quality will not last long as safety, and to make sure that the product will be accepted by the consumers. As we have known, Cambodia is a tropical country which temperature can be reached to 40 °C. Therefore, to maintain quality and safety acceptance, the ready-to-cook Cambodian Korko soup should be last for 2 years at room temperature.

4.8 Sensory evaluation

4.8.1 Original ready-to-cook Korko soup base prototype

The ready-to-cook Korko soup base prototype was taken to cook Korko soup by weighing 90g to cook with 1.5 L of freshwater. The prototype was conducted alone not against any sample by 32 untrained panelists. The preference or liking score was indicated by rating on the 6-Hedonic scale to evaluate the acceptance of the prototype, also 5-point JAR for a guideline exploring the same attributes.

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Figure 4.6 Original product of ready-to-cook Cambodian Korko soup powder

4.8.1.1 The preference score of the original ready-to-cook Korko soup base prototype

The result of the preference (Table 4.22) shows the liking score by the 6-point hedonic scale on the Korko soup cooking by the ready-to-cook Korko soup base by using 90 g sample with 1.5 L freshwater, fresh vegetables and chicken were added into the soup.

Table 4.22 The preference score by original prototype (n=32)

Sample	Overall liking	Appearance		Flavor	
		Color	Texture	Aroma	Taste
First prototype of ready-to-cook Korko soup base	4.1±1.32	4.89±0.65	4.46±0.48	3.19±1.12	4.08±0.97

Range of scores: 6-point Hedonic scale (1= Extremely dislike, and 6= Extremely like)

4.8.1.2 The improved attributes of the original prototype

The result of the Just-About-Right (JAR) evaluation, Table 4.23, shows the necessary and non-necessary attributes for product development by sensory evaluated principles. The net score principle was used for improved guideline determination in this study (Anprung, 2008 and Chompreeda, 2013). The percentage of JAR level showed texture, and color attributes of the commercial product were represented by 84.03% and 86.6%, respectively. Hence, both attributes were indicated to be quality-developed benchmarks in the next step while more than 70% was accepted by the descriptive test method. However, aroma and flavor attributes were found 67.57% and 67.92% which less than 70%, therefore, they had to be increased.

The net score result found that the product prototype was too weak in aroma, and flavor, however, too strong in texture and color. Hence, the net score indicated that the standard formula that needed to be increased was aroma and flavor.

Table 4.23 JAR evaluation result of the original prototype (n=32)

Attributes	JAR Percentages			Net score
	Weak	JAR	Strong	
Texture	7.18	84.03	8.79	1.61
Color	6.3	86.6	7.1	0.8
Aroma	18.65	67.57	13.78	-4.87
Flavor	17.55	67.92	14.53	-3.02

Range of scores: 6-point hedonic scale (1= extremely dislike, and 6= extremely like) and 5- point JAR (1= Too weak, 3=JAR, and 5=Too strong)

In summary, the product prototype had the potential to be improved. However, the product prototype required improvement on some attributes such as increasing aroma and flavor, therefore, the ingredients related with aroma and flavor were added. The improved formula shows in Table 4.24. Some ingredients have increased such as lemongrass, kefir lime leaves, garlic, galangal, and MSG to 16.73, 2.23, 3.49, 2.13, and 3.55% respectively.

Table 4.24 Improved formula of original prototype

Ingredients	Original formula (%)	Improved formula (%)
Lemongrass	12.73	16.73
Kefir lime leaves	1.37	2.23
Garlic	2.89	3.49
Galangal	1.96	2.13
Turmeric	0.27	0.27
Fermented fish	21.3	21.3
Roasted rice flour	18.76	18.76
Salt	10.71	10.71

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Sugar	20.79	20.79
Monosodium	2.55	3.55

4.8.2 Improved ready-to-cook Korko soup base prototype (second prototype)

The second prototype evaluation (n=30) of the improved formulation was conducted. This sensory evaluation was aimed to ensure the improved prototype acceptability and no need to adjust the attributes that may affect the consumer liking.

The result of the 6-point hedonic preference (Table 4.25) shows the ready-to-cook Korko soup base sample overall liking score, 4.67±1.01.

Table 4.25 The preference score of the second prototype (n=30)

Sample	Overall liking	Appearance		Flavor	
		Color	Texture	Aroma	Taste
Second prototype of ready-to-cook Korko soup base	4.67±1.01	5.09±0.65	4.59±0.48	4.65±1.12	4.57±0.97

Range of scores: 6-point hedonic scale (1=dislike extremely, and 6=like extremely)

4.8.2.1 The improved attributes of the second prototype

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From the result shows in Table 4.26, the percentage of JAR level shows the color, texture, flavor was indicated and accepted more than 70%. However, the net score of aroma showed the attributes that need to be increased such as too weak in kefir lime leaves.

Table 4.26 JAR evaluation result of the improved product prototype (n=30)

Attributes	**JAR Percentages**			**Net score**
	Weak	**JAR**	**Strong**	
Texture	7.18	78.54	8.79	1.61
Color	6.3	85.63	7.1	0.8
Aroma	18.65	68.98	13.78	-4.87
Flavor	17.55	72.43	14.53	-3.02

In summary, the improvement on the attributes after the sensory evaluation of the second ready-to-cook Korko soup base prototype was the aroma, the improved formula was acceptable by 4.67 overall liking score. The accepted formula of lemongrass, kefir lime leaves, garlic, galangal, turmeric, fermented fish, roasted rice, salt, sugar and, MSG were 16.3, 2.23, 3.49, 2.13, 0.27, 21.3, 18.76, 10.71, 20.79, 3.55%, respectively. Some ingredients needed to be increased related to aroma especially kefir lime leaves.

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CHAPTER V
CONCLUSION

5.1 Conclusion

Within this research, the ready-to-cook Cambodian Korko soup base was developed by using different drying temperatures to select a better temperature that is suitable to maintain the quality of the final product. Based on the experiments, 60 ºC is suitable for drying herbs because it can prevent the loss of antioxidant properties better than higher temperatures. Besides, dried fermented fish from 30 ºBrix fermented fish was selected due to the lowest histamine content compared to 45 and 60 ºBrix after drying. Temperature of 70 ºC is a suitable temperature for drying 30 ºBrix fermented fish due to the slower and lower increase in histamine. In short, the drying process can affect the quality, and safety of the product as the high temperature leads to a decrease in DPPH, FRAP, and an increase in histamine. The product was preferred in powder form based on the consumer survey.

The physicochemical properties of the ready-to-cook Korko soup base (tried form) during storage at different temperatures such as 30 ºC, 40 ºC, and 50 ºC were slightly different during 6 weeks storage. The result of physicochemical showed that increasing water activity, rancidity, moisture content and decreasing solubility were significant differences ($p \leq 0.05$), also caking was not occurred on the product during the storage. The color coordinates which *L**, *a**, and *b** were slightly decreased during storage duration while the microbial (i.e., total plate count, yeasts, and molds) was determined and within limitation stated in the regulations from FDA. In addition, the expected shelf life of the ready-to-cook Korko soup base was almost 4 years (a_w based) and more than 5 years (TPA based) storing at 30 ºC and it significantly decreases when storing at 40 ºC and 50 ºC. Therefore, this product should be store at 30 ºC for better safety, stability, and quality. Besides, the physicochemical properties of the final product such as moisture content % (w.b.), water activity (a_w), ash, fat, protein, crude fiber, carbohydrate (g/100g), *L**, *a**, *b**, histamine (ppm), and solubility (%) were found at 2.79, 0.375, 22.39, 2.63, 10.22, 4.27, 62.97, 62.07, 2.37, 20.55, 32.56, and 60.82, respectively. The microbial load was within the safe limitation to consume.

According to the sensory evaluation of prototype, the panelists had positive liking scores (4.67) on the second prototype. The attributes such as texture, color, aroma, and flavor were 78.54, 85.63, 68.98, and 72.43 % respectively and accepted by the consumers. Only the aroma attribute was needed to improve a bit more to pass 70 %.

5.2 Suggestions

In the present study, the ready-to-cook Korko soup base was performed and accepted on the prototype or laboratory level. However, there is some limitation that needs further research to provide a better understanding. Further research should focus on the following points:

- To study about paste based form of the ready-to-cook Korko soup base without using drying process.
- To study about pricing, packaging size, serving amount, and buying attention of the product.

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APPENDIX A

Calculation of shelf life

$$Q_{10} = \frac{K_{T+10}}{K_T}$$

$$\text{Shelf life} = \frac{A\,(max\ aw) - Ao\,(initial\ aw)}{K}$$

Quality factor	Temp. (ºC)	Regression equation	Correlation coefficient	K	A_o (initial a_w)	A (max a_w)	Q10	t (predicted days)	Shelf life (years)
a_w	30	y = 0.00018x + 0.3365	0.9479	0.00018	0.34	0.6	1.11	1444.44	3.96
	40	y = 0.0002x + 0.3417	0.9243	0.00020	0.34	0.6	1.11	1300.00	3.56
	50	y = 0.00022x + 0.3415	0.8790	0.00022	0.34	0.6	1.10	1181.82	3.24

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APPENDIX B

Related approved document

AF 01-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Group I, Chulalongkorn University
Jamjuree 1 Building, 2th Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel: 0-2218-3202, 0-2218-3049 E-mail: eccu@chula.ac.th

COA No. 149/2021

Certificate of Approval
Exemption for Ethics Review

Study Title No. 104.1/64 : DEVELOPMENT OF READY-TO-COOK CAMBODIAN KORKO SOUP BASE

Principal Investigator : MENGSREANG LOEM

Place of Proposed Study/Institution : Faculty of Science, Chulalongkorn University

This Research proposal is exempted for ethics review in compliance with the Office for Human Research Protections (OHRP Exempt Categories) 45 CFR part 46.101(b).

Certified under condition: To conduct this research project, the researcher (s) must strictly adhere to research proposal approved by the committee. If there is any amendment, it must be sent to the committee for review before carrying on the project.

Signature: Prida Tasanapradit
(Associate Professor Prida Tasanapradit, M.D.)
Chairman

Signature: Raveenan Mingpakanee
(Assistant Prof. Raveenan Mingpakanee, Ph.D.)
Secretary

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PUBLICATION	Mengsreang Loem and Chaleeda Borompichaichartkul. 2021. Development of ready-to-cook Cambodian Korko soup base. Full paper in the Proceedings of the Food Innovation Asia Conference 2021 (FIAC2021), 17-18 June 2021.

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