EFFECT OF GLYCEROL AND SODIUM CHLORIDE ON THE SHELF-LIFE OF FRESH NOODLE

NORAZLIN BINTI SUPANDI

BACHELOR OF CHEMICAL ENGINEERING UNIVERSITI MALAYSIA PAHANG

EFFECT OF GLYCEROL AND SODIUM CHLORIDE ON THE SHELF-LIFE OF FRESH NOODLE

NORAZLIN BINTI SUPANDI

Thesis is submitted in partial fulfilment of the requirements for the award of the degree of Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering UNIVERSITI MALAYSIA PAHANG

DISEMBER 2016

UNIVERSITI MALAYSIA PAHANG

Author's Full Nam	Norazlin Binti Supandi			
Date of Birth	. 20 March 1994			
Title	The Effect of Glycerol and Sodium Chloride on			
	The Shelf-Life of Fresh Noodle			
Academic Session	Semeseter 1 (2016/2017)			
I declared that this thesis i	is classified as:			
	(Contains confidential information under the Official Secret Act 1972)*			
RESTRICTED	(Contains restriction information as specified by the organization where research was done)*			
OPEN ACCESS	I agree that my thesis to be published as online open access (Full text)			
I acknowledge that Univer	sity Malaysia Pahang reserve the right as follows:			
1. The Thesis is the Prop	erty of University Malaysia Pahang.			
2. The Library of Univer purpose of research or	sity Malaysia Pahang has right to make copies for the ly.			
3. The Library has the rig	ght to make copies of the thesis for academic exchange.			
Certified By:				
(Student's Signatur	e) (Supervisor's Signature)			
	Dr. Zatul Iffah Bt Mohd Arshad			
940320-01-5026				

NOTES : *If the thesis is CONFIDENTIAL or RESTRICTICTED, please attach with the letter from the organization with period and reasons for confidentiality or restriction

SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

Signature	:
Name of main supervisor	: DR. ZATUL IFFAH BT MOHD ARSHAD
Position	: LECTURER
Date	: 15 DECEMBER 2016

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree

Signature:Name: NORAZLIN BINTI SUPANDIID Number: KC13030Date: 15 DECEMBER 2016

To my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake and to my mother, who taught me that even the largest task can be accomplished if it is done one step at time and face it patiently.

ACKNOWLEDGEMENT

I would like to express my special appreciation and thanks to my supervisor, Dr. Zatul Iffah. You have been a brilliant mentor for me. I would like to thank you for your neverending support during my tenure as research student under your guidance, for giving insightful comments and suggestions of which without it, my research path would be a difficult one . Your advice on my research has been valuable.

A special thanks to my family. Words cannot express how grateful I am to my mother, father, and my siblings for the love and support throughout these years. Your prayer for me was what sustained me thus far. I am also indebted to the Ministry of Higher Education and Universiti Malaysia Pahang for funding my study.

I would also like to thank all of my friends who supported me in writing, and motivate me to strive towards my goal. I am sincerely grateful to the staffs of Chemical Engineering and Natural Resources Faculty who helped me in many ways and made my stay in UMP pleasant and unforgettable.

ABSTRACT

Fresh noodles are wheat flour product that become staple food in Malaysian cuisine. The world consumption of noodle product has increase in recent years due to the ease of transportation, and cooking purposes. Nevertheless, the fresh noodle is easily perishable due to the high water content and improper storage condition. Most of the chemical preservatives used in fresh noodle nowadays are linked to several problem such as digestive problems, cancer, neurological conditions, attention deficit hyperactivity disorder, heart disease, and obesity. Thus, the objective of this study is to investigate the effects of sodium chloride and glycerol to the shelf-life extension of fresh noodles. The microbial growth, pH, moisture content and sensory attributes are the process parameters evaluated during the days of storage time at the room temperature. Glycerol was added to decrease the water activity content of the noodles. In addition, salt was also used in this experiment to inhibit the enzyme activities and the growth of microorganisms in fresh noodles, thus extending their shelf life. The sensory attributes such as odour, colour, viscoelasticity, and texture were used as the characteristics evaluated using quantitative descriptive analysis (QDA). Integral scores were given from 1 to 25 in which 25, denotes as excellent quality and 1 as completely deteriorated quality. The data obtained were expressed as the mean and standard deviation from three replicates and were analysed using SPSS software version 23. There are two modified sample used in this studied with the addition of glycerol (GL) and sodium chloride (S) as additive according to ratio of GL:S 2:4 for sample A and 4:2 for sample B. Initial TPC for both sample A and B were log 5 CFU/mL and significantly increased to log 7 CFU/mL at the end of the storage period for 7 days. Linear regression analysis between storage time and pH for sample B showed the strongest correlation ($R^2 = 0.959$ and p = <0.001). The correlation between storage time and moisture content for sample A demonstrated strong correlation (R^2 = 0.956 and $p = \langle 0.001 \rangle$. The high composition of glycerol in sample B give the best result for all the process parameters studied.

ABSTRAK

Mi segar adalah produk daripada tepung gandum yang menjadi makanan ruji dalam masakan Malaysia. Penggunaan produk mi di dunia telah meningkat dalam beberapa tahun kebelakangan kerana pengangkutan, dan untuk tujuan masakan. Walau bagaimanapun, jangka-hayat mi segar adalah singkat disebabkan oleh kandungan air yang tinggi dan mudah rosak jika tidak disimpan di bawah keadaan sejuk beku. Kebanyakan bahan pengawet kimia yang digunakan dalam mi segar kini berkait dengan beberapa masalah seperti masalah penghadaman, kanser, keadaan saraf, gangguan hiperaktiviti defisit perhatian, penyakit jantung dan obesiti. Oleh itu, objektif kajian ini adalah untuk menyiasat kesan natrium klorida dan glycerol untuk perlanjutan jangka-hayat mi segar. Pertumbuhan mikrob, pH, kelembapan atribut kandungan dan deria adalah parameter proses yang dinilai sepanjang waktu simpanan di bawah suhu bilik. Glycerol telah ditambah bagi mengurangkan kandungan air di dalam mi. Di samping itu, garam juga digunakan di dalam eksperimen ini untuk menghalang aktiviti enzim dan pertumbuhan mikroorganisma dalam mi segar, sekali gus memanjangkan jangka hayat mi. Sifat-sifat deria seperti bau, warna, viscoelasticity dan tekstur digunakan sebagai ciri-ciri penerimaan, dan dinilai menggunakan analisis deskriptif kuantitatif (QDA). Markah diberi dari 1 hingga 25 dimana 25 menandakan kualiti yang sangat baik dan 1 benar-benar rendah kualiti. Data yang diperolehi telah direkodkan sebagai min dengan sekurangkurangnya tiga kali sampel penentuan dan sisihan piawai (SD) dengan menggunakan perisian SPSS. Nilai microb awal bagi kedua-dua sampel A dan B adalah sekitar log 5 CFU/mL dan meningkat dengan ketara ke log 7 CFU/mL pada akhir tempoh penyimpanan. Analisis regresi linear antara waktu simpanan dan pH bagi sampel B menunjukkan hubung-kait yang kuat ($R^2 = 0.959$ dan p = < 0.001). Hubung kait antara waktu simpanan dan kandungan lembapan untuk sampel A menunjukkan hubung-kait yang kuat ($R^2 = 0.956$ dan p = < 0.001). Penambahan lebih banyak glycerol dalam sampel B memberikan hasil yang terbaik untuk semua parameter dalam kajian ini berbanding sampel A.

TABLE OF CONTENTS

			Page
SUPI	ERVISC	DR'S DECLARATION	iv
STU	DENT'S	DECLARATION	\mathbf{v}
ACK	NOWL	EDGEMENT	vii
ABS'	ГRACT		viii
ABS'	ГRAK		ix
TAB	LE OF	CONTENTS	х
LIST	OF TA	BLES	xii
LIST	OF FIC	GURES	xiii
LIST	OF syn	nbolS	xiv
LIST	OF AB	BREVIATIONS	xv
СНА	PTER 1	INTRODUCTION	1
1.1	Backgr	ound of the Study	1
1.2	Motiva	tion	2
1.3	Problem	n Statement	2
1.4	Objecti	ves	3
1.5	Scopes	of Study	3
СНА	PTER 2	LITERATURE REVIEW	4
2.1	Introdu	ction	4
2.2	Food d	eterioration	5
2.2.1	Microb	ial as a cause spoilage of food	6
2.3	Food P	reservatives	7
2.4	Charac	terization of fresh noodle	9
CHA	PTER 3	6 METHODOLOGY	10
3.1	Introdu	ction	10
3.2	Materia	als	10
3.3	Overvi	ew of overall experiment	11
	3.3.1	Preparation of fresh noodle	12
	3.3.2	Determination of moisture content	12
	3.3.3	Determination of pH	12
	3.3.4	Determination of Total Plate Count (TPC)	13
	3.3.5	Sensory evaluation	13

			xi
	3.3.6	Statistical analysis	13
CHA	PTER 4	RESULTS AND DISCUSSION	14
4.1	Introduct	ion	14
4.2	Effect of	moisture content	14
4.3	pH chang	ges	15
4.4	TPC cha	nges	17
4.5	Sensory	analysis	18
4.6	Compari	son on costing of noodle production	21
CHA	PTER 5	CONCLUSION AND RECOMMENDATION	22
5.1	Conclusi	on	22
5.2.	Recomm	endation	23
REFE	ERENCE	S	24
Appe	ndix		27

LIST OF TABLES

Table No.	Title	Page
Table 2.1 : Potential natural an	timicrobial for noodle preservation	8
Table 4.1 : Sensory quality of 1	fresh noodles	20
Table 4.2 : Comparison of pric	e on noodle production	21

LIST OF FIGURES

Figure No. Title	Page
Figure 3.1 : Flowchart of the overall experimental study	11
Figure 4.1 : Moisture content for different sample during storage time	15
Figure 4.2 : Changes of pH in fresh noodles during storage time	16
Figure 4.3 : Changes of microbiological in fresh noodles during storage time	17
Figure 4.4 : (A) Sample contain (2% (w/w) of GL + 4% (w/w) of S)	
after few days storage	18
(B) Sample contain (4% (w/w) of $GL + 2\%$ (w/w) of S)	
after few days storage	18
(C) Sample without additives after few days storage	18

LIST OF SYMBOLS

°C degree Celcius

% percentage

LIST OF ABBREVIATIONS

GL	Glycerol
S	Sodium Chloride (Salt)
TPC	Total Plate Count
CFU	Colony forming unit
SD	Standard deviation
MC	Moisture content

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Fresh noodle is a traditional wheat-based food from China and has been popular among Asian nations like Korea, Thailand, Japan, Malaysia and Indonesia. It has been estimated that at least 12 per cent of global wheat production is used for processing Asian noodle product (Li et al., 2011). There are two major types of Asian noodles, which are white salted noodles and yellow alkaline noodles. These two noodles are differ greatly in various aspect such as formulation, production procedures, and sensory attributes (Li et al., 2017). Noodle quality is typically evaluated on the basis of color, surface appearance, texture, taste and cooking loss, with noodle firmness, cohesiveness, tensile strength, and sensory appreciation as the main discriminating factors (Rombouts et al., 2014).

However, the shelf-life of fresh noodle is very short and will increase the wastage in the industry and can be a potential source of food poisonings. There are many studies that have been done to prolong the shelf-life of fresh noodle including the use of various food additives. According to Li et al., (2012), the shelf-life of fresh noodle made from ozone treated wheat flour was largely extended, in relation to microbial growth and darkening rate. The investigation on the effect of water activity lowering agents on the shelf-life of fresh noodle showed that it could be extended for more than 7 times (Li et al., 2011).

Food additives are added to food mainly to affect the flavor, to enhance the taste, and to produce a good appearance. In the late 19th century, many commercialized foods appear and it make the use of food additives increased immensely. The use of traditional additive also been apply in noodle making such as potassium bromate and benzoyl

peroxide which act as dough strengtheners and to give chewy texture to noodles (Ginting et al., 2015). Phosphate salts were added to noodle as additive to impart a bright appearance and smooth texture to the products (Wang et al., 2017).

Glycerol is widely used as formulating aid in food, drug, cosmetic and also in pharmaceutical formulations. It serves in food as a humectant and sweeter which help to preserve foods. It is added in noodle to decrease the water activity content of the noodle. Addition of salt in noodle can enhanced the formed network of noodle dough and impart a pliable mouthfeel to the products (Li et al., 2014). Salt also can inhibit enzyme activities and growth of microorganisms in fresh noodles. For these reasons, it is necessary for salt to be added as additive in fresh noodle.

In this study, the use of glycerol and sodium chloride provide another option on prolonging the shelf-life of fresh noodles will be investigated. Microbial growth, pH, moisture content and sensory characteristics were evaluated to monitor the properties of fresh noodles during the storage time under room temperature. The process parameters mentioned is used in this study due to their potential as spoilage indicators in the fresh noodle after a few days storage time. The same experiment were also applied for commercial noodle from market noodle without additive.

1.2 Motivation

The shelf-life of fresh noodles are very short due to the high water content and improper storage. Due to the aforementioned problems, chemical additives are used to prolong the shelf-life of fresh noodle which is harmful to human health. Hence, the addition of glycerol and sodium chloride in the fresh noodle can prolong its shelf-life of as these additive have been proven to be safe by the FDA.

1.3 Problem Statement

The use of boric acid as additive in fresh noodle products is to control starch gelatinization, enhance colour, texture and flavor. Besides, boric acid is effective against

yeasts, and to a much lesser extent, against moulds and bacteria, they can be used to preserve food products (Pang et al., 2008). Unfortunately, the use of boric acid in food can cause many health problems such as kidney failure, nausea and even death. Besides, based on Huang et al., 2007, the use of chitosan and xylose as preservation in noodle also can prolong the shelf-life of fresh noodle. However, the cost of these preservative is high. Therefore, the use of glycerol and sodium chloride provide the safe and cheapest additive to preserve the noodle.

1.4 Objectives

The aim of this study is to investigate the effects of sodium chloride and glycerol to extend the shelf-life of fresh noodles.

1.5 Scopes of Study

- The effect of glycerol and sodium chloride at the ratio of 2:4 and 4:2 on the process parameters of pH,MC,TPC of fresh noodles was investigated and compared with the fresh noodles without additive and from the market.
- 2) The sensory attributes of noodle samples will be performed by 10 panellists using hedonic test and the data will be statistically using SPSS software version 23.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Noodles are generally made from common wheat flour. It was originated in China 4000 years ago and are mostly consumed in Asian countries (Li et al., 2014). The traditional form of Chinese noodles, fresh noodle is now attracting more people for its unique flavor and taste (Li et al., 2012). Noodles are an important diet in many countries of eastern and south eastern Asia, accounting for approximately one third of Australia wheat and about 40% of wheat consumed in Asia countries (Zhu et al., 2010). Noodle flour quality varies dramatically throughout the Asia region.

Major types of Asian noodles are white salted noodles and yellow alkaline noodles. The classification of noodles is based on the ingredients and processing methods. The two noodle types differ greatly in various aspects such as formulation, production, procedures, and also the sensory attributes. For the quality of the noodles, it has various aspect includes the appearance, eating quality, taste, cooking properties and odor. Color is considered as a major determinant of noodle marketability, dark or gray colors are negative attributes (Li et al., 2016). Fresh noodles are extremely liable to darken due to the accelerated enzymatic and nonenzymatic darkening reactions by high water content (Choo et al., 2010).

Texture is one of primary concern for consumers of noodle product (Rombouts et al., 2014). The hardness, adhesiveness, springiness, and chewiness of fresh noodles changed to different extents as the storage time increased (Li et al., 2017). Most of the edible gums can be used in pasta and noodles for maintaining the dough structure and give a palatable mouthfeel to the products (Luo et al., 2015). The incorporation of guar gum, algin, xanthan gum, locus bean gum, konjac powder and so on into the noodles

dough matrix and thus, affect the cooking and eating qualities of the products. (Li et al., 2014).

2.2 Food deterioration

The shelf-life of fresh noodles is very short due the high contents of water and nutrient substances, and they will deteriorate quickly if not stored under refrigeration. The most common spoilage organisms in fresh noodles are bacteria, yeast, and molds (Li et al., 2011). One of the most important factors leading to the perishability of fresh noodles is the high level of microorganisms in wheat flour, which is the main raw material for noodle making (Li et al., 2012). The short shelf-life of fresh noodle has resulted in high levels of wastage in food industry, and it might also become a potential source of food poisonings (Ghaffar, 2009). Therefore, it is important to control the loading of food additives on prolonging the shelf-life of fresh noodle.

At present, the introduction of commercialized preservative derived from chemical such as potassium sorbate, sodium dehydroacetate and calcium propionate into fresh noodle has increased immensely (Li et al., 2012). Nowadays consumers are increasingly concern about the safety of food additives and becoming interested in natural "green" food. Some food additives, especially chemical synthesis ones, have been linked to digestive problems, cancer, neurological conditions, attention deficit hyperactivity disorder, heart disease, or obesity (Li et al., 2014). For that reason, various antimicrobial agents derived from natural ingredients, such as flaxseed, Chitosan and xylose have been added to noodles. Nevertheless, these natural preservatives, only able to prolong the shelf-life of fresh noodles for a few extra days (Li et al., 2011).

The primary factors associated with food spoilage are intrinsic food properties and contamination during processing in combination with temperature abuse (Del Nobile et al., 2009). For fresh foods, the primary quality changes may be categorized as (i) bacterial growth and metabolism resulting in possible pH-changes and formation of toxic compounds, off-odours, gas and slime-formation, (ii) oxidation of lipids and pigments in

fat-containing foods resulting in undesirable flavours, formation of compounds with adverse biological effects or discoloration. (Sirichokworrakit et al., 2015).

2.2.1 Microbial as a cause spoilage of food

Food deterioration can be said as a result of growth of microorganisms that usually appear in processed food. Microorganisms are sometimes also named as microbes or germs that included in the group of bacteria and fungi (involving yeast and mould). Various types of these organisms may cause changes in the character of food that can be classified as positive or negative impact (Yadav et al., 2014). Positive microbial transformation can produce good products such as cheese, yoghurt, and also wine.

Negative impact of microbial growth include food spoilage and food poisoning which mainly caused by different widespread bacteria. As they grow, microorganisms will release their own enzymes into the liquid surrounding them and absorb the products for external digestion (Tan et al., 2009). This is the basis microbial food spoilage, which lowers the nutritional value of food. The bacterial contamination can precipitate major public health hazard and economic loss in term of food poisoning. Bacteria and moulds also produce waste food products that can act as poisons or toxins, thus causing the renowned ill-effects.

Spoilage is most rapid in proteinaceous foods slice such as meat, poultry, fish, shellfish, milk and some dairy product. These foods are highly nutritional, possess a neutral or slightly acid pH and high moisture content and can permit growth of microorganism (Halley et al., 2005). Yeasts and moulds can be found in a wide variety of environments, such as in plants, animal products, soil, water and insects. This can be explained by the fact that yeasts and moulds can utilize a variety of substrates such as pectines and other carbohydrates, organic acids, proteins and lipids (Chanasattru et al., 2007) Other than that, yeasts and moulds are relatively tolerant to low pH, low water activity, low temperature and the presence of preservatives. It is also notable that yeasts can utilize food ingredients, such as organic acids like lactic, citric and acetic acids, that are generally considered to have an effect on the growth of many microorganisms (Gabriel, 2008).

Spoilage induced by yeasts and moulds can be recognize in the product's appearance by the production of slime, pigmented growth on the surface, and off-flavours. In addition to visible spoilage, moulds can also spoil foods through the formation of mycotoxins (Li et al., 2016).

2.3 Food Preservatives

Historically these noodle products originated in northern China, from where they were introduced to other parts of Asia by traders, seafarers and migrants. Noodles gradually became a staple food for many consumers and this was facilitated by the introduction of dried noodles (Ginting et al., 2015). Nowadays, consumers demand certain quality standards for the noodle, such as firm, elastic, smooth, chewy texture, as acceptable taste, nutritional qualities, and functional properties (Loubes et al., 2016). There are many chemical additives used in noodles products such as calcium propionate, sodium benzoate and sodium dehydroacetate that function as microbial inhibition in noodles (Andrade et al., 2013). Some of these chemical additives are forbidden todays as consumer awareness about food additives and safety has increased (Li et al., 2011). Table 1 below show the potential natural antimicrobials for noodle preservation.

Potential natural	Current application	References	
preservatives			
Organic acid (malic acid	Juices, jam, fresh noodles	Fu,(2008)	
lactic acid, sorbic acid,			
citric acid, and others)			
Natural alcohol (ethanol,	Fresh noodles, bread, steamed	Fu,(2008);	
sorbitol, glycerol)	bread	Li et al.,(2011)	
Herbs and spice extracts	Bread, pasta, and noodles	Tiwari et al.,(2009)	
Essential oil (eugenol,	Sweet cherry, fresh pasta,	Nielsen et al.,(2000)	
thymol, menthol)	bread		

Table 2.1 : Potential natural antimicrobial for noodle preservation

Tea and tea extracts	Fresh noodles, juices	Li et al.,(2012)	
Monoacylglycerols	Soy sauce, miso, sausages,	Fu et al.,(2008)	
	cakes, and noodles		
Chitosan and its Maillard	Fresh noodles, meat products	Xu et al.,(2008)	
reaction products			
Basic protein and peptides	Noodles, bread, milk, sausage	Li et al.,(2011)	

Salt (sodium chloride) is an important ingredient in noodle processing with the amount added is about 1-3% of flour weight. Salt has three principle functions in noodles processing. Firstly, salt have a strengthening and tightening effect on the gluten of dough. This is because, salt can inhibits proteolytic enzyme activities and significantly improves sheeting properties of dough, especially at high water absorption levels. Second function of salt is flavour enhancing and texture improving effects. Salt enhances flavours including imparting great fullness to "mouth feel", masking possible off-tastes and improving the flavour balance. Noodles with added salt have a shorter cooking time and have softer and elastic textures than those without salt. Lastly, salt can inhibit the enzyme activities and growth of microorganisms. It can slows down the oxidative discolouration process and spoilage under high temperature and humidity environments, thereby it can extending the shelf-life of fresh noodles (Fu, 2008).

Glycerol is a colourless, odourless, viscous liquid that widely used in the pharmaceutical formulations. In foods and beverages, it serves as a humectant and sweetener which help to preserve foods. Glycerol is a safe and widely used as formulating aid in food, drug, cosmetic, tobacco industries as well as used as filler in commercially prepared low-fat foods (Chen et al., 2016). The addition of glycerol give great influence in the performance of food matrixes, the interfacial tension, optimum curvature of emulsion-based systems and solubility characteristics of matrices (Saberi et al., 2013).

Besides, glycerol is also an excellent co-solvent for hydrophobic nutraceuticals to improve the solubility and reduces the risk of degradation of the bioactive molecule (López-Rubio & Lagaron, 2011). In industry, glycerol is also used in the production of dry pet foods to retain the moisture and enhance palatability. Indirect use of glycerol in food processing is represented by monoglycerides, the glycerol esters of fatty acids, which are emulsifiers and stabilizers for many product (Rahn et al., 2010). The monoglycerides help to maintain moisture balance in a product and permit richer formulations with longer shelf-life. When it is added to margarine, it can increase plasticity and to promote dispersion of fat. It is also used in salad dressings, candy, frozen desserts, and food coatings. Patel et al., (2014) stated that glycerol, as co-solvent for both hydrophobic corn protein (zein) and carotenoid molecules, was used to form oil-inglycerol (O/G) emulgels enriched with β -carotene to be used as margerine alternatives for cakes and have same characterization with the commercial one.

2.4 Characterization of fresh noodle

Food like fresh noodle normally will have short shelf-life due to its high nutritional content. They will deteriorate quickly during storage, which therefore limits the mas' production (Bui et al., 2007). Several factors can limit the growth of microorganisms in foods; such as moisture content, pH and temperature (Akhigbemidu et al., 2015). Ensuring the quality retention in fresh noodles is challenging (Li et al., 2017). It is because, during storage time, fresh noodle will undergo physiochemical and biochemical changes at different rates and causing significant quality losses that lead to final deterioration of the noodles (Li et al., 2016).

(Ghaffar, 2009) concluded that the microbial growth and decrease in pH value were the most obvious characterizations during the deterioration of fresh noodles. A food may start with a pH with precludeial growth of microbe but as a result of the metabolism of other microorganisms such as yields and molds, pH shifts may occur and permit further bacterial growth. (Akhigbemidu et al., 2015).

Sensory characteristics such as odor, taste, appearance, texture and combining all of the characteristics could be the main spoilage indicator in noodles (Holley et al., 2005). According to (Li et al., 2011) odour and colour were chosen in the study as spoilage indicators for shelf-life determination, since they were the two most important characteristics perceived by panellists and consumers, compared to other attributes.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The research methodology of this research was cover on the preparation of fresh noodles and the analysis of the fresh noodle. The preparation of fresh noodle had five steps from the mixing of flour with distilled water until the fresh noodle was formed at the last step. After the fresh noodle is ready, it will be used in the analysis step. The fresh noodle was analysed on the moisture content, pH, total plate count (TPC), and sensory attribute. The SPSS software was used to analyse sensory attributes in the sensory evaluation. All the parameter was also been tested on the commercial noodle from industry and fresh noodle without additive.

3.2 Materials

High protein white wheat flour and the table salt (NaCl) were obtained from the local market. Glycerol with purity of 99 % and nutrient agar were purchased from Sigma Aldrich. All the chemicals used were of analytical grade.

3.3 Overview of overall experiment

Figure 3.1 show the flowchart of the making process of fresh noodle and the parameter that will be analysed in this study.

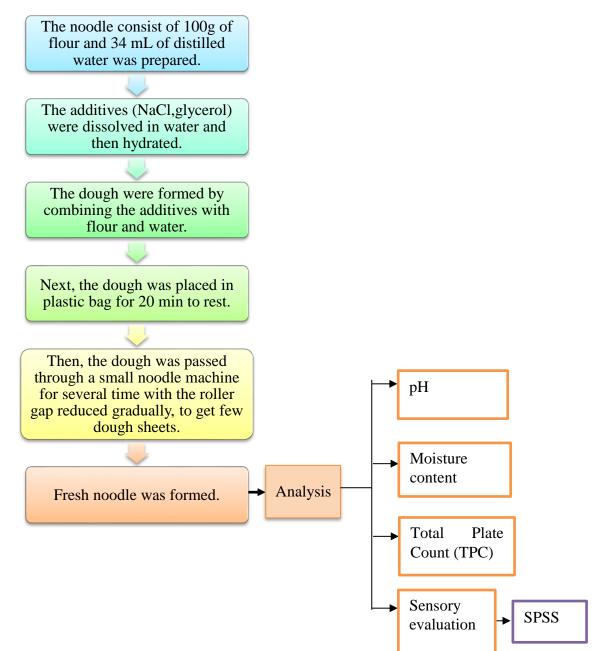


Figure 3.1: Flowchart of the overall experimental study

3.3.1 Preparation of fresh noodle

100g of wheat flour and 34mL of distilled water were prepared for each batch of dough. The additives NaCl (S) and glycerol (GL) with the composition of S:GL at ratio of 2:4 and 4:2 were dissolved in water first and added to the flour over a period of 30 sec in the mixer set using speed one. After that, the speed of the mixer will be increased to set two and allowed for further mix for about 5 min. The dough was formed after 6 min in the mixing. Then, the prepared dough was rested in a plastic bag for 20 min at room temperature. Next, the dough was passed through a small noodle machine for several time with the roller gap reduced gradually in order to get a uniform sheet of a single dough sheet. The noodle strands was cut into 20 cm lengths before boiled for 30 sec in boiling water. After boiling, the noodles were washed using distilled water. The products was sterilized at 100°C for 30 min and stored in seal plastic bag. (Xiaowei et al., 2015).

3.3.2 Determination of moisture content

Ten grams of each sample was weighted and placed in air/humidity tight container. The sample was dried in oven at $130\pm 3^{\circ}$ C for 1 hour. The weight of each sample was weighted using an analytical balance. The process of drying, cooling and weighing were repeated until a constant weight was obtained. The loss in weight was used to calculate the moisture content of the sample using the equation (1):

$$Moisture \ content \ (\%) = \frac{Loss \ in \ weight \ of \ sample \ upon \ drying}{Initial \ weight \ of \ sample} \times 100$$
(1)

3.3.3 Determination of pH

Ten grams of sample was homogenized with 90 mL of distilled water. The homogenates was centrifuged and the supernatants obtained was used as sample for pH measurement using pH meter (Mettler Toledo-320)

3.3.4 Determination of Total Plate Count (TPC)

Total plate count (TPC) were examined according to (Code of National Standard of China, 2008). The 20g of samples was mixed with normal saline solution in the blender machine and was blended for 60s in ratio of 1:10 with saline solution. Ten times serial dilutions were prepared using normal saline solution. At sixth dilution, 100 μ L of sample were spread onto sterile petri plates containing plate count agar (PCA). Then, the plate were incubated at 36±1°C for 48±2hrs (Li et al., 2011).

3.3.5 Sensory evaluation

Sensory evaluation was evaluated using 10 panelists (Ghaffar et.al 2009). The noodles was evaluated for appearance, odor, texture, viscoelasticity, and overall liking of the sample using a 1 to 25 point hedonic test, where 25 = excellent and 1 = unacceptable. The scores for each characteristic for a given specimen were tabulated in table and the mean value will representing the judgement of the panel on the sensory attribute of the noodles.

3.3.6 Statistical analysis

The data collected in this study were expressed as the mean of three replicates determinations. Standard deviation (SD) were assessed with one-way ANOVA followed by Turkey's multiple comparison test for significant differences by using the SPSS software (Huang et al., 2007).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter present all the experimental result and discussion of this study. Firstly, the production of noodle with different composition of additive has been analysed on the effect of moisture content during the storage time of fresh noodle. Secondly, to prove the effect of additive, pH changes of the noodle during storage time for all sample been analysed to indicate the spoilage on the fresh noodle. Besides, the sample also has been analysed on the total plate count, TPC as it also can be a potential spoilage indicator in the fresh noodle. Other than that, sensory analysis also been tested on the noodle which all the data were analysed using SPSS software version 23.

4.2 Effect of moisture content

Based on Figure 4.1, when the storage time is increased, the moisture content of the noodle samples also increased which can drive to the increased of the water activity. Different concentration of glycerol and NaCl was used in which sample A contain (2% (w/w) of GL + 4% (w/w) of S) and sample B contain (4% (w/w) of GL + 2% (w/w) of S). Both samples (A and B) have low moisture content compared to market sample, sample D. Sample B contain more glycerol than sample A, thus it had low moisture content. The moisture content for a sample were increased significantly with increasing water activity (Farahnaky et al., 2009) . Moisture content for sample C, which is non additive sample, quickly increased to more than 30 % after 2 days storage. At day 5, the graph showed that both combination (sample A and B) have moisture content more than 20 %, thus indicated that the sample already spoiled at that day. Li et al. (2011) stated that at the moisture

content of 26 % can trigger the bacterial growth. Maltini et al. (2003) stated that water activity microorganisms growth and well related with most of degradation reactions of chemical, enzymatic and physical nature in the food product. Glycerol (GL) and sodium chloride (S) were used to reduce the water activity. Lombard et al. (2000) reported that glycerol at the concentrations of 150 and 180 g/kg flour could produce water activity levels of 0.908 and 0.880 in South African Steamed Bread respectively, which were sufficient to inhibit bacteria, yeast and molds growth in the products.

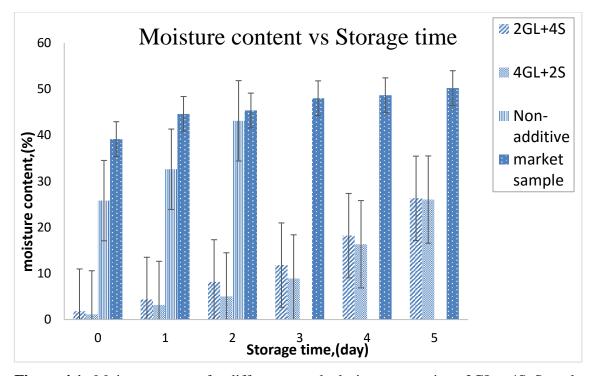


Figure 4.1 : Moisture content for different sample during storage time. 2GL + 4S, Sample A (2%(w/w) of GL + 4%(w/w) of Salt); 4GL + 2S, Sample B(4%(w/w) of GL + 2%(w/w) of Salt); non-additive sample, Sample C; market sample, Sample D. *GL=glycerol;*S=salt (NaCl).

4.3 pH changes

The pH changes during storage time were shown in Figure 4.2. It was found that the pH values of all groups showed a continuous drop trend. The initial pH for sample A contain (2% (w/w) of GL + 4% (w/w) of S) and sample B contain (4% (w/w) of GL + 2% (w/w) of S) were, 5.74 and 6.00 respectively. Sample A, B and D indicate the correlation of ($R^2 = 0.911$), ($R^2 = 0.959$), ($R^2 = 0.928$) respectively at (P<0.05).These show that all

samples A,B and D have relationship between pH changes and the storage time for the fresh noodle. The pH value of sample A that contain (2% (w/w) of GL + 4% (w/w) of S) decreased more slowly than sample B contain (4% (w/w) of GL + 2% (w/w) of S). Sample B that contain (4% (w/w) of GL + 2% (w/w) of S) have the lowest pH values compared to other sample from initial storage until day 5.A significant (P<0.01) correlation ($R^2 = 0.94414$) also was observed between the pH value and TPC for sample A. Ghaffar (2009) reported that the pH changes in foods was due to the activity of microorganisms in food that contain both of carbohydrates and protein. It is because, the microorganisms will utilize the carbohydrates, and produce acids, hence will reduces the pH value of noodle. Based on the graph, it can be seen that the market sample which is alkaline noodles have high pH at initial storage. The color of alkaline noodles is due to the present of apigenin-C-diglycosides compounds that found in wheat grain and flour are colorless at neutral or acid pH but turned yellow at higher pH (Asenstorfer et al., 2006).

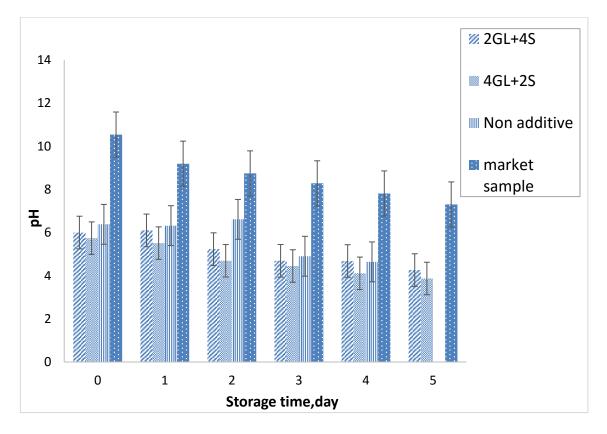


Figure 4.2 : Changes of pH in fresh noodles during storage time.2GL + 4S, Sample A (2%(w/w) of GL + 4%(w/w) of Salt); 4GL + 2S, Sample B(4%(w/w) of GL + 2%(w/w) of Salt); market sample, Sample C; non-additives of fresh noodle, Sample D. *GL=glycerol; *S=salt (NaCl)

4.4 TPC changes

The effect of TPC for the different noodles sample during storage time was shown in Figure 4.3. From the figure, it can be seen, the control sample which was non additive fresh noodle, sample C quickly deteriorated after two days, when TPC reading was over 10^6 CFU/g. Ghaffar (2009) reported that the level at 10^6 CFU/g in fresh noodles was considered as the cutoff point between spoiled and unspoiled. Thus, the analysis of the sample was stopped when the TPC was over 10^6 CFU/g. At initial storage time, both sample A and B have same TPC reading which is 10^4 CFU/g. Microbial quality is said to be the degree of acceptability of the total number of microbes present in a given food (Luo et al., 2015. Market sample, sample D that contain kansui (mixture of potassium carbonate and sodium carbonate) and alkaline salt have the longer storage time compared to the other sample. The application of alkaline salts in a noodle formula is mainly for extending the shelf life due to the mold-inhibiting effect of the high pH values.(Lü et al., 2014). Sample A that contain (2% (w/w) of GL + 4% (w/w) of S) have lower TPC reading at day 5 compared to sample B that contain (4% (w/w) of GL + 2% (w/w) of S).

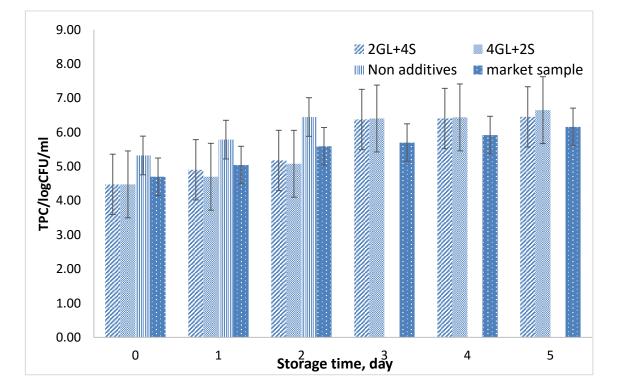


Figure 4.3 : Changes of microbiological in fresh noodles during storage time.2GL + 4S, Sample A (2%(w/w) of GL + 4%(w/w) of Salt); 4GL + 2S, Sample B(4%(w/w) of GL + 2%(w/w) of Salt); market sample, Sample C; non-additives of fresh noodle, Sample D. *GL=glycerol; *S=salt (NaCl)

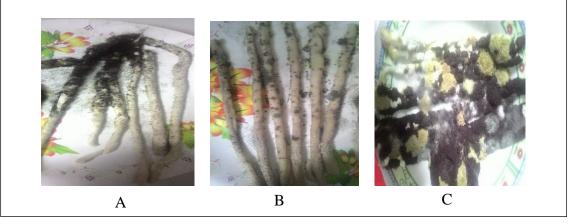


Figure 4.4 :(A) Sample contain (2% (w/w) of GL + 4% (w/w) of S) after few days storage
(B) Sample contain (4% (w/w) of GL + 2% (w/w) of S) after few days storage
(C) Sample without additives after few days storage

Figure 4.4 show the fresh noodle samples with different composition of additive and without additive after few days storage. All the samples are spoiled with mold spots on the surface and have unpleasant color and odor. Akhigbemidu et al., (2015) in their study stated that microbial analysis on noodle showed the presence of *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Aeromonas* and *Streptococcus* was the most frequently isolated while the five genera of fungi isolated were *Aspergillus*, *Mucor*, *Penicillum*, *Rhodotorula* and *candida*. The presence of all the bacteria are pathogenic and give health risk to man. The presence of these pathogens even in small numbers could render even a beverage unsuitable for human consumption (Akhigbemidu et al., 2015). Most researchers indicated that bacteria, fungi and yeasts may exert their phatogenic action either through infection of body, or as a source of toxic substances demonstrated in contaminated foods. The presence of fungi such as *Mucor*, *Rhodotorula* and *candida* is of major health concern and it is reported that these may also produce mycotoxins in foods. *Penicillum* sp. have been reported to produce aflatoxins which have been isolated from grains, fruuts, meats, spices, cheeses, milk, corn and cotton seeds (Li et al., 2017).

4.5 Sensory analysis

As shown in Table 4.1, the sensory scores (overall acceptability) by the consumer were constructed. According to the study of Ghaffar (2009), sensory characteristic could

be the main spoilage indicators in noodles. Growth of microorganisms in foods causes spoilage by producing an unacceptable change in odor, taste, appearance, texture and combining all of the characteristics (Li et al., 2016). Based on the test, market noodle score the lowest on the odor characteristics. The panels give more marks for noodle sample B which contain more glycerol (GL) and make it more viscoelasticity and also on the texture. Compared to sample A that contain (2% (w/w) of GL + 4% (w/w) of S), sample B that contain (4% (w/w) of GL + 2% (w/w) of S) get more mark from all the panels based on its appearance which have more light colour and smooth surface. The colour of food products is an important trait that strongly influences consumer acceptance of the products and is considered as a major determinant of noodle marketability (Li et al., 2016).

 19 ± 3.46 24 ± 1.73 24 ± 1.73 24 ± 1.73 Panel 9 18±2.36 23±2.00 21±0.00 22 ± 3.32 Panel ø, 21±2.83 22 ± 3.32 14±3.32 33 ± 3 Panel 60 20 ± 3.317 22±3.32 21 ± 2.83 24 ± 1.73 Panel t. **Overall acceptability score** 17±0.00 18 ± 3.317 24±1.73 21 ± 0.00 Panel Ś 16 ± 3.32 22±1.73 10±1.73 Panel 33 ± 2 цą. 21±0.00 14 ± 1.73 21 ± 2.83 24±1.73 Panel πł. 15±2.00 21±2.83 24 ± 1.73 20 ± 3.32 Panel ς÷γ 23±2.00 20±4.36 17±2.83 12 ± 3.32 Panel e-it 14±4.36 19±3.46 21 ± 2.83 20±1.73 Panel Sample Q 角 Α ЧĊ,

Table 4.1 : Sensory quality of fresh noodles

Results are presented as means \pm standard deviation (n=4).

- Sample A contain (2%(w/w) of GL + 4%(w/w) of S)
- Sample B contain (4%(w/w) of GL + 2%(w/w) of S)
 - Sample C is market sample
- Sample C contain no additive

*GL=glycerol *S=salt

4.6 Comparison on costing of noodle production

In previous study by Li et al., (2011), the addition of glycerol and sodium chloride is an effective and low-cost method to prolong the shelf-life of fresh noodles at room temperature, and will not produce any negative changes in sensory attributes. Table 4.2 showed the costing of noodles with different composition of additives in 500g flour and also price of market noodle.

Ingredients	Sample A	Sample B	Market
Wheat flour	RM 0.80	RM 0.80	-
Sodium Chloride	RM 0.10	RM 0.10	-
Glycerol	RM 0.58	RM 1.16	-
Total Price	RM 1.58	RM2.06	RM1.50

Table 4.2 : Comparison of price on noodle production

Based on the table above, there is only a little different for the total price between noodle of sample A and market price. Thus, proven that the modified noodle with glycerol and sodium chloride as additives have a reasonable price which is affordable to be bought by the consumer.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Noodles are staple foods in many countries all over the world. However, like other food, noodles also contains sufficient nutrients to support microbial growth and make it have short shelf-life. Therefore, in this paper, alternative ways to prolong the shelf life of fresh noodle have been discussed. In order to achieve the objective of the research, two scope were drawn in this thesis. Firstly, the addition of different amount of salt and glycerol at the ratio of 2:4 and 4:2 are considered in this experiment. Secondly, all samples were tested on pH, moisture content, microbiological changes which is TPC and also the sensory attributes and compared with fresh noodles from market and without additives.

Among the samples, sample B that contain (4% (w/w) of GL + 2% (w/w) of S)was proven to be best composition to prolong the shelf-life of fresh noodle based on its low pH value and low moisture content at day 5 and indicate slow changes in TPC reading between storage time compared to sample A that contain (2% (w/w) of GL + 4% (w/w))of S). Based on sensory evaluation, all panel give high score for sample B that contain (2% (w/w) of GL + 4% (w/w) of S) for its characterization on the appearance, smell, viscoelasticity and the texture of the noodle.

5.2. Recommendation

Based on the results obtained in this research study, the following recommendations were proposed:

- i. This study only covers the references from outside journal. It is suggested to further study with the additive that been used in rural area that produce their own fresh noodle and their effectiveness.
- Since the nutrition composition in the noodle can be one of the potential spoilage. It is suggested to do necessary testing like FTIR analysis that can give the composition of the ingredient of the fresh noodle.

REFERENCES

- Akhigbemidu, W., Musa, A., & Kuforiji, O. (2015). Assessment of the microbial qualities of noodles and the accompanying seasonings. *Nigerian Food Journal*, *33*(1), 48-53.
- Bui, L. T. T., & Small, D. M. (2007). The influence of formulation and processing on stability of thiamin in three styles of Asian noodles. *Food Chemistry*, 102(4), 1394-1399.
- Chanasattru, W., Decker, E. A., & McClements, D. J. (2007). Modulation of thermal stability and heat-induced gelation of β -lactoglobulin by high glycerol and sorbitol levels. *Food Chemistry*, 103(2), 512-520.
- Chen, X. W., Fu, S. Y., Hou, J. J., Guo, J., Wang, J. M., & Yang, X. Q. (2016). Zein based oil-in-glycerol emulgels enriched with beta-carotene as margarine alternatives. *Food Chem*, 211, 836-844.
- Choo, C. L., & Aziz, N. A. A. (2010). Effects of banana flour and β -glucan on the nutritional and sensory evaluation of noodles. *Food Chemistry*, 119(1), 34-40.
- Del Nobile, M. A., Di Benedetto, N., Suriano, N., Conte, A., Lamacchia, C., Corbo, M. R., & Sinigaglia, M. (2009). Use of natural compounds to improve the microbial stability of Amaranth-based homemade fresh pasta. *Food Microbiology*, 26(2), 151-156.
- Farahnaky, A., Ansari, S., & Majzoobi, M. (2009). Effect of glycerol on the moisture sorption isotherms of figs. *Journal of Food Engineering*, 93(4), 468-473.
- Fu, B. X. (2008). Asian noodles: History, classification, raw materials, and processing. *Food Research International*, 41(9), 888-902.
- Gabriel, A. A. (2008). Estimation of water activity from pH and degrees Brix values of some food products. *Food Chem*, *108*(3), 1106-1113.
- Ghaffar, S., Abdulamir, A.S., Bakar, F. A., Karim, R., & Saari (2009). Microbial Growth, Sensory Characteristic and pH as Potential Spoilage Indicators of Chinese Yellow Wet Noodles from Commercial Processing Plants. *American Journal of Applied Sciences*, 6(6), 1059-1066.
- Ginting, E., & Yulifianti, R. (2015). Characteristics of Noodle Prepared from Orangefleshed Sweet Potato, and Domestic Wheat Flour. *Procedia Food Science*, 3, 289-302.
- Holley, R. A., & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*, 22(4), 273-292.

- Huang, J.-r., Huang, C.-y., Huang, Y.-w., & Chen, R.-h. (2007). Shelf-life of fresh noodles as affected by chitosan and its Maillard reaction products. LWT - Food Science and Technology, 40(7), 1287-1291.
- Li, M., Ma, M., Zhu, K. X., Guo, X. N., & Zhou, H. M. (2017). Delineating the physicochemical, structural, and water characteristic changes during the deterioration of fresh noodles: Understanding the deterioration mechanisms of fresh noodles. *Food Chem*, 216, 374-381.
- Li, M., Zhu, K.-X., Guo, X.-N., Brijs, K., & Zhou, H.-M. (2014). Natural Additives in Wheat-Based Pasta and Noodle Products: Opportunities for Enhanced Nutritional and Functional Properties. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 347-357.
- Li, M., Zhu, K., Guo, X., Peng, W., & Zhou, H. (2011). Effect of water activity (aw) and irradiation on the shelf-life of fresh noodles. *Innovative Food Science & Emerging Technologies*, *12*(4), 526-530.
- Li, M., Zhu, K. X., Sun, Q. J., Amza, T., Guo, X. N., & Zhou, H. M. (2016). Quality characteristics, structural changes, and storage stability of semi-dried noodles induced by moderate dehydration: understanding the quality changes in semidried noodles. *Food Chem*, 194, 797-804.
- Li, M., Zhu, K. X., Wang, B. W., Guo, X. N., Peng, W., & Zhou, H. M. (2012). Evaluation the quality characteristics of wheat flour and shelf-life of fresh noodles as affected by ozone treatment. *Food Chem*, *135*(4), 2163-2169.
- López-Rubio, A., & Lagaron, J. M. (2011). Improved incorporation and stabilisation of β-carotene in hydrocolloids using glycerol. *Food Chemistry*, *125*(3), 997-1004.
- Loubes, M. A., Flores, S. K., & Tolaba, M. P. (2016). Effect of formulation on rice noodle quality: Selection of functional ingredients and optimization by mixture design. *LWT - Food Science and Technology*, 69, 280-286.
- Lü, Y.-g., Chen, J., Li, X.-q., Ren, L., He, Y.-q., & Qu, L.-B. (2014). Study on processing and quality improvement of frozen noodles. *LWT - Food Science and Technology*, 59(1), 403-410.
- Maltini, E., Torreggiani, D., Venir, E., & Bertolo, G. (2003). Water activity and the preservation of plant foods. *Food Chemistry*, 82(1), 79-86.
- Patel, A., & Singh, S. (2014). A green and sustainable approach for esterification of glycerol using 12-tungstophosphoric acid anchored to different supports: Kinetics and effect of support. *Fuel*, 118, 358-364. doi:10.1016/j.fuel.2013.11.005
- Rombouts, I., Jansens, K. J. A., Lagrain, B., Delcour, J. A., & Zhu, K.-X. (2014). The impact of salt and alkali on gluten polymerization and quality of fresh wheat noodles. *Journal of Cereal Science*, 60(3), 507-513.

- Saberi, A. H., Fang, Y., & McClements, D. J. (2013). Effect of glycerol on formation, stability, and properties of vitamin-E enriched nanoemulsions produced using spontaneous emulsification. J Colloid Interface Sci, 411, 105-113.
- Sirichokworrakit, S., Phetkhut, J., & Khommoon, A. (2015). Effect of Partial Substitution of Wheat Flour With Riceberry Flour on Quality of Noodles. *Procedia - Social* and Behavioral Sciences, 197, 1006-1012.
- Tan, H.-Z., Li, Z.-G., & Tan, B. (2009). Starch noodles: History, classification, materials, processing, structure, nutrition, quality evaluating and improving. *Food Research International*, 42(5–6), 551-576.
- Wang, Z., Wu, J., Zhu, L., & Zhan, X. (2017). Characterization of xanthan gum produced from glycerol by a mutant strain Xanthomonas campestris CCTCC M2015714. *Carbohydrate Polymers*, 157, 521-526.
- Xiaowei, F., Bin, H., Wu, D., & Wenping, Y. (2015). Effect of Organic-Acid-Soaking on the Extension of the Shelf Life of Fresh Noodles. *American Journal of Food Technology*, 10(5), 215-222.
- Yadav, B. S., Yadav, R. B., Kumari, M., & Khatkar, B. S. (2014). Studies on suitability of wheat flour blends with sweet potato, colocasia and water chestnut flours for noodle making. *LWT - Food Science and Technology*, 57(1), 352-358.

APPENDIX

Raw Data

1) pH

Day	No of exp.	Sample A	Sample B	Sample C	Sample D
0	1	5.70	5.70	6.40	10.50
	2	5.90	5.73	6.30	10.54
	3	6.20	5.68	6.44	10.58
	Mean	6.00	5.74	6.38	10.50
	1	6.20	5.53	6.40	9.10
1	2	6.10	5.40	6.30	9.20
1	3	6.00	5.60	6.26	9.27
	Mean	6.10	5.51	6.32	9.19
	1	5.20	4.70	6.70	8.70
2	2	5.24	4.57	6.65	8.50
2	3	5.25	4.80	6.48	9.02
	Mean	5.23	4.69	6.61	8.74
	1	4.70	4.35	5.10	8.30
2	2	4.60	4.45	4.90	8.40
3	3	4.69	4.45	4.70	8.14
	Mean	4.69	4.45	4.90	8.28
	1	4.64	4.00	4.60	7.70
4	2	4.80	4.10	4.70	7.83
4	3	4.60	4.23	4.62	7.90
	Mean	4.68	4.11	4.64	7.81
	1	4.40	3.70		7.20
5	2	4.20	3.90		7.40
5	3	4.18	4.00		7.30
	Mean	4.26	3.87		7.30

- Sample A contain (2%(w/w) of GL + 4%(w/w) of S)
- Sample B contain (4%(w/w) of GL + 2%(w/w) of S)
- Sample C is market sample
- Sample C contain no additive

*GL=glycerol *S=salt

2) Moisture content

Day	No of exp.	Sample A	Sample B	Sample C	Sample D
0	1	1.85	1.12	26.00	39.11
	2	1.83	1.15	25.60	39.20
U	3	1.82	1.14	25.70	39.15
	Mean	1.83	1.13	25.80	39.14
	1	4.34	3.16	33.00	45.00
1	2	4.33	3.18	32.40	44.50
1	3	4.37	3.20	32.60	44.30
	Mean	4.36	3.18	32.60	44.60
	1	8.17	5.04	43.00	45.30
2	2	8.15	5.00	43.10	45.35
4	3	8.18	5.03	43.20	45.37
	Mean	8.17	5.02	43.10	45.34
	1	11.80	8.87		47.50
3	2	11.79	8.93		48.00
3	3	11.85	8.96		48.50
	Mean	11.81	8.92		48.00
	1	18.00	16.30		48.70
4	2	18.50	16.31		48.64
4	3	18.10	16.40		48.61
	Mean	18.20	16.34		48.65
	1	26.31	26.06		50.45
5	2	26.27	25.97		50.20
3	3	26.29	26.03		50.00
	Mean	26.29	26.02		50.20

- Sample A contain (2%(w/w) of GL + 4%(w/w) of S)
- Sample B contain (4%(w/w) of GL + 2%(w/w) of S)
- Sample C is market sample
- Sample C contain no additive

*GL=glycerol *S=salt

3) Total Plate Count (TPC)

Additive		Day						
Glycerol (w/w%)	Salt (w/w%)	0	1	2	3	4	5	std deviation
2	4	4.48	4.90	5.18	6.37	6.40	6.45	0.882185
4	2	4.48	4.70	5.08	6.40	6.44	6.65	0.978139
Non-additives		5.32	5.79	6.45				0.565387
Market Sample		4.70	5.04	5.59	5.70	5.92	6.16	0.548995