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Reduced Sodium Delivery Through Submicronization of Sodium Chloride, Its Use In The Manufacture of Surface Salted Cheese Crackers and The Evaluation of Physico-Chemical, Microbiological and Sensory Characteristics of Cheese Crackers

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REDUCED SODIUM DELIVERY THROUGH SUBMICRONIZATION OF SODIUM
CHLORIDE, ITS USE IN THE MANUFACTURE OF SURFACE SALTED CHEESE
CRACKERS AND THE EVALUATION OF PHYSICO-CHEMICAL, MICROBIOLOGICAL
AND SENSORY CHARACTERISTICS OF CHEESE CRACKERS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Animal Sciences

by

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May 2014

For my parents: Wilfredo Moncada (my angel in heaven) and Domitila Reyes

I would thank them from the bottom of my heart for their love and unconditional support

For my Brothers and Sisters: Melba, Wilberg, German, Roberto, Johana, David, Karen, Nestor
Bayardo (from the stars), Wilfredo and Alexis

For believing in me and constant encouraging words

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ABSTRACT

Reducing the particle size of sodium chloride crystal would increase its dissolution rate leading to a more efficient transfer of the ions to the taste buds and hence perhaps a saltier perception of foods. The objective of this study was to develop submicrosalt by using a nanospray drying method, its use in surface salted cheese crackers and evaluating their physico-chemical, microbiological and sensory characteristics. The cheese cracker treatments consisted of 3 different salt sizes (regular, microsalt and submicrosalt) and 3 different concentrations (2, 1.5 and 1%). The 9 (3 sizes x 3 concentrations) different cheese cracker treatments were tested for salt concentration and sodium content at week 1. Water activity, yeast and mold counts, texture-fracturability, color, and consumer test were determined at week 1 and 4 months of storage. Completely Randomized Design (CRD) with repeated measures was used for water activity, yeast and mold counts, texture-fracturability, and color. A Balanced Incomplete Block Design was used to conduct the consumer analysis of cheese crackers for submicrosalt (2, 1.5 and 1%), microsalt (2, 1.5 and 1%) and regular 2% (control as used by industry) using 476 participants. Submicrosalt treatments (2, 1.5 and 1%) had positive effect in yeast reduction at 4 months compared to regular salt (2, 1.5 and 1%). There was no mold growth in all treatments at all times. The L^* , b^* , C^* and h^* values in all treatments increased significantly ($P < 0.05$) from 1 week to 4 months. At 4 months, submicrosalt treatments (2, 1.5 and 1%) resulted in having significantly ($P < 0.05$) more preferred saltiness scores compared to control (regular 2%). At 4 months, submicrosalt (1.5 and 2%) showed significantly ($P < 0.05$) more preferred just about right saltiness scores compared to control. The reduction of 25 and 50% salt content in cheese cracker through use of submicro particulated salt maintained low counts in yeasts, no counts in molds and did not adversely influence sensory color, aroma, crunchiness, overall liking and

acceptability scores, which were the same compared to control and microsalt treatments (2, 1.5 and 1%). Reduction in sodium chloride particle size 1000 fold from regular salt to submicrosalt increased saltiness but reduction in salt size 10 fold from microsalt to submicrosalt did not increase the saltiness of surface salted cheese crackers.

CHAPTER 1: INTRODUCTION

1.1. Importance of Salt in the Food

Historically between 5000 to 10,000 years ago, the main reason for the addition of salt to food was for preservation and it was the driver of high sodium consumption of all societies in the early days (He and MacGregor 2007). However, it also has had function in flavor and processability.

Taste is not the only reason for the continued use of high levels of sodium in foods (Henney et al., 2010). For some foods, sodium still plays a role in reducing the growth of pathogens and organisms that spoil products and reduce their shelf life (Kurlansky, 2002). In other applications, sodium levels remain high because salt plays additional functional roles, such as improving texture. Many of other sodium-containing compounds are also used for increasing the safety and shelf life of foods or creating physical properties desired.

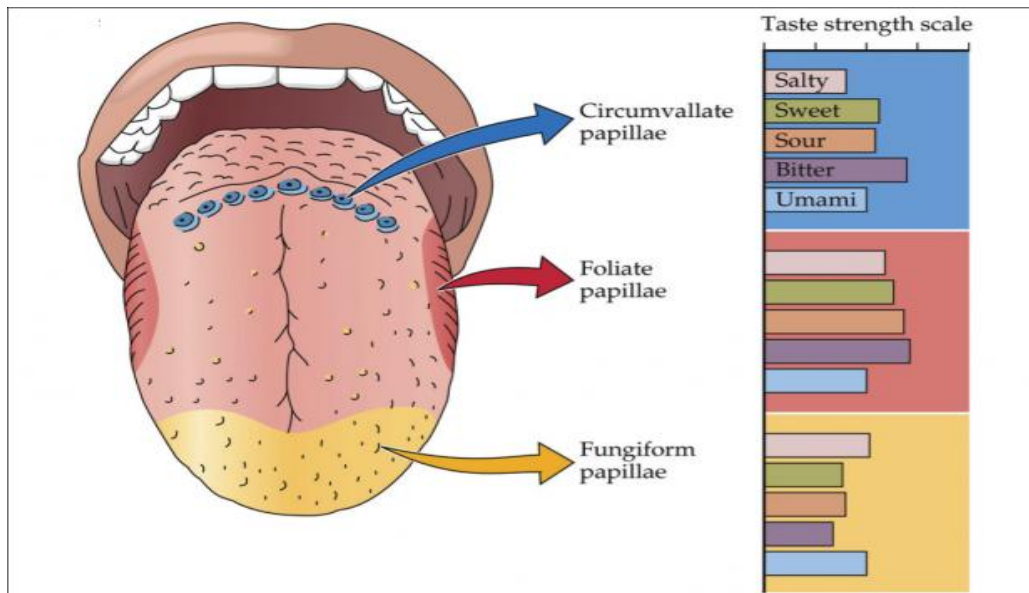
Salt has an effect on prevent microorganisms growth reducing the water activity of the food and an antimicrobial action due the ion (Kurlansky, 2002). In other words, the water will not be as accessible to microbial growth and reduce the onset of potential pathogens. The cells will lose water in a high salt environment and divert energy to accumulate solutes in the cell to reach a balance with the conditions in the food (Kurlansky, 2002). Additionally, the activity of microorganisms and enzymes in dairy products maturation process may is influenced by salt, as is the water activity.

Salt adds the typical salty flavor to the food. The cation portion of the salt or the sodium portion of salt gives salt the typical salty perception (PRNewswire, 2012). Salt contributes to the taste of

many foods, and also contributes more generally to overall flavor contributions from other components (Henney et al., 2010). In addition, salt can reduce the perception of other stimuli, such as bitter compounds.

The processing functions of salt have important effects on gluten development, reducing the sticky texture; improve water holding capacity in dairy products and increases binding in shredded ingredients (Desmond, 2006). Sodium chloride also contains more cations than any other salt product, thus the salty perception is hard to replace. According to PRNewswire, (2012) potassium chloride is very similar in molecular weight to sodium chloride and will give the next best salty perception in a dairy product when comparing salts. However, potassium chloride also will add a bitter or metallic note in addition to the salty note to the cheese. Lowering its sodium content represents a challenge as well as an opportunity to improve the nutritional value without sacrificing food sensory attributes (Henney et al., 2010).

The way that sodium chloride is absorbed start when the food product is taken; subsequently the sodium and chloride ions are released into the mouth depending on the structure and composition of food, mastication and salivation (Keast and Roper, 2007). The ions are transported to the taste buds (contain most of the taste receptor cells), which are clustered on three types of papillae (fungiform, foliate, and circumvallate) located on the tongue (at the apical top end of the taste receptor cells) (Keast and Roper, 2007; Neyraud et al., 2003) (Figure 1). Sodium and chloride ions are required for the activation of the salt receptor, while sodium salts with larger anions do not provide much saltiness (Keast and Roper, 2007).



<http://www.mindsmachine.com/summary06.html>

Figure 1. Human Taste Buds

1.2. Dietary Sodium Intake

The amount of sodium a person could consume each day varies from individual to individual and from culture to culture; some people eat as little as 2 g/day, some as much as 12 grams. The Departments of Agriculture and Health and Human Services recommend daily intake of less than 5.8 g of salt (2.3 g of sodium), with a lower target of 3.7 g of salt per day for most adults (CDC, 2011). Despite these guidelines, the average man in the United States was estimated to have consumed 10.4 g of salt per day and the average woman 7.3 g per day during the period from 2005 through 2006. An increased consumption of processed convenience foods, with high salt contents, has had serious implications on the nutritional status of consumer's diets and a negative impact on health. High intakes of processed foods, such as ready meals, pizzas, savory snacks and bread have led to an excessive consumption of dietary salt (He et al., 1999).

He and Wheelock, (1997) identified a positive relationship between dietary intake of sodium and blood pressure across populations as well as within populations. The high blood pressure is a strong risk factor for coronary heart disease and stroke, a high dietary sodium intake might be predicted to increase the risk of cardiovascular disease (He et al., 1999). Additionally, He and Whelton, (1997) reported that some studies have found a positive relationship between average population dietary sodium intake and mortality due to stroke.

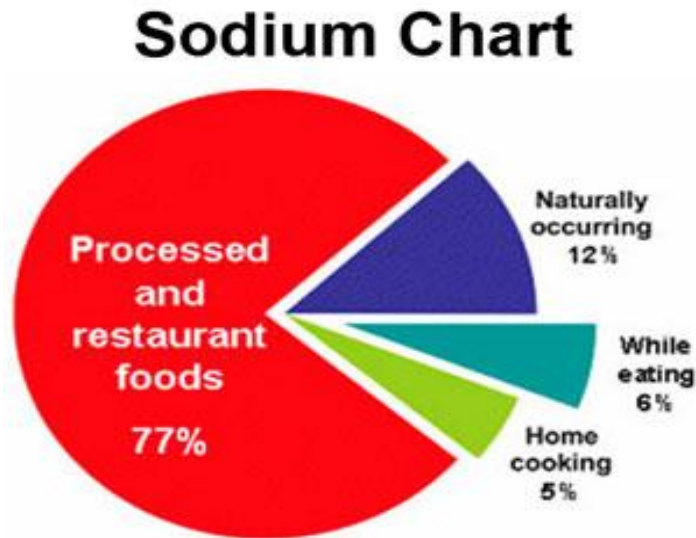
Most individual's blood pressure is reduced as a response in lowering of sodium intake (He and Whelton, 1997). Small reduction in salt consumption in the population could reduce the blood pressure and it should likely to result in substantial health benefits such as: approximately 2.5 million deaths a year could be prevented worldwide and many strokes and heart attacks (He and Whelton, 1997). However, 75 to 80% of the salt in the U.S. diet comes from processed foods, and only 5% is added during food preparation or consumption (Figure 2) (Hooper et al., 2004).

Another important factor to take in consideration for stopping hypertension is the lifestyle modification, which it consists in changes in the diet, and therefore the individuals may reduce blood pressure, prevent incidence of hypertension, enhance antihypertensive drug efficacy, and decrease cardiovascular risk (Sacks et al. 2001).

1.3. Health benefits

Departments of Agriculture and Health and Human Services recommend daily intake of less than 5.8 g of salt (2.3 g of sodium), with a lower target of 3.7 g of salt per day for most adults. Reducing dietary salt by 3 g per day is estimated to reduce the annual number of cases of high blood pressure by 11 million, coronary heart disease (CHD) cases up to 120,000, stroke up to

66,000 and would save up to 392,000 quality-adjusted life-years and save \$10 billion to \$24 billion in health care costs annually (Bibbins-Domingo et. al., 2010).



<http://www.precisionnutrition.com/all-about-high-blood-pressure>

Figure 2. Sodium sources in the US diet

The population from the different segments would obtain benefit; specifically the African-American segment benefiting proportionately more, women benefiting particularly from stroke reduction, older adults from reductions in CHD events, and younger adults from lower mortality rates (Bibbins-Domingo et al., 2010). A regulatory intervention designed to achieve a reduction in salt intake of 3 g per day would save up to 400,000 quality-adjusted life-years and up to \$24 billion in health care costs annually (Palar and Sturm, 2009).

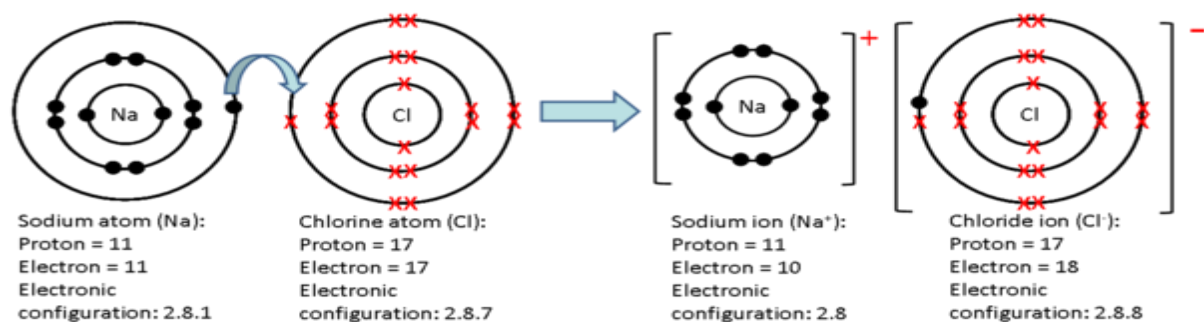
1.4. Sodium Chloride Properties

Sodium chloride also known as salt, common salt, or table salt, is an ionic compound with the formula NaCl, representing 60.663% elemental chlorine (Cl) and 39.337% sodium (Na) (Salt Institute, 2013) (Figure 3). The physical properties of NaCl are the following: isometric, cubic

crystal form, orthorhombic crystal system, clear to white color, a refraction index of 1.5442, melting point of 2,669 °F, boiling point of 2,669°F, hardness of 2.5 and a critical humidity of 75.3% at 68°F (Salt Institute, 2013).

Sodium chloride is the salt responsible for the salinity of the ocean and of the extracellular fluid of many multicellular organisms. Humans, and all animals, possess an inherent appetite for salt. For many million years ago the evolutionary ancestors of humans ate a diet that contained less than 0.25 g/day of salt (Eaton and Konner 1985). Sufficient sodium exists in natural foods to ensure that mammals could develop away from the sea. Moreover, the salt has played an important role in the development of civilization (MacGregor and de Wardener 1998). It was first found to have the magical property of preserving foods, when it was discovered that meat soaked in saline solution could be preserved for a long time.

Salt brings to food more than one of the five basic taste sensations (sweet, salty, sour, bitter, and umami) (Salt Institute, 2013). It is necessary for the normal physiological function of human beings, aids digestion by providing chlorine for hydrochloric acid (Salt Institute, 2013). Furthermore, salt has the most prevalent cation (sodium) in extracellular fluid, and decreases in sodium levels result in decreases in blood volume and pressure that can be fatal (CDC, 2011).



BBC, 2011

Figure 3. Sodium chloride chemical structure

1.5. Challenges in Reducing Salt

The most notable sensory changes resulting from a reduction in salt is often an increase in bitterness, which can result from either a loss of bitterness inhibition by salt, or bitterness inherent in some salt replacers (Heidolph, et al., 2011). Perceived bitterness is considered to be a negative and undesirable attribute in many food products.

Sensory challenges arising from salt reduction relate not only to maintaining an acceptable salt perception but also relate to the additional sensory properties (Henney, 2010). Ideally, salt reduction should not adversely change the characteristic flavor and mouthfeel of a product (Henney, 2010).

1.6. Improvement in Salt Reduction

In the past few decades, the reductions of sodium in foods have had the attention. The World Health Organization who has called on food manufacturers to lower the sodium content in food products. The WHO recommends a daily salt intake of 5 g per day and this will require large reductions from current salt intake levels (WHO, 2007).

The acceptability of reduced salt foods has been improved in the last 7 years, leading the development of numerous salt replacing ingredients and compounds to enhance the salty flavor of salt without affecting the sodium content; however they do not have the preservative characteristics of salt and other attributes (Brandsma, 2006).

Sodium chloride replacers impart a salty flavor to food but the flavor profile is different. Potassium chloride (KCl), ammonium chloride, calcium chloride and magnesium sulphate are most frequently used though deliver undesirable flavors to foods (Heidolph, et al., 2011).

Katsiari et al., (2008), reported that potassium chloride may maintain the salty taste of sodium chloride in foods up to 25% without losses in palatability. However, a reminder of bitter taste was attributed to the excessive addition of potassium chloride. Brandsma (2007) concluded that potassium chloride can generally only substitute up to 30% of salt in majority of food products, because at higher levels, potassium chloride has a perceptible metallic flavor. Potassium chloride's metallic flavor along with consumers avoiding potassium for health reasons limits its use as a salt replacer in food products (Liem, et al., 2011). Additionally, the uses of sodium chloride replacers are more effective in hot savory products than the rest of food products (Katsiari et al., 2008).

The incorporation of taste enhancers (yeast extracts and monosodium glutamate (MSG)) are used in the investigation of improving the quality of reduced sodium products (Kilcast, and den Ridder 2007). The main effect in foods is the increase of flavor due to activating taste buds linked to the umami taste receptors (Brandsma, 2007). Furthermore, negative organoleptic and health problems are associated with some of taste enhancers. The monosodium glutamate has been associated with possible health implications including stomachache, hyperactivity, and sickness (Kilcast, and den Ridder 2007).

The sodium reduction by stealth approach involves the gradual reduction of salt in processed foods over an extended period of time that is unnoticeable to consumers, large reductions could be achieved (Liem, et al., 2011). It is thought that small reductions in salt content over a period of time, enables the consumer not to detect any changes to the organoleptic qualities of products, and at the same time as reducing the salt content and the consumer's sensitivity to the saltiness of a product (Liem, et al., 2011). Additionally, Grigis et al., (2003), gradually reduced the sodium

content in 25% of white bread over a period of 1.5 months (6 weeks) and consumers were not able to notice a difference in flavor.

The salt reduction includes the adaptation of the food matrix in finished products (Grigis et al., 2003). This method involves altering the food matrix when reducing salt by applying the salt at different levels during the finished product (Morris, et al., 2009). It is speculated that a continuous delivery of salt from food can lead to a gradual decline of taste sensitivity (Morris, et al., 2009; Grigis et al., 2003).

Salt crystals size reduction has the ability to influence the delivery of the salty taste (Johnson et al., 2011). It is speculated that the smaller the particle size, the faster the rate of dissolution and therefore the rate of perception of salt is increased (Johnson et al., 2011). The modification of the structures as an approach for sodium reduction is to optimize the delivery of sodium ions to the taste buds and therefore to cause maximum stimulation of the taste receptors without increasing the sodium content of the product (Noort et al., 2012). Additionally, Noort et al., (2012) concluded that the key for optimizing the delivery of sodium ions is that during mastication a portion of the sodium in the product could not be detected by the taste receptors and consequently does not contribute to the saltiness of the product. To obtain acceptable product structures (salt particles), it is important to take in consideration the interactions between the shape and distribution of the salt in the food and the breakdown of the food in the mouth (Noort et al., 2010). All the interactions include salt distribution within the product, salt dissolution and mixes in saliva, how it is released from the food product and transported to the taste buds (Noort et al., 2010).

1.7. Submicro and Nanoparticles Production

A nanometer is a thousandth of a thousandth of a thousandth of a meter (10^{-9} m). One nanometer is about 60,000 times smaller than a human hair in diameter or the size of a virus, a typical sheet of paper is about 100,000 nm thick, a red blood cell is about 2,000 to 5,000 nm in size. Nanoparticles are generally accepted as those with a particle size below 100 nanometers where unique phenomena enable novel applications and benefits (Morris and Parker, 2008). Submicron and nanomaterials on which most of the researches have been carried out are normally powders composed of nanoparticles which exhibit properties that are different from powders of the same chemical composition, but with much bigger particles (IOM, 2009).

1.8. Nanostructures in Food

Many natural foods contain nanoscale components and their properties are determined by their structure (Nanotechnology and the food industry, 2013). These have been eaten safely for generations. In fact, some of food's most important raw materials (proteins, starches, and fats) undergo structural changes at the nanometer and micrometer scales during normal food processing (Nanotechnology and the food industry, 2013). The number of food products using nanotechnology is relatively small because this field is still only a promise for research and development of new food products (Morris and Parker, 2008) and consequently, may revolutionize the food system and influence the science of food in a positive way.

The dairy proteins like native beta-lactoglobulin (3.6 nm) could be denatured via pressure, heat, and pH and the denatured components reassemble to form larger structures (yogurt) (Semo et al., 2007). Casein micelles could be used as for entrapment, protection and delivery of sensitive hydrophobic nutraceuticals within other food products (Semo et al., 2007). Starch granules

expand when heated and hydrated releasing biopolymers that can be recrystallized into nanosized and micro-sized structures (10-20 nm); dextrans and other degradation products of extrusion can be used to encapsulate bioactive substances in microregions (Bugusu et al., 2009). Also, Mulder and Walstra (1974) reported that fats, monoglycerides, can self-assemble into the nanoscale level (homogenized milk = 100 nm), and hierarchically structured into triglycerides can be crystallites (10-100 nm).

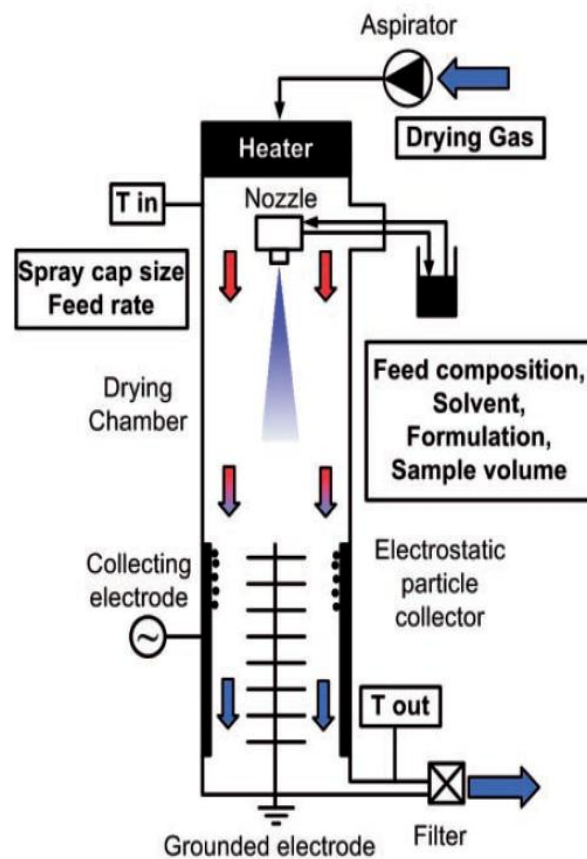
Mulder and Walstra (1974) and Semo et al., (2007) concluded that the dairy industry utilizes micro-sized and nanosized basic structures (casein micelles, fat globules, whey proteins) to produce different products in emulsions form such as whipped cream, butter, and ice cream.

Additionally, functionalized nanostructure materials could have potential applications in many sectors of the food and pharmaceutical industry that are under investigation, such as nanosensors, new packaging materials with improved mechanical and barrier properties (smart packages), and delivery systems of targeted nutrients (Nanotechnology and the food industry, 2013). A better understanding of the benefits of this technology will help to increase the acceptance of nanotechnology by the food industry as another alternative to produce food.

1.9. Nanospray Dryer

The Nano Spray Dryer B-90 is based on a new spray drying concept and is described in the Figure 4. The drying gas enters the apparatus from the top, heating up to the setting inlet temperature, then flows through the drying chamber, exiting the spray dryer at the bottom outlet, and is fine filtered before (Buchi, 2012). The new Nano Spray Dryer B-90 utilizes a vibrating mesh technology for fine droplets generation (before evaporation). This is a revolution in spray drying technology, making it possible to produce powders in the submicron size range with very

narrow distributions and high formulation yields (Buchi, 2012). The piezoelectric crystal driven spray head is incorporated with a small spray cap that contains a thin perforated membrane (spray mesh) having an array of precise micron sized holes (4 μm , 5.5 μm or 7 μm). When the piezoelectric actuator is driven at an ultrasonic frequency (60 kHz), the mesh will vibrate upwards and downwards, injecting millions of precisely sized droplets from the hole and generating the aerosol and subsequently the production of powder Nanoparticles (Buchi, 2012). For the purpose of this research the particles obtained were called submicrosalt particle due the particle range was very wide (80% of the submicrosalt was between 500 nm to 1900 nm).



Buchi, 2012

Figure 4. Nanospray Dryer B-90

1.10. Savory Cheese Crackers Trend

The food coding scheme of the US Department of Agriculture (2008) claims that milk products was estimated to contribute about 8 to 8.9 % of sodium to the diet of consumers over 20 years of age. Cheeses represent 50% of the sodium in that category.

The growing importance of health and wellness has significantly changed consumption and buying behaviors. The consumer demand for convenience has always been a driving factor in the food industry.

Tully and Holland (2010), reported that the cracker market for 2008 in the U.S. is approaching \$4B in sales dominated by three companies, Kraft, Kellogg and Pepperidge Farm. Currently these big three control greater than 75% of total cracker sales. However, focused cracker producers are taking advantage of different trends and creating increased consumer interest in areas such as natural and organic, new flavor varieties and gourmet flavors. The broader food industry theme of natural and organic products has been targeted by leading brands and smaller, more focused brands alike. Additionally, Whitaker, (2013) reported that cracker sales in 2012 grew 4.1% to 4,468.1 million (\$4.47 billion).

1.11. Justification

In the last 10 years the high sodium consumption has been linked to the increase in cardiovascular disease (CVD) and stroke cases in the American society (American Heart Association, 2013). In 2010 were affected 700,000 American by stroke (CDC, 2011). And 67 million American had high blood pressure (1 in 3 American adult) and more than half (36 million) did not have it under control, (CDC, 2011). The United States health care services in 2010 spent in direct medical expenses for CVD and stroke \$286 billion (Heidenreich et al.,

2011). The World Health Organization (2012) and the World Heart Federation, (2013) reported that in 2011 about 970 million individuals had hypertension and 15 million suffered a stroke around the world. Approximately 7.1 million deaths per year were caused due to hypertension and 6 million by stroke and another 5 million were left permanently disabled (American Heart Association, 2013; WHF, 2013). Departments of Agriculture and Health and Human Services recommend daily intake of less than 5.8 g of salt (2.3 g of sodium), with a lower target of 3.7 g of salt per day for most adults. Reducing dietary salt by 3 g per day is estimated to reduce the annual number of cases of high blood pressure by 11 million, coronary heart disease (CHD) cases up to 120,000, stroke up to 66,000 and would save up to 392,000 quality-adjusted life-years and save \$10 billion to \$24 billion in health care costs annually (Palar and Sturm, 2009).

The sodium levels, especially in processed foods, remain high. The tastes and flavors associated with historical salt use have come to be expected, and the relatively low cost of enhancing the palatability of processed foods has become a key rationale for the use of salt in food.

Cheese cracker is one of the most popular snack (sales \$4.47 in 2012) products in North America, with an excellent acceptability and well-liked dairy products (Whitaker, 2013). The alternative to reduce the sodium content in cheese crackers without using sodium chloride replacer is to maintain constant the sodium chloride composition and at the same time modifying the physical aspects of salts. It has been speculated that lowering the particles size of nutritional salts would lead to enhanced saltiness (Noort et al., 2012).

This theory is based on the fact that the smaller the salt grains the faster the dissolution rate of salt in saliva. Faster dissolution rate inherently would lead to a more efficient transfer of the ions to the taste buds and hence a saltier perception of the food product. According to Noyes and

Whitney (1897) the size reduction leads to an increased surface area and to an increased dissolution velocity. Therefore, micronization is a suitable way to successfully enhance the bioavailability of particles where the dissolution velocity is the rate limiting step. By moving from micronization further down to submicronization and nanonization, the particle surface is further increased and thus the dissolution velocity increases too (Junghanns and Müller 2008). Additionally, the saturation solubility (point of maximum concentration) is also a function of the particle size. The saturation solubility increases with decreasing particle size below 1000 nm (Junghanns and Müller 2008).

The hypothesis was whether 25 and 50% reduced amounts of sodium chloride use in submicro particulated form would alter physico chemical, microbiological and sensory characteristics including saltiness of surface salted cheese cracker.

1.12. Objectives

- Developments of NaCl submicro particles by using nano spray drying method.
- Incorporation of the developed NaCl submicro particles in surface salted cheese cracker and it's the evaluation of physico-chemical, microbiological, and sensory characteristics.

CHAPTER 2: MATERIALS AND METHODS

2.1. Sodium Chloride Submicro Particles Manufacture

A solution of salt in deionized water (3% w/w) was prepared, completely dissolved and filtered through a Whatman Number 2 filter paper (Clifton, NJ) and subsequently was processed by Nanospray drying (Nanospray dryer B-90, BÜCHI Labortechnik AG, Flawil, Switzerland). The sodium chloride solutions were sprayed through the nozzle size of 4 µm. The air flow (125 l/min), pressure (38 millibars), head temperature (95°C) and spray percentage (90%) were kept constant in all treatments. The resulting submicron particles were analyzed by Scanning Electron Microscopy (SEM) to visually analyze shape and size and by Dynamic Light Scattering to analyze the particle size distribution.

2.2. Experimental Design

The sodium chloride submicro particles were used in the cheese cracker sodium reduction treatments. The treatments consisted in 3 different salt sizes (Regular, microsalt and submicrosalt) and 3 different concentrations (2, 1.5 and 1%). The 9 (3 sizes x 3 concentrations) different cheese cracker treatments were tested for salt concentration and sodium content at week 1. Water activity, yeast and molds, texture-fracturability, and color at week 1 and 4 months of storage. The experimental design for salt concentration and sodium content was a Completely Randomized Design (CRD), and the experimental design for water activity, yeast and molds texture-fracturability, and color was a Completely Randomized Design (CRD) with repeated measures. For the sensory analysis of cheese crackers were analyzed 7 treatments; 3 concentrations of submicrosalt (2, 1.5 and 1%), 3 concentrations of microsalt (2, 1.5 and 1%) and

1 concentration of regular salt 2% (control as used by industry) at week 1 and 4 months and the sensory test was conducted and analyzed as a Balanced Incomplete Block Design (BIBD).

2.3. Particle Size Distribution

The submicrosalt and microsalt particle size measurement was analyzed in the Microtrac S3500 by wet measurement (isopropyl alcohol-mobile phase). 50mg of submicrosalt were added in 100ml IPA and sonicated for 180 seconds. The particle sizes were measured 3 times by detecting the low angle region to almost the entire angular spectrum (approximately zero to 160 degrees).

2.4. Scanning Electron Microscopy

The scanning electron microscopy analysis was conducted by a JSM-6610 high-performance scanning electron microscope (SEM) (JEOL Ltd., Tokyo, Japan) with an acceleration voltage of 5 kV was used for fast characterization, imaging and measurement of powder structures. A thin layer of submicrosalt and microsalt was placed on aluminum SEM stubs with sticky tabs, coated with platinum in an EMS 550X sputter coater and imaged with JSM-6610 High Vacuum mode SEM (JEOL Ltd., Tokyo, Japan) with a vacuum of 1×10^{-1} mbar using 25 mA during 2 min. Prior to SEM images were recorded, 5 field observations were taken.

2.5. Cheese Cracker Manufacture

The cheese crackers formulation is showed in the Table 1 and the manufacturing process is found in the Figure 5. Subsequently, the cheese crackers were packaged in modified atmosphere packaging (BOPPT/VMCPP-Biaxially-Oriented Polypropylene-Plastics technology/Cast Polypropylene) bags.

Table 1. Cheese cracker formulation

Ingredients	Percentages (%)
Unsalted fresh cheese	61
Unsalted butter	14
Wheat flour	18
Water	5
Cayenne pepper	2

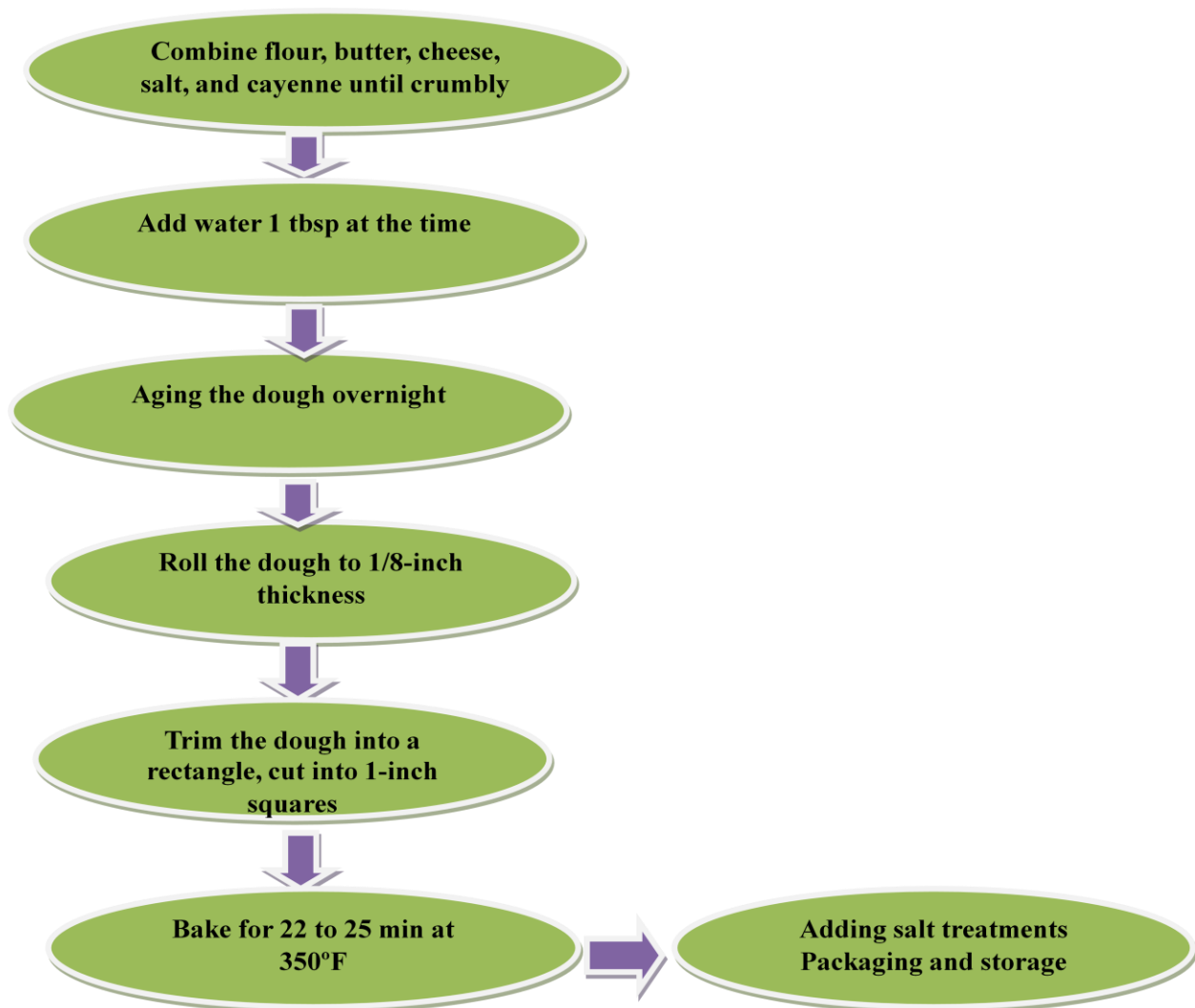


Figure 5. Cheese cracker manufacturing process

2.6. Salt Concentration

The cheese crackers salt concentration was analyzed by the method of Korkmaz, (2012) with slightly modification; in which it determined the potentiometric titration with silver nitrate. The cheese cracker samples were evaluated at week 1. The 25 g of cheese crackers were weighted and transferred to a 500 ml Erlenmeyer flask with 250 ml deionized water. The solution was mixed for 1-3 minutes and subsequently 10 ml of the solution were pipetted into a 250 ml volumetric flask with 25 ml of deionized water. 7 drops of Potassium Chromate were added to the solution. Then the solution was titrated with 0.1 N Silver Nitrate until the solution changes the color (bright red). The percentage of salt concentration was determined using the equation.

$$\text{NaCl} = C \times 5.83$$

$$C = \text{ml of silver nitrate used}$$

2.7. Sodium Content

Specifically sodium content was determined by the mathematic calculation from the salt content at week 1. The percentage of salt content was multiplied by number of g of sample used; subsequently that result was multiplied by the Na percentage in the NaCl molecule and then multiplied by 1000 (mg/g) and finally was obtained the result of sodium content in mg/g.

2.8. Water Activity (A_w)

Water activity (A_w) of the cheese cracker samples was determined using a Hygrolab Rotronic 3 (Rotronic, Bassersdorf, Switzerland) at week 1 and 4 months of storage. The cheese crackers were grinded with a mortar and pestle and filled up to 75% of the volume of 14 mm disposable PS-14 A_w cups. The cheese cracker treatments were measured using the standard function of the device which keeps measuring constant values of A_w . Subsequently, for each cheese cracker

sample A_w was determined at the point when the sample was stable in the graph available on the software HW4. All of the measurements were carried out in triplicate.

2.9. Yeasts and Molds

The cheese crackers were tested for yeast and molds before conducting the sensory evaluation using the method by 3M Company (2005), with slight modifications. The enumeration of yeasts and molds was conducted the week 1 and 4 months of room temperature storage. These analyses were determined by making serial dilutions and duplicated in peptone water (0.1% wt/v) and plated in 3M™ petrifilms for yeasts and molds (3M Microbiology, St. Paul MN). The petrifilms were placed on a flat surface and 1 ml of the cheese crackers dilution was placed on the center of the bottom film. The inoculum was covered by the top film and spread to an area of 20 cm² using the plastic spreader supplied. The 3M™ Petrifilms plates were incubated for 72 hours at 22°C. After the incubation period, the colonies were counted.

2.10. Texture-Fracturability

The analysis was performed with a texture analyzer Stable Micro Systems model TA.XT. plus (Texture Technologies Corp., New York, USA) with a HDP/BS blade. The weight calibration was performed daily with a 2000 g weight standard and the height calibration was set at 15 mm. The set up used in the TPA included: pretest speed of 1 mm/ sec, test speed of 5 mm/sec and posttest speed of 2 mm/sec, the trigger system used “force”, and defining 2.4 mm of distance. The cheese cracker samples had uniform surface. At the day of the analysis the samples were removed from MAP (Modified Atmosphere packaging) sealed bag. 5 samples from each treatment were analyzed. The parameter evaluated was fracturability (g).

2.11. Color Analysis

Evaluation of color was performed on a group of cheese cracker surface at 1 week and 4 months of storage (room temperature), using a Hunter MiniScan XE Plus, portable color spectrophotometer (Hunter Associates Laboratory Inc. Reston, VA, USA). The instrument was calibrated using the black and white standard tiles that came along with the instrument. The operating conditions were 10° observer, D65 illuminant and 45/0 sensor. An average of three values was taken per sample. An optical aperture of 1.7 cm was used. L^* , a^* , b^* , C^* and h values were recorded (with L^* representing the lightness on a scale of 0 (dark) to 100 (white), $+a^*$ for redness, $-a^*$ for greenness, $+b^*$ for yellowness and $-b^*$ for blueness), C^* is the derived quantities saturation (chroma) defined as $(a^{*2}+b^{*2})^{1/2}$ and hue angle (h) equals $\tan^{-1} (b^*/a^*)$. Mean L^* , a^* , and b^* values at each time point measured were used to calculate the magnitude of the total color difference (ΔE) using the following equation (HunterLab 2001):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

2.12. Consumer Study

The sensory study was approved by the LSU Institutional Review Board with the IRB exempt number of HE13-15 (Appendix A). A Balanced Incomplete Block Design was used to conduct the consumer analysis of cheese crackers using 476 participants in total at week 1 and 4 months later. The cheese crackers containing the seven treatments were placed in a plastic plates; a 3 digits random number code was used to label the plastic plates. Three treatments samples were provided to each participants along with the evaluation questionnaire which consisted of a 9-points rating scale (1= Dislike extremely, 9 = Like extremely), and acceptability and purchase intent questions (yes/no questions). Panelists were asked to evaluate each cheese cracker for the

following attributes: color, aroma, crunchiness, saltiness, overall liking, acceptability and purchase intent (Appendix B).

2.13. Statistical Analysis

The cheese cracker data from the physical and chemical analysis were analyzed using Proc Mixed of Statistical Analysis System (SAS[®]) and the data from the sensory analysis were analyzed using the Proc Glimmix of Statistical Analysis System (SAS[®]). The analysis of change on purchase intent was performed by the McNemar's Test using SAS[®] 9.3. Differences of least square means were used to determine significant differences at $P < 0.05$ for main effects (treatment and time) and their interaction effects (treatment * time). Data are presented as mean \pm standard deviation of the means. Significant differences were determined at $\alpha = 0.05$. Significant difference ($P < 0.05$) among the main effects analyzed using Tukey's adjustment and Macro program to determine differences between treatments.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Particle Size Distribution for Microsalt and Submicrosalt

Microsalt particle size distribution was showed in the Figure 6. There was a wide range of particle sizes from 3.27 μm to 148 μm . In the figure 6 is observed that approximately the 93% of the particles were in the size of 11 μm to 148 μm .

Submicrosalt particle size distribution is shown in the Figure 7. There was a range of particle sizes from 500 nanometers to 5500 nanometers; accordingly, the 80% of the submicro particles analyzed were between 500 nm to 1900 nm respectively (Figure 5). Similar results for sodium chloride as a calibration solution for the equipment were obtained by Li et al., (2010) using the nanospray dryer B-90 with slight modification in the operating conditions. The particle size distribution was from 517 to 993 nm by increasing the solid concentration from 0.1 to 1% w/w. Furthermore, they analyzed the furosemide powder (diuretic drug) and were obtained size measured of 1.24 μm using a 1.25% concentration. Additionally, Buchi, (2012) described that the nanospray dryer B-90 is able to produce narrowly distributed particles in the range of 300 nm to 5 μm using 4 μm spray cap.

Schmid et al., (2009), analyzed trehalose (protein stabilizer) in the nanospray dryer B-90 (spray and obtained particle size range was between 600 nm to 1.2 μm at 1 wt % concentration. Blasi et al., (2010) evaluated the sodium alginate (emulsifier and immobilization agent) in the nanospray dryer B-90 and found that the particle size distribution was 761 nm to 1.2 μm for the spray cap of 4 μm and 5.5 to 7.6 μm for the spray capo of 7.0 μm .

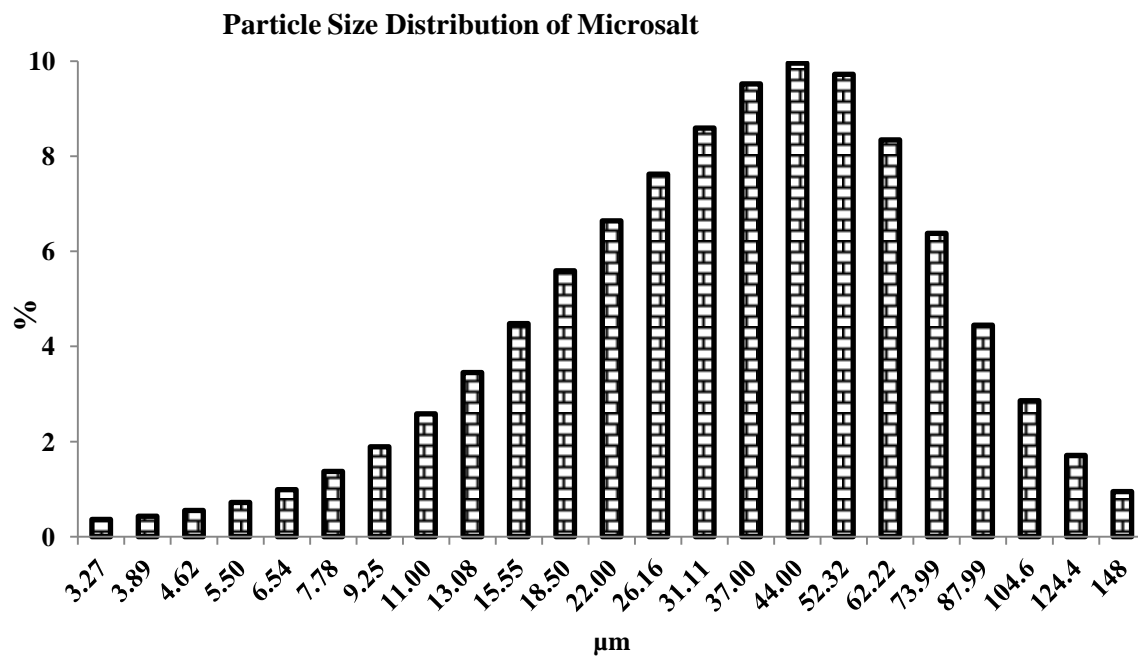


Figure 6. Particle size distribution of microsalt

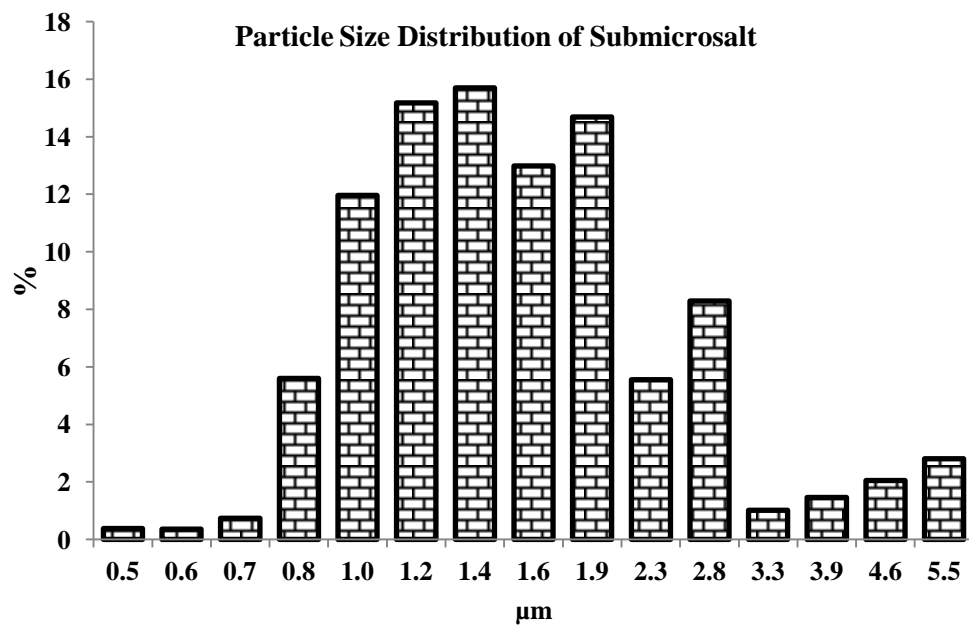


Figure 7. Particle size distribution of submicrosalt

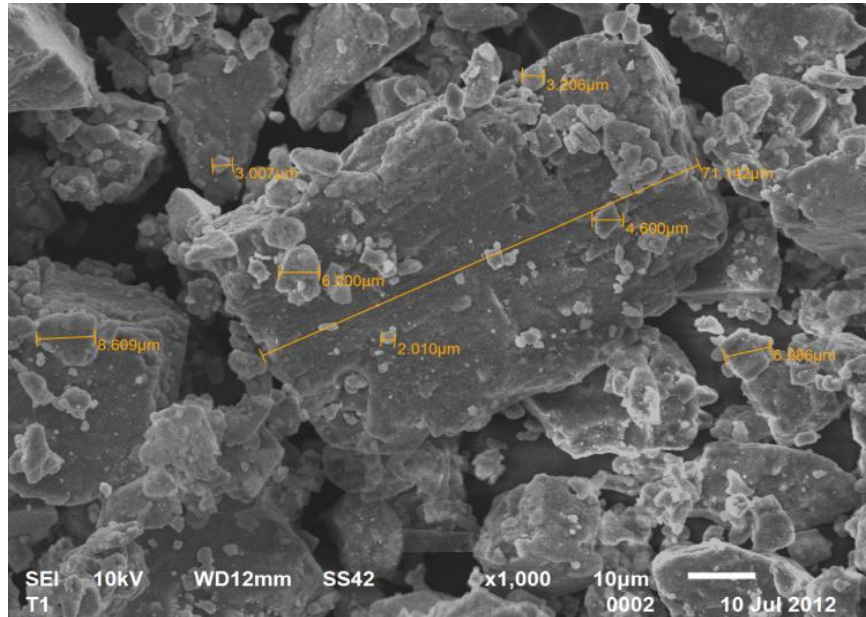
3.2. Scanning Electron Microscopy of Surface Salted Cheese Cracker

Scanning electron microscopy (SEM) was used to observe the structure of submicrosalt and microsalt (Figure 8 and 9). Microsalt particles were more irregular in shape and sizes (Figure 8A). Its shape was non spherical, irregular squares and different dimensions (Figure 8B). According to the manufacturer Cargill (2012), this microsalt was produced using a grinding method (ball mill), so the differences in shape could be attributed to the production process. However, ball milling provide many advantages including ease of handling, large-scale production capabilities, applicability to a variety of materials and reliable control over parameters, to achieve.

The structure of submicrosalt in size and shape could be affected by several factors in the process of nanospray drying (Figure 9A). The particle sizes observed through SEM photomicrographs for microsalt and submicrosalt were 3-75 μm and 520-1300 nm, respectively (Figure 8B and 9B). The scanning electron microscopy images indicated that the submicrosalt yielded mostly irregular submicro particle shapes and with slight agglomeration (Figure 9A).

Changes of submicro particle size and morphology during nanospray drying are related to moisture content, drying temperature and nozzle size (Buchi, 2012). In the first stage of nanospray drying or constant drying stage, the hot air causes an increase of the droplet temperature, which promotes quick liquid evaporation from the droplet surface and a corresponding reduction of the droplet and subsequently resulting in the submicron and nanoparticles (Buchi, 2012).

A



B

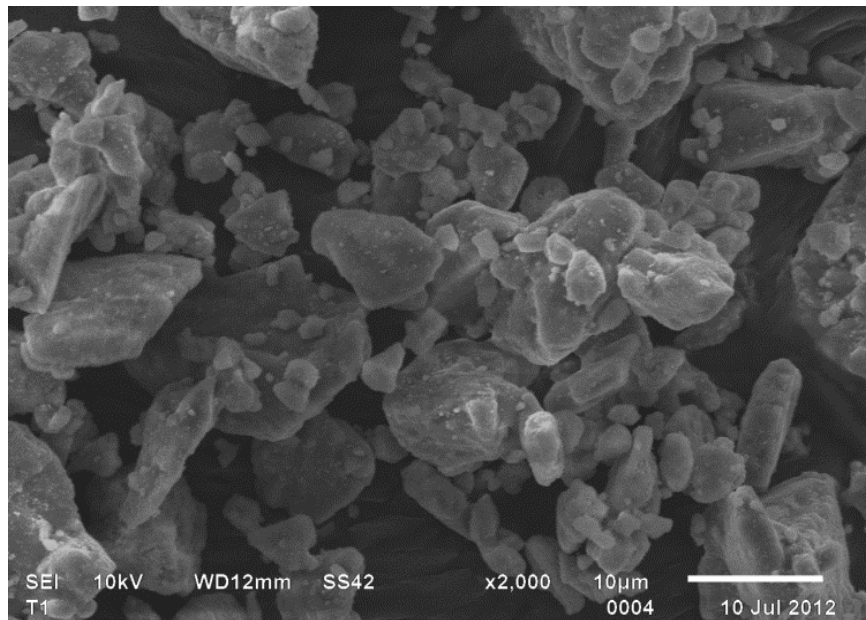
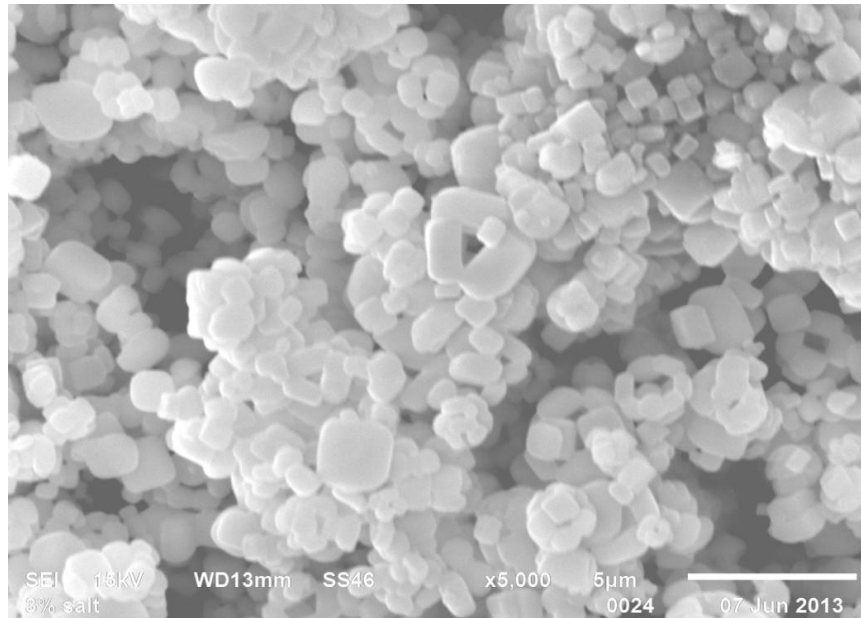


Figure 8. Scanning electron microscopy images of microsalt

A



B

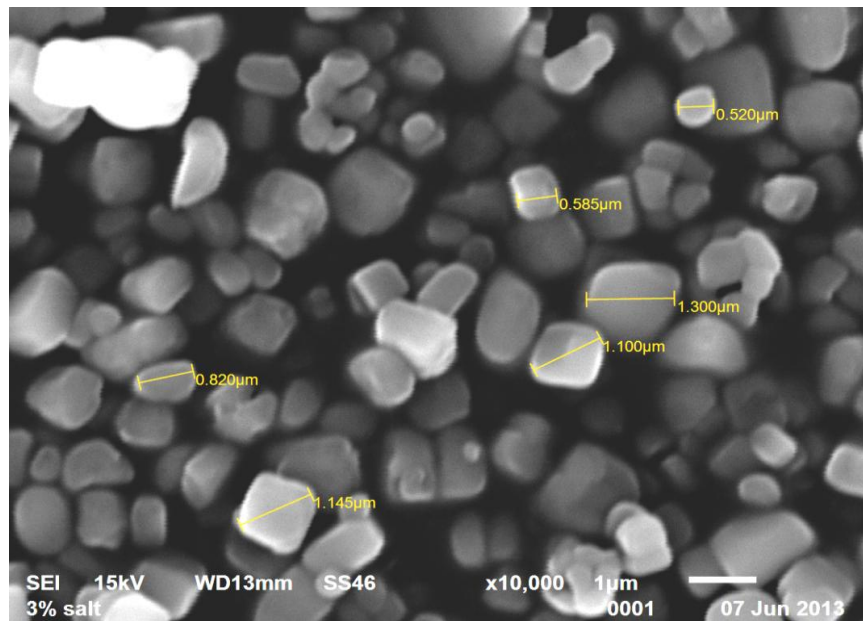


Figure 9. Scanning electron microscopy images of submicrosalt

3.3. Salt Content

The salt content of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Table 2. There were significant ($P < 0.05$) differences between different amounts (2, 1.5 and 1%) of salt (Table 3). There was no significant ($P > 0.05$) difference in salt content between regular 2%, microsalt 2% and submicrosalt 2% (Table 2). Submicrosalt 1.5% showed no differences in salt content between microsalt 1.5% and regular 1.5% (Table 2). Additionally, there was not significant ($P > 0.05$) difference in salt content between regular 1%, microsalt 1% and submicrosalt 1% (Table 2).

Table 2. Least Square Means for salt content of surface salted cheese cracker as influenced by treatment

EFFECT	Salt Content (%)
	1 Wk
Regular 2%	2.00 ^A
Regular 1.5%	1.50 ^B
Regular 1%	1.01 ^C
Microsalt 2%	2.02 ^A
Microsalt 1.5%	1.52 ^B
Microsalt 1%	1.03 ^C
Submicrosalt 2%	2.02 ^A
Submicrosalt 1.5%	1.52 ^B
Submicrosalt 1%	1.03 ^C

^{ABC} LSMeans with the different letter within the attribute are significantly different

Table 3. Probability > F of different particle size treatments for the salt content and sodium of surface salted cheese cracker

EFFECT	Salt content	Sodium cont
	Pr > F	Pr > F
TRT	0.0317	<0.0001

3.4. Sodium Content

The sodium content of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Table 4. There were significant ($P<0.05$) differences between different amounts (2, 1.5 and 1%) of salt (Table 3). There was no significant ($P>0.05$) difference between regular 2%, microsalt 2% and submicrosalt 2% in sodium content (Table 4). Submicrosalt 1.5% showed no differences in sodium content between microsalt 1.5% and regular 1.5% (Table 4). Additionally, there was no significant ($P>0.05$) difference in sodium between regular 1%, microsalt 1% and submicrosalt 1% (Table 4). Nurul et al., (2010) found sodium content in fish crackers samples in the ranges of 1184-1888 mg/100 g. This sodium in fish crackers was the results of the addition of salt and monosodium glutamate during the manufacturing process which contributed to the sodium content in the fish crackers.

Table 4. Least Square Means for sodium content of surface salted cheese cracker as influenced by treatment

EFFECT	Sodium Content (mg/30g)
	1 Wk
Regular 2%	240 ^A
Regular 1.5%	180 ^B
Regular 1%	121.2 ^C
Microsalt 2%	242.4 ^A
Microsalt 1.5%	182.4 ^B
Microsalt 1%	123.6 ^C
Submicrosalt 2%	242.4 ^A
Submicrosalt 1.5%	182.4 ^B
Submicrosalt 1%	123.6 ^C

^{ABC} LSMeans with the different letter within the attribute are significantly different

3.5. Water Activity (A_w)

Water activity (a_w) is defined as: $A_w = p/p_0$, where p is the vapor pressure of water in the substance, and p_0 is the vapor pressure of pure water at the same temperature (Rockland and Beuchat, 1987). Water activity is a measure of the energy status of the water in a system (part of physico-chemical reaction) (Rockland and Beuchat, 1987).

The water activity of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 10. There was a significant ($P<0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 5). Water activity in all treatments increased significantly ($P<0.05$) from 1 week to 4 months (Table 6). Similar results in A_w were reported in crackers analyzed every month for 4 months by Hozova et al., (1997), which at day one A_w was 0.16 and at the end of storage (4 months) A_w achieved the maximum of 0.34. Submicrosalt 2% had significantly ($P<0.05$) lower A_w compared to microsalt 2% and regular 2% at 4 months (Table 6). Submicrosalt 1% had significantly lower A_w compared to microsalt 1% and regular 1% at 4 months (Table 6). However, the A_w results found in this study did not reach a limit which would be required for the growth of yeast that could adversely affect the sensory characteristics. The water activity in the cheese crackers was less in average (at 1 week = 0.16 and 4 months = 0.3) than the external percent relative humidity (75-85% in Louisiana). Moisture permeated into the package raising the cracker's A_w . The rate of gain or loss of moisture is a function of film permeability and the area to volume (solid weight) of the food contained (Barbosa-Cánovas et al., 2008). However, the package used had low oxygen permeability (0.9 ml/m². day) (Metalized polyester and polyethylene bonded film) (Uline, 2013). Additionally, Adams and Moss, (2000) concluded that salt when is ionized completely in water into sodium and chloride ions, make the water unavailable for microbial growth due to the ion hydration, and

therefore is more effective at reducing the water activity of food than other ingredients like sucrose. However, in this study in all the treatments at all the times the salt crystals were not ionized (kept the original molecule).

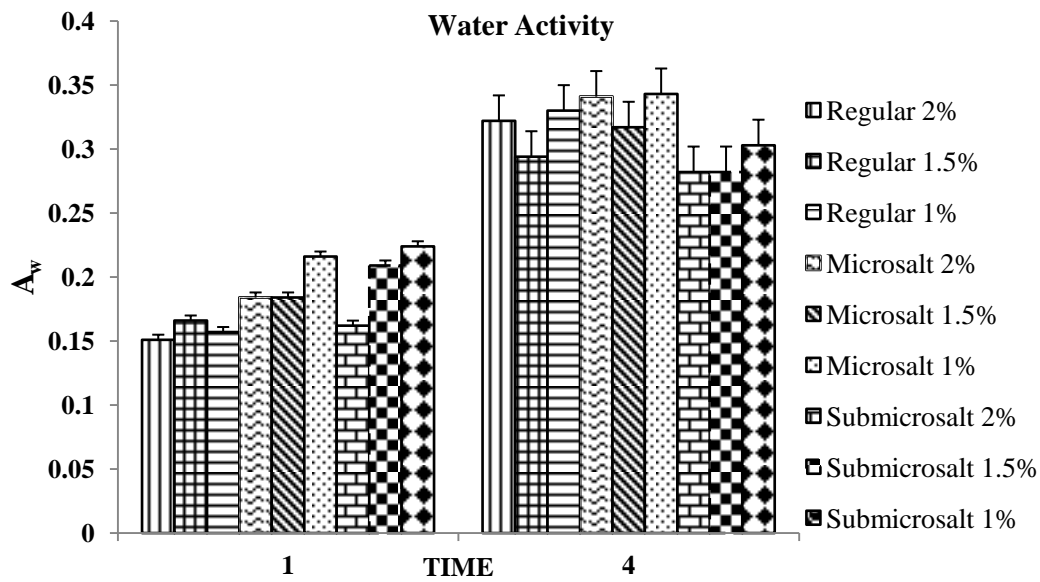


Figure 10. Water activity of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 5. Probability > F of different particle size treatments, time and their interaction for the water activity (A_w), yeast and mold counts, of surface salted cheese cracker

EFFECT	Water activity	Yeast	Mold	Texture
	Pr > F	Pr > F	Pr > F	Pr > F
TRT	<0.0001	<0.0001	0.0000	<0.0001
TIME	<0.0001	<0.0001	0.0000	<0.0001
TRT*TIME	<0.0001	<0.0001	0.0000	<0.0001

Time = 1 week and 4 month

Table 6. Least Square Means for A_w of surface salted cheese cracker as influenced by time of storage

EFFECT	A_w	
	1 Wk	4 Mos
Regular 2%	0.151 ^H	0.322 ^B
Regular 1.5%	0.166 ^H	0.294 ^{DE}
Regular 1%	0.157 ^H	0.330 ^{AB}
Microsalt 2%	0.184 ^G	0.341 ^A
Microsalt 1.5%	0.184 ^G	0.317 ^{BC}
Microsalt 1%	0.216 ^F	0.343 ^A
Submicrosalt 2%	0.162 ^H	0.282 ^E
Submicrosalt 1.5%	0.209 ^F	0.282 ^E
Submicrosalt 1%	0.224 ^F	0.303 ^{CD}

^{ABCDEFGH} LSMeans with the different letter within the attribute are significantly different

3.6. Yeast Analysis

The yeast growth of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 11. There was a significant ($P<0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 5). Yeast counts in all treatments increased significantly ($P<0.05$) from 1 week to 4 months (Table 7). This increased probability was because A_w increased from 1 week to 4 months (Table 6). The effect of storage time showed that submicrosalt 2% and microsalt 2% at 4 months had significantly ($P<0.05$) lower yeast counts than regular 2% at 4 months (Table 7). Submicrosalt 1.5% at 4 months was significantly ($P<0.05$) lower in yeast counts compared to regular 1.5% (Table 7). Submicrosalt 1% at 4 months showed significantly ($P<0.05$) the lowest yeast counts compared to microsalt 1% and regular 1% at 4 months (Table 6). These results indicated that the submicrosalt treatments (2, 1.5 and 1%) had positive effect in yeast reduction at 4 months compare to regular salt (2, 1.5 and 1%). Saddozai and Kalil (2009), analyzed yeast in snacks food (crackers and potato chips) at

different schools and colleges and the results showed absence of yeast in all the different places. Additionally Hozova et al., (1997) analyzed yeast in amaranth crackers every month for 4 months and they reported absence of yeast counts until the end of the storage.

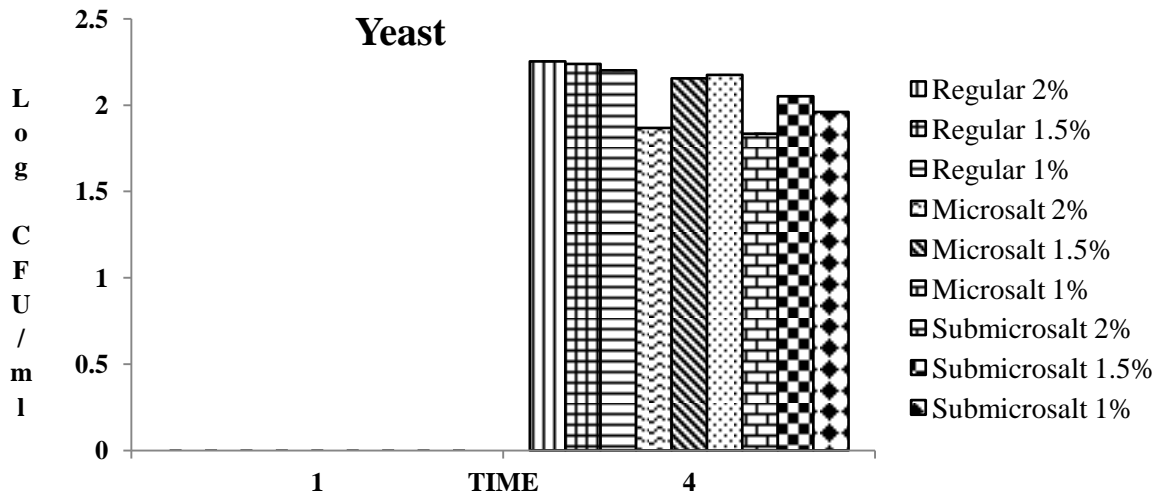


Figure 11. Yeast growth of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 7. Least Square Means for yeast counts of surface salted cheese cracker as influenced by time of storage

EFFECT	Yeast (Log cfu/ml)	
	1 Wk	4 Mos
Regular 2%	0 ^E	2.254 ^A
Regular 1.5%	0 ^E	2.238 ^A
Regular 1%	0 ^E	2.202 ^{AB}
Microsalt 2%	0 ^E	1.867 ^D
Microsalt 1.5%	0 ^E	2.156 ^{AB}
Microsalt 1%	0 ^E	2.175 ^{AB}
Submicrosalt 2%	0 ^E	1.835 ^D
Submicrosalt 1.5%	0 ^E	2.052 ^{BC}
Submicrosalt 1%	0 ^E	1.960 ^{CD}

^{ABCDE} LSMeans with the different letter within the attribute are significantly different

The effect of having submicrosalt crystal on the surface salted cheese cracker increased the surface area in 1000 times compared to regular salt crystals; therefore there were reduced area available for yeast growth. Beside of the dehydration effect, the direct effect of the chloride ion, reduced oxygen tension and interference with the action of enzymes as water availability decreases all contribute to the preservative action of salt (Dams and Moss 2000). All organisms with a semi permeable membrane are subject to osmotic pressure, or the effect of water moving in and out of the cell (Wassenaar, 2013). If the cell did not have a cell wall, this could cause the cell to burst. Salt work as a preservative because when the outside environment around a cell is salty, then the concentration of water in the solution is less than inside the cell and water tends to leave the cell (Wassenaar, 2013). In our study the surface salted may had attributes to cause yeasts dehydration and therefore the reduction in yeasts growth by subjecting them to a salty environment.

3.7. Mold Analysis

The mold growth of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in the Table 5. There was no significant ($P>0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 5), since there was no mold growth in all treatments at all times. Saddozai and Kalil (2009), analyzed molds in snacks food (crackers and potato chips) at different schools and colleges and the results showed absence of molds in all the different places. Moreover, Hozova et al., (1997) evaluated mold in amaranth crackers every month for 4 months and they reported absence of mold counts until the end of 4 months.

3.8. Texture Analysis

The texture of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 12. There was a significant ($P<0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 5). Submicrosalt 1.5%, microsalt 1.5% and regular 1% significantly ($P<0.05$) decreased in fracturability from 1 week to 4 months (Table 8). Microsalt 1.5% had significantly ($P<0.05$) lower fracturability compared to regular 1.5% at 4 months (Table 8).

Nakamura et al., (2012) studied the quality deterioration of rice crackers over time using the one bite test with tensipresser and concluded that the hardness of cracker markedly increased during storage of 20 days at 35°C. Its changes were attributed to the retrogradation rice starch over time. In this study we did not use rice starch.

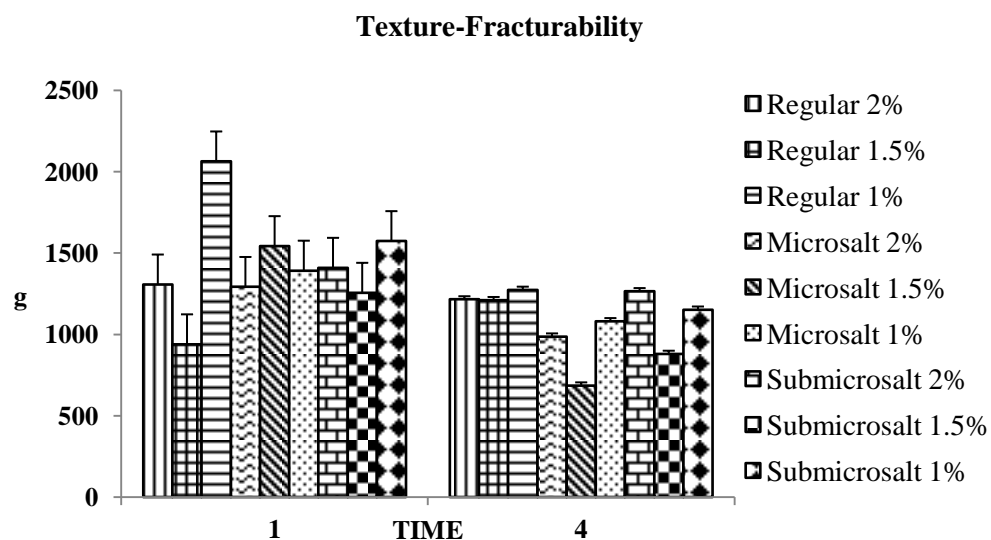


Figure 12. Texture of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 8. Least Square Means for texture of surface salted cheese cracker as influenced by time of storage

EFFECT	Texture-Fracturability (g)	
	1 Wk	4 Mos
Regular 2%	1306.3 ^{BCD}	1214.5 ^{BCD}
Regular 1.5%	938.8 ^{CDE}	1210.0 ^{BCD}
Regular 1%	2062.5 ^A	1273.3 ^{BCD}
Microsalt 2%	1291.3 ^{BCD}	986.1 ^{CDE}
Microsalt 1.5%	1542.1 ^B	685.2 ^E
Microsalt 1%	1391.4 ^{BC}	1080.3 ^{BCDE}
Submicrosalt 2%	1408.9 ^{BC}	1264.7 ^{BCD}
Submicrosalt 1.5%	1255.4 ^{BCD}	879.8 ^{DE}
Submicrosalt 1%	1572.9 ^{AB}	1151.4 ^{BCDE}

^{ABCDE}LSMeans with the different letter within the attribute are significantly different

3.9. Color Analysis

3.9.1. L*

The L* values are from 0 to 100, 100 represents a perfect reflecting diffuser (white) and zero represents black. The L* (lightness) of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 13. There was a significant ($P < 0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 9). L* values in all treatments increased significantly ($P < 0.05$) from 1 week to 4 months (Table 10). This increase in lightness was attributed to the ingredients effects (cayenne pepper and cheese) in the cheese cracker (Table 1). The dried red pepper (cayenne pepper) powder loses a part of its pigments (capsorubin, capsanthin, zeaxanthin, lutein and β -carotene) in the storage, attributed for the time and temperature of storage, oxygen of air, light, moisture content of the product and kind of packaging (Malchev et al., 1982). At 1 week and 4 months, submicrosalt 1.5% was significantly

($P < 0.05$) higher in lightness values compared to regular 1.5% (Table 10). At 4 months, submicrosalt 2% had significant ($P < 0.05$) lower lightness values compared to microsalt 2% and regular 2% (Table 10). At 4 months, there were no significant differences ($P > 0.05$) in lightness between treatments of 1% (Table 10).

Mohamed et al. (1989) studied the type of flour used in fish crackers and concluded that it affected the clarity (color) of the fish crackers. Another factor that could also affect the color of fish crackers is the maillard reaction (sugar in the formulation). This reaction occurs between the free amino group of lysine and other amino acids and the carbonyl groups of reducing sugars such as glucose, fructose, lactose and maltose (Camire et al., 1990). Additionally, Camire et al., (1990) reported that for the high temperatures and low moisture conditions used during the industrial treatments, some chemical reactions occur such as maillard reaction in carbohydrates in food (glucose, lactose and maltose).

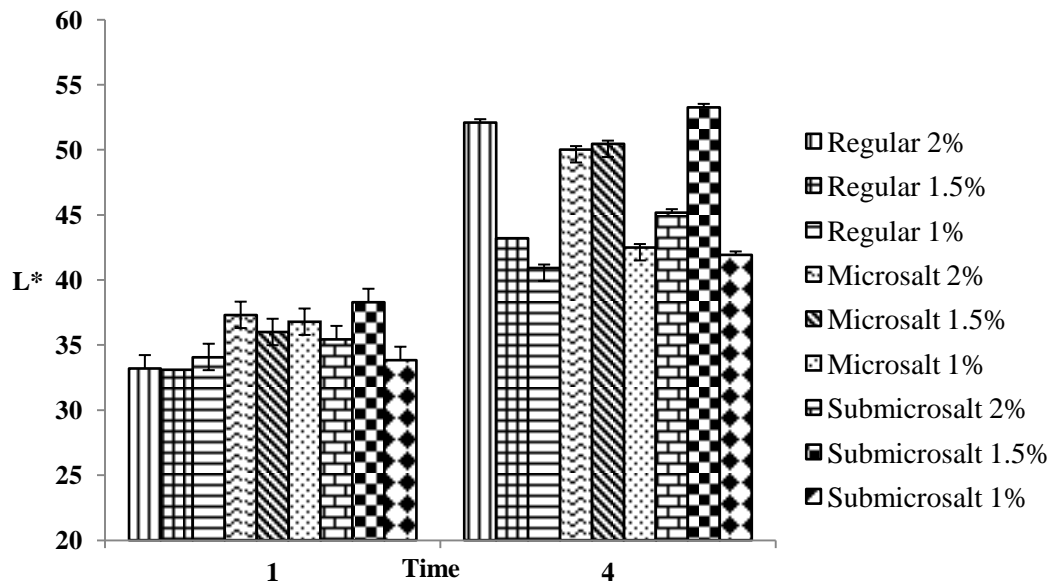


Figure 13. Measurement of lightness (L^*) of surface salted cheese cracker at different salt particle sizes/concentrations

Table 9. Probability > F of different particle size treatments, time and their interaction for the color attributes of surface salted cheese crackers

EFFECT	L*	a*	b*	C*	h
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
TRT	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
TIME	<0.0001	<0.0001	0.0329	<0.0001	<0.0001
TRT*TIME	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Time = 1 week and 4 months

Table 10. Least Square Means for color of surface salted cheese cracker as influenced by time of storage

EFFECT	L*		a*		b*		C*		h*	
	1 Wk	4 Mos	1 Wk	4Mos	1 Wk	4Mos	1 Wk	4Mos	1 Wk	4Mos
Regular 2%	33.193 ^{HI}	52.087 ^{AB}	10.430 ^{EFGH}	13.660 ^A	17.020 ^F	30.913 ^A	22.580 ^{HI}	33.643 ^A	62.490 ^D	67.020 ^{AB}
Regular 1.5%	33.093 ^I	43.197 ^{CD}	10.287 ^{FGH}	11.643 ^{CD}	19.840 ^{EF}	28.013 ^{ABC}	22.457 ^I	30.600 ^{CD}	62.740 ^D	67.877 ^A
Regular 1%	34.063 ^{GHI}	40.920 ^D	10.097 ^{FGH}	11.337 ^{CDE}	20.753 ^{EF}	27.537 ^{ABC}	22.793 ^{GHI}	29.987 ^{DE}	64.003 ^{CD}	68.043 ^A
Microsalt 2%	37.297 ^{EF}	50.020 ^B	9.970 ^{GH}	11.937 ^{BC}	21.073 ^{EF}	29.533 ^{ABC}	23.340 ^{GHI}	31.867 ^{BC}	64.757 ^C	67.970 ^A
Microsalt 1.5%	35.983 ^{FG}	50.440 ^B	10.010 ^{GH}	12.607 ^B	21.820 ^{DE}	30.490 ^{AB}	23.930 ^{GHI}	33.413 ^{AB}	65.283 ^{BC}	68.250 ^A
Microsalt 1%	36.770 ^{EF}	42.493 ^D	10.276 ^{FGH}	10.927 ^{DEF}	22.003 ^{DE}	26.987 ^{ABC}	24.247 ^{GH}	29.113 ^{DEF}	65.097 ^C	68.103 ^A
Submicrosalt 2%	35.433 ^{FGH}	45.173 ^C	10.527 ^{EFGH}	10.660 ^{EFGH}	21.407 ^E	26.350 ^{BC}	23.760 ^{GHI}	28.463 ^{EF}	64.763 ^C	67.723 ^A
Submicrosalt 1.5%	38.287 ^E	53.263 ^A	10.273 ^{FGH}	10.713 ^{EFGH}	22.120 ^{DE}	25.810 ^{BC}	24.397 ^G	28.023 ^F	65.140 ^C	67.373 ^A
Submicrosalt 1%	33.833 ^{GHI}	41.927 ^D	9.927 ^H	10.837 ^{DEFG}	20.343 ^{EF}	26.357 ^{BC}	22.697 ^{HI}	28.490 ^{EF}	63.587 ^D	67.790 ^A

ABCDEFghi LSMeans with the different letter within the attribute are significantly different

Hempel et al., (2005) evaluated wafer crackers made with ultra filtered syrup and different type of flours and found that its color was more bright (lightness). They evidently attributed the results to the reduction of reducing sugar monomers in the syrup (isomerization). Opposite results were found by Nurul et al., (2009) evaluated the effect of different ratios of fish meat to tapioca flour in fish cracker and they found a decrease in lightness (L^*) with an increase in the ratio of fish meat to tapioca flour. In our study the protein source was not fish meat, but cheese and wheat flour.

The presence of the phosphate radical in milk during heating caused darkening in the lactose color, subsequently an increase of the water activity in milk powder increased lipid oxidation, and lactose crystallization (O'Brien, 1997). Additionally, Millard reactions in dry systems are generally conclude to progress faster at increased water activities (O'Brien, 1997). However, in this product (surface salted cheese cracker) the presence of lactose in the cheese and butter was low.

3.9.2. a^*

The a^* axes (red- green axis) have numerical values from -60 to 60. Positive a^* is red and negative a^* is green. The a^* of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 14. There was a significant ($P<0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 9). At 4 months, submicrosalt treatments (2 and 1.5%) were significantly ($P<0.05$) lower in a^* values compared to the regular 2 and 1% (Table 10). For fish cracker manufacture using different ratios of fish meat to tapioca flour, the redness (a^*) tended to decrease with an increase in the ratio of fish meat to tapioca flour (Nurul et al., 2009). Malchev et al., (1982) conclude that the red component of the

pigment complex has been degraded at more accelerated rates than the yellow component. Additionally the capsorubin, capsanthin, zeaxanthin β lutein and β and α carotene showed a higher stability in the dried red pepper as compared to the red pepper powder during six months of storage.

3.9.3. b^*

The b^* axes (blue- yellow axis) have numerical values from -60 to 60. Positive b^* is yellow and negative b^* is blue. The b^* of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 15. There was a significant ($P<0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 9). The b^* values in all treatments increased significantly ($P<0.05$) from 1 week to 4 months (Table 10). This increase in yellowness was attributed to the ingredients effects in the cheese cracker (Table 1). At 4 months, submicrosalt treatments (2, 1.5 and 1%) were significantly ($P<0.05$) lower in b^* values compared to regular 2% (Table 10). Riboflavin and carotenoids contribute to the yellowish color of cheese, and the degradation of these compounds during light exposure may result in discoloration of the product (Bosset, et al., 1995). Nurul et al., (2009) evaluated the effect of different ratios of fish meat to tapioca flour in fish cracker and they reported that yellowness (b^*) tended to be higher in samples with low amounts of fish meat. Additionally, Kristensen et al., (2000) reported that the havarti cheese packaged in modified atmosphere packaging (25% CO_2 and 75% N_2) exposed to light and stored for up to 21 days at 51°C, had a significant decrease in yellowness values.

3.9.4. C^*

The C^* (chroma/saturation) of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 16. There was a significant ($P<0.05$) effect for

treatment * time interaction, treatment effect and time effect (Table 9). The C* values in all treatments increased significantly ($P<0.05$) from 1 week to 4 months (Table 10). This increase in (C*) values was attributed to the ingredients effects/changes in the cheese cracker (Table 1). At 1 week, submicrosalt 1.5% was significantly ($P<0.05$) lower in C* (chroma) values compared to regular 1.5 and 2% (Table 10). At 4 months, submicrosalt treatments (2, 1.5 and 1%) showed significantly ($P<0.05$) lower C* (chroma) values compared to regular 2 and 1.5% (Table 10). Hempel et al., (2005) conclude that the wafer crackers made with ultrafiltered syrup were less saturated (C*). They attributed this effect in C* to the reduction of reducing sugar monomers in the syrup (isomerization), therefore resulting in an intensity of non enzymatic browning changes.

3.9.5. h*

The h* (hue) of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 17. There was a significant ($P<0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 9). The h values in all treatments increased significantly ($P<0.05$) from 1 week to 4 months (Table 10). This increase in hue values was attributed to the ingredients effects/changes in the cheese cracker (Table 1). At 1 week, submicrosalt 2% and 1.5% had significantly ($P<0.05$) lower hue values compared to regular 2 and 1.5% (Table 10). However, at 4 months there were not significant ($P>0.05$) differences in hue values between all treatments (Table 10). Hempel et al., (2005) evaluated wafer crackers made with ultra filtered syrup and different types of flours affect the hue values (higher values- lightness). Those changes in hue values were attributed to the reduction of reducing sugar monomers in the syrup).

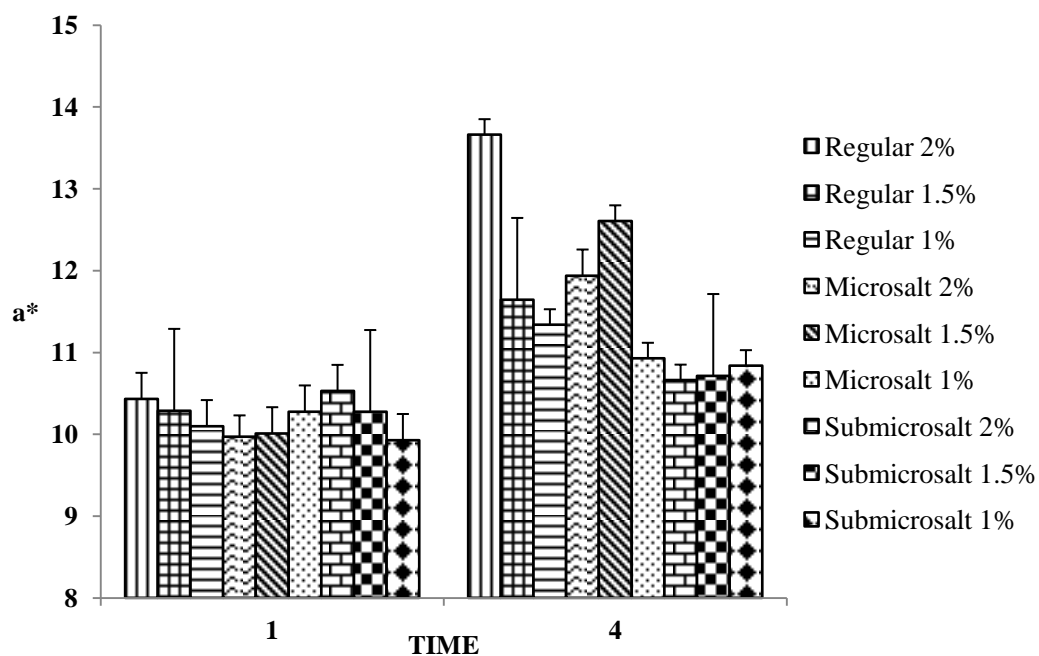


Figure 14. Measurement of redness (a^*) of surface salted cheese cracker at different salt particle sizes/concentrations

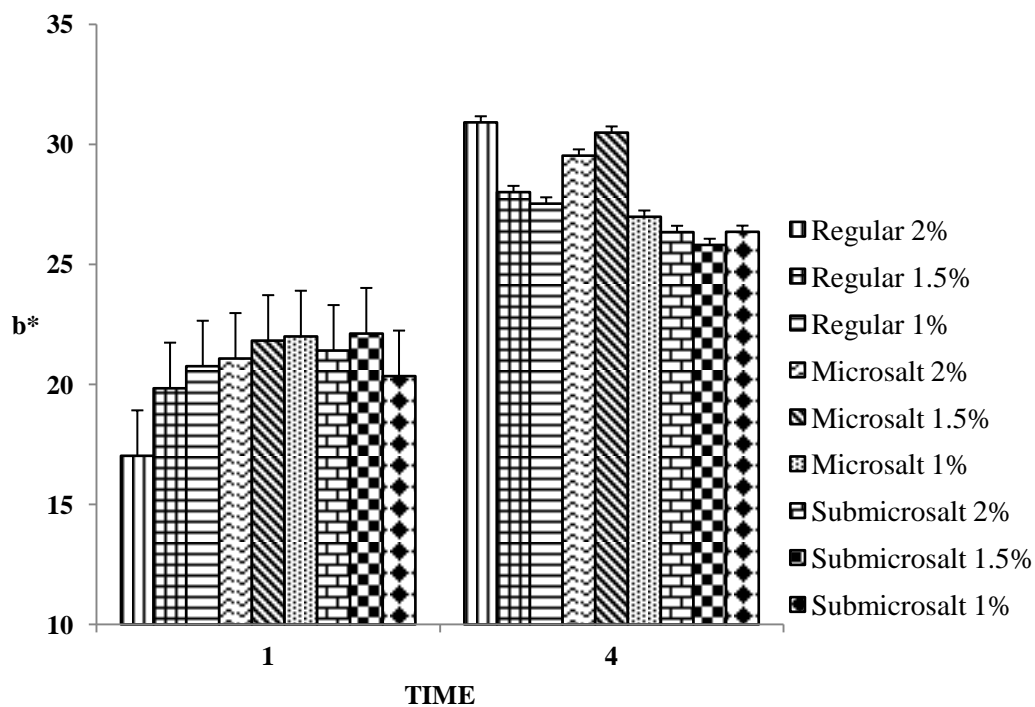


Figure 15. Measurement of yellowness (b^*) of surface salted cheese cracker at different salt particle sizes/concentrations

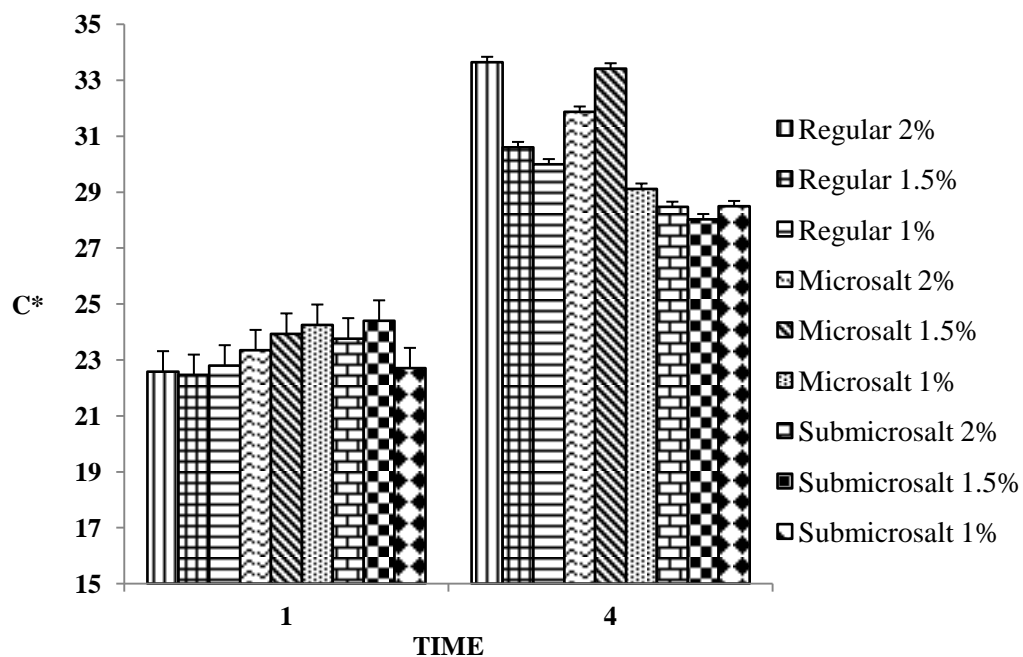


Figure 16. Measurement of chroma (C^*) of surface salted cheese cracker at different salt particle sizes/concentrations

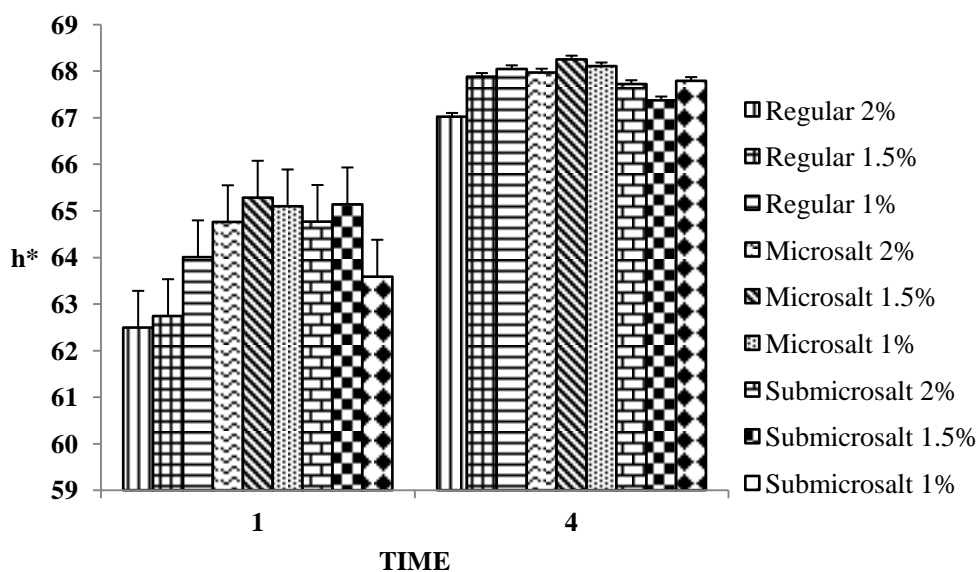


Figure 17. Measurement of hue (h) of surface salted cheese cracker at different salt particle sizes/concentrations

3.10. Sensory Study of Surface Salted Cheese Cracker

Since the main question was whether the sensory saltiness of submicrosalt 1% and 1.5% was similar to regular 2% (control) (as currently used by industry), all contrasts were made to regular 2% (control).

3.10.1. Color

The sensory study for color of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 18. There was a significant ($P < 0.05$) effect for treatment * time interaction and time effect (Table 11). The contrasts for submicrosalt 1%, 1.5% and 2% were significantly ($P < 0.05$) different compared to control (regular 2%) (Table 12). However, the contrast for submicrosalt treatments was not significant ($P = 0.2108$) different compared to microsalt treatments (Table 12). At 1 week, submicrosalt 1% had significantly ($P > 0.05$) higher sensory scores compared to control (regular 2%) (Table 13). However, at 4 months there were no significantly ($P > 0.05$) differences in sensory color scores between all treatments (Table 13).

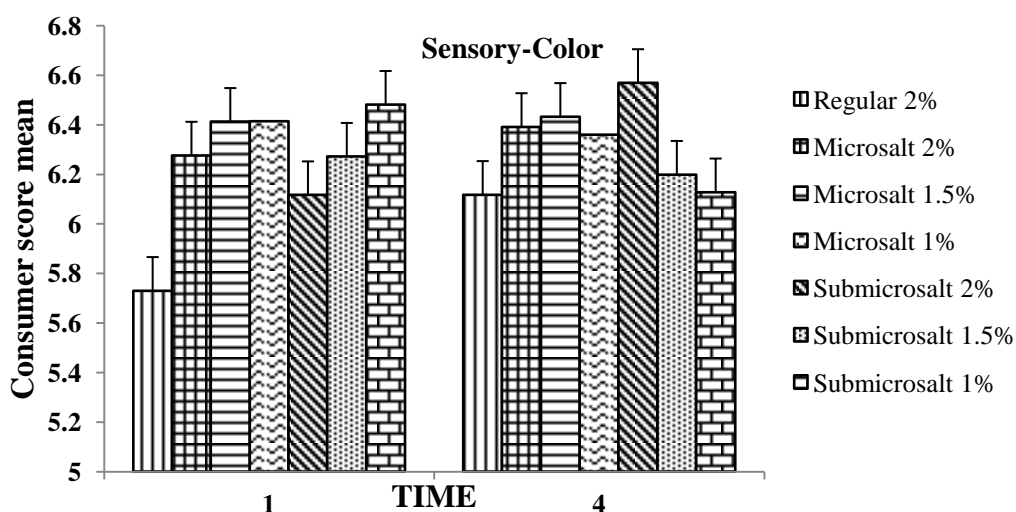


Figure 18. Sensory study for sensory color of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 11. Probability > F of different particle size treatments, time and their interaction for the sensory color, aroma, saltiness, just about right (JAR) saltiness, crunchiness, overall like and acceptability of surface salted cheese cracker

EFFECT	Color	Aroma	Saltiness	JAR-Saltiness	Crunchiness	Overall like	Acceptability
	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
TRT	0.5088	0.9588	0.0153	0.2439	<.0001	0.0177	0.0072
TIME	0.0011	<.0001	<.0001	<.0001	0.0012	<.0001	<.0001
TRT*TIME	0.0125	0.0003	0.0173	0.0131	<.0001	0.0002	0.0223

Table 12. Probability > F of sensory color of cheese cracker at different particle size treatments to control (Regular 2%)

Contrast	Pr > F
Submicrosalt 1% vs Regular 2%	0.0019
Submicrosalt 1.5% vs Regular 2%	0.0002
Submicrosalt 2% vs Regular 2%	0.0002
Micro 1% vs Regular 2%	0.0019
Micro 1.5% vs Regular 2%	0.0002
Micro 2% vs Regular 2%	0.0002
Micro vs Regular 2%	<.0001
Submicrosalt vs Regular 2%	0.0002
Micro vs Submicrosalt	0.2108

Table 13. Least Square Means for sensory color of surface salted cheese cracker as influenced by time of storage

EFFECT	Color	
	1 Wk	4 Mos
Regular 2%	5.7299 ^B	6.1168 ^{AB}
Microsalt 2%	6.2759 ^{AB}	6.3908 ^A
Microsalt 1.5%	6.4120 ^A	6.4316 ^A
Microsalt 1%	6.4143 ^A	6.3595 ^{AB}
Submicrosalt 2%	6.1161 ^{AB}	6.5683 ^A
Submicrosalt 1.5%	6.2713 ^{AB}	6.1980 ^{AB}
Submicrosalt 1%	6.4809 ^A	6.1271 ^A

^{AB} LSMeans with the different letter within the attribute are significantly different

3.10.2. Aroma

The sensory study for aroma of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 19. There was a significant ($P < 0.05$) effect for treatment * time interaction and time effect (Table 11). The contrasts for submicrosalt 1%, 1.5% and 2% were significantly ($P < 0.05$) different compared to control (regular 2%) (Table 14). Additionally, the contrasts between microsalt and submicrosalt treatments were no significant ($P = 0.5560$) different compared each other (Table 14). At 1 week, microsalt and submicrosalt treatments had significantly ($P < 0.05$) higher aroma score compared to control (regular 2%) (Table 15). At 4 months were no significantly ($P = 0.5560$) differences between submicrosalt treatments (2, 1.5 and 1%) compared to control (regular 2%) (Table 15).

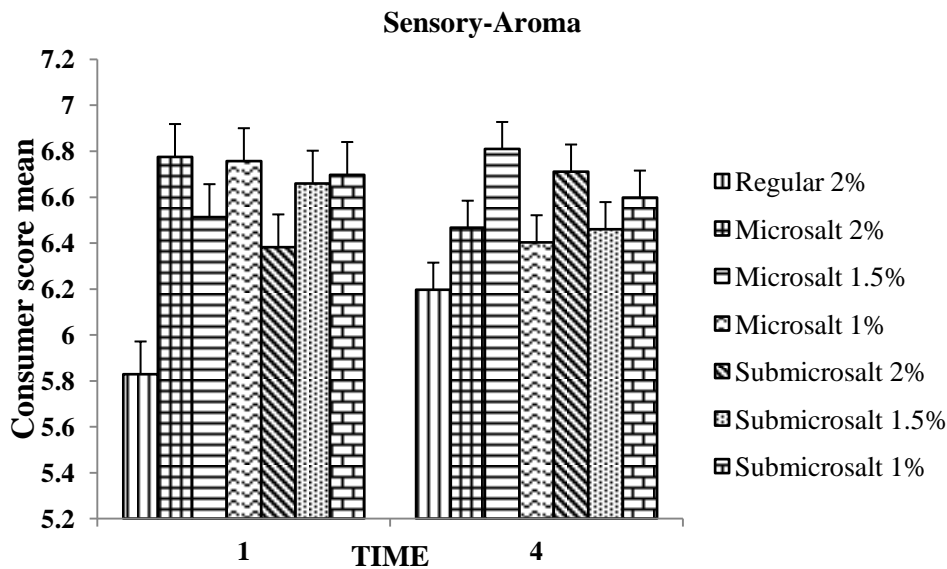


Figure 19. Sensory study for aroma of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 14. Probability > F of aroma of cheese cracker at different treatments to control (Regular 2%)

Contrast	Pr > F
Submicrosalt 1% vs Regular 2%	<.0001
Submicrosalt 1.5% vs Regular 2%	<.0001
Submicrosalt 2% vs Regular 2%	<.0001
Micro 1% vs Regular 2%	<.0001
Micro 1.5% vs Regular 2%	<.0001
Micro 2% vs Regular 2%	<.0001
Micro vs Regular 2%	<.0001
Submicrosalt vs Regular 2%	<.0001
Micro vs Submicrosalt	0.5560

Table 15. Least Square Means for aroma of surface salted cheese cracker as influenced by time of storage

EFFECT	Aroma	
	1 Wk	4 Mos
Regular 2%	5.8283 ^C	6.1966 ^{BC}
Microsalt 2%	6.7752 ^{AB}	6.4663 ^{AB}
Microsalt 1.5%	6.5134 ^{AB}	6.8092 ^A
Microsalt 1%	6.7571 ^{AB}	6.4030 ^{AB}
Submicrosalt 2%	6.3816 ^{AB}	6.7110 ^{AB}
Submicrosalt 1.5%	6.6591 ^{AB}	6.4603 ^{AB}
Submicrosalt 1%	6.6970 ^{AB}	6.5975 ^{AB}

^{ABC} LSMeans with the different letter within the attribute are significantly different

3.10.3. Saltiness

The sensory study for saltiness of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 20. There was a significant ($P<0.05$) effect for treatment * time interaction, treatment effect and time effect (Table 11). The contrasts for the submicrosalt treatments of 1%, 1.5% and 2% were significantly ($P<0.05$) different compared to

control (regular 2%) (Table 16). However, the contrasts for submicrosalt treatments were no significant ($P = 0.7872$) different compared to microsalt treatments (Table 16).

At 1 week and 4 months, submicrosalt treatments resulted in having significantly ($P < 0.05$) more preferred saltiness scores compared to control (regular 2%) (Table 17). These results are encouraged because cheese cracker industry uses regular 2% salt. The consumer tended to prefer more the saltiness of surface salted cheese cracker with submicrosalt 1% (25% less salt) than control (regular 2%).

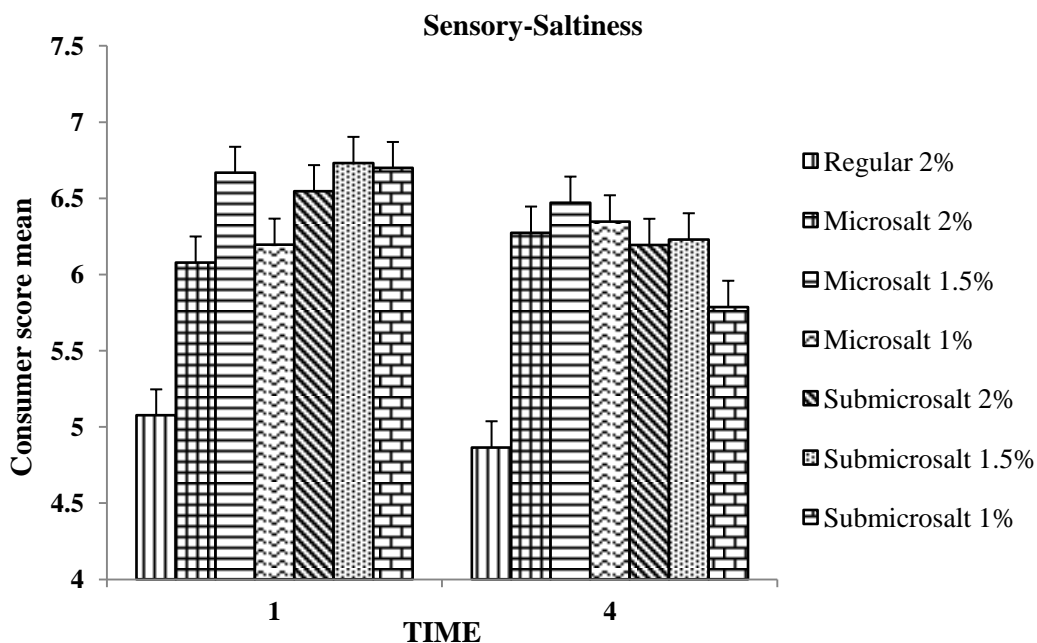


Figure 20. Sensory study for saltiness of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 16. Probability > F of saltiness of cheese cracker at different particle size treatments to control (Regular 2%)

Contrast	Pr > F
Submicrosalt 1% vs Regular 2%	<.0001
Submicrosalt 1.5% vs Regular 2%	<.0001
Submicrosalt 2% vs Regular 2%	<.0001
Micro 1% vs Regular 2%	<.0001
Micro 1.5% vs Regular 2%	<.0001
Micro 2% vs Regular 2%	<.0001
Micro vs Regular 2%	<.0001
Submicrosalt vs Regular 2%	<.0001
Micro vs Submicrosalt	0.7872

Table 17. Least Square Means for Saltiness of surface salted cheese cracker as influenced by time of storage

EFFECT	Saltiness	
	1 Wk	4 Mos
Regular 2%	5.0752 ^{CD}	4.8648 ^D
Microsalt 2%	6.0782 ^{AB}	6.2734 ^{AB}
Microsalt 1.5%	6.6665 ^A	6.4698 ^{AB}
Microsalt 1%	6.1952 ^{AB}	6.3473 ^{AB}
Submicrosalt 2%	6.5463 ^{AB}	6.1931 ^{AB}
Submicrosalt 1.5%	6.7315 ^A	6.2294 ^{AB}
Submicrosalt 1%	6.6982 ^A	5.7865 ^{BC}

^{ABCD} LSMeans with the different letter within the attribute are significantly different

3.10.4. Just About Right (JAR) Saltiness

To further understand the consumer's saltiness score, the consumers attending the sensory study were asked to select the saltiness of the surface salted cheese cracker in 3 categories too weak (1), just about right (JAR) (2) and too strong (3). The JAR saltiness of surface salted cheese cracker as influenced by different salt particle sizes/concentrations was showed in Figure 21.

There was a significant ($P < 0.05$) difference for treatment * time interaction and time effect

(Table 11). The contrasts for the submicrosalt of 1%, 1.5% and 2% were significantly ($P<0.05$) different compared to control (regular 2%) (Table 18). Additionally, the contrasts for submicrosalt treatments were no significantly ($P<0.05$) different compared to microsalt treatments (Table 18).

At 1 week, submicrosalt and microsalt treatments had significantly ($P<0.05$) more preferred JAR saltiness scores compared to regular 2% (Table 19). At 4 months, submicrosalt (1.5 and 2%) and microsalt treatments (2, 1.5 and 1%) showed significantly ($P<0.05$) more preferred saltiness JAR score compared to control (regular 2%) (Table 19). These results obtained in just about right saltiness are positive because cheese cracker industries use regular 2% salt. The consumer had more preferred score for the JAR saltiness of surface salted cheese cracker with submicrosalt 1% (25% salt content reduction) and submicrosalt 1.5% (50% less salt) than control (regular 2%).

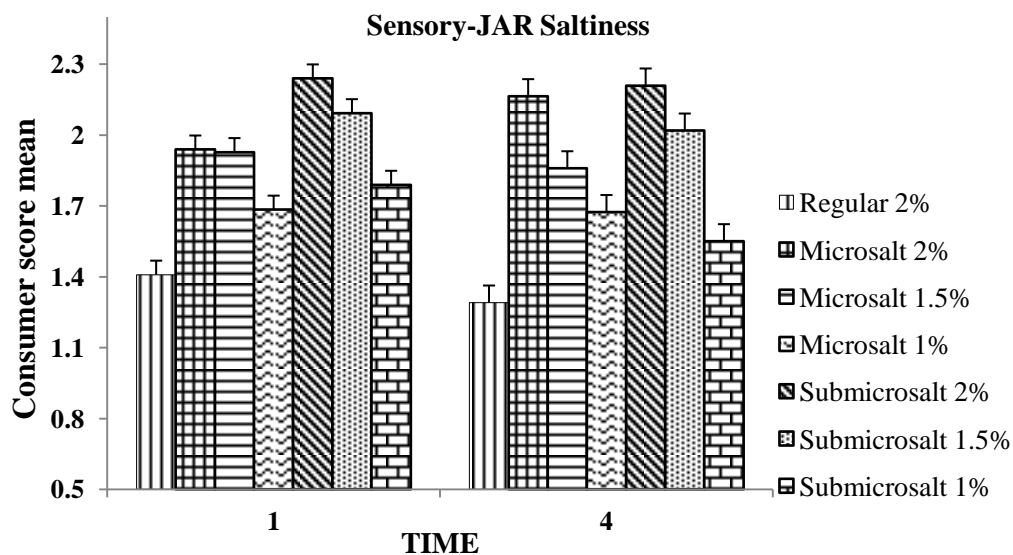


Figure 21. Sensory study for JAR-saltiness of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 18. Probability > F of JAR-saltiness of cheese cracker at different particle size treatments to control (Regular 2%)

Contrast	Pr > F
Submicrosalt 1% vs Regular 2%	<.0001
Submicrosalt 1.5% vs Regular 2%	<.0001
Submicrosalt 2% vs Regular 2%	<.0001
Micro 1% vs Regular 2%	<.0001
Micro 1.5% vs Regular 2%	<.0001
Micro 2% vs Regular 2%	<.0001
Micro vs Regular 2%	<.0001
Submicrosalt vs Regular 2%	<.0001
Micro vs Submicrosalt	0.0009

Table 19. Least Square Means for JAR-saltiness of surface salted cheese cracker as influenced by time of storage

EFFECT	JAR-Saltiness	
	1 Wk	4 Mos
Regular 2%	1.4083 ^{GH}	1.2900 ^H
Microsalt 2%	1.9380 ^{BCDE}	2.1633 ^{AB}
Microsalt 1.5%	1.9271 ^{BCDE}	1.8583 ^{CDE}
Microsalt 1%	1.6835 ^{EF}	1.6729 ^{EFG}
Submicrosalt 2%	2.2389 ^A	2.2086 ^{AB}
Submicrosalt 1.5%	2.0917 ^{ABC}	2.0179 ^{ABCD}
Submicrosalt 1%	1.7887 ^{DEF}	1.5496 ^{FGH}

^{ABCDEF}LSMeans with the different letter within the attribute are significantly different

3.10.5. Crunchiness

The crunchiness of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 22. There was a significant ($P<0.05$) difference for treatment * time interaction, treatment effect and time effect (Table 11). The contrasts for the submicrosalt 1.5 and 2% had a significant ($P<0.05$) difference compared to control (regular 2%).

Additionally, the contrasts for submicrosalt treatments were no significantly ($P = 0.3949$) different compared to microsalt treatments (Table 20).

At 1 week, submicrosalt 1% and microsalt 1 and 1.5% had significantly ($P < 0.05$) more preferred crunchiness score compared to control (regular 2%) (Table 21). At 4 months, there was no a significant ($P > 0.05$) difference in crunchiness score between control (regular 2%) and submicrosalt treatments (Table 21). Also, at 4 months there was no a significant ($P > 0.05$) difference in crunchiness preference between control and microsalt treatments (Table 21).

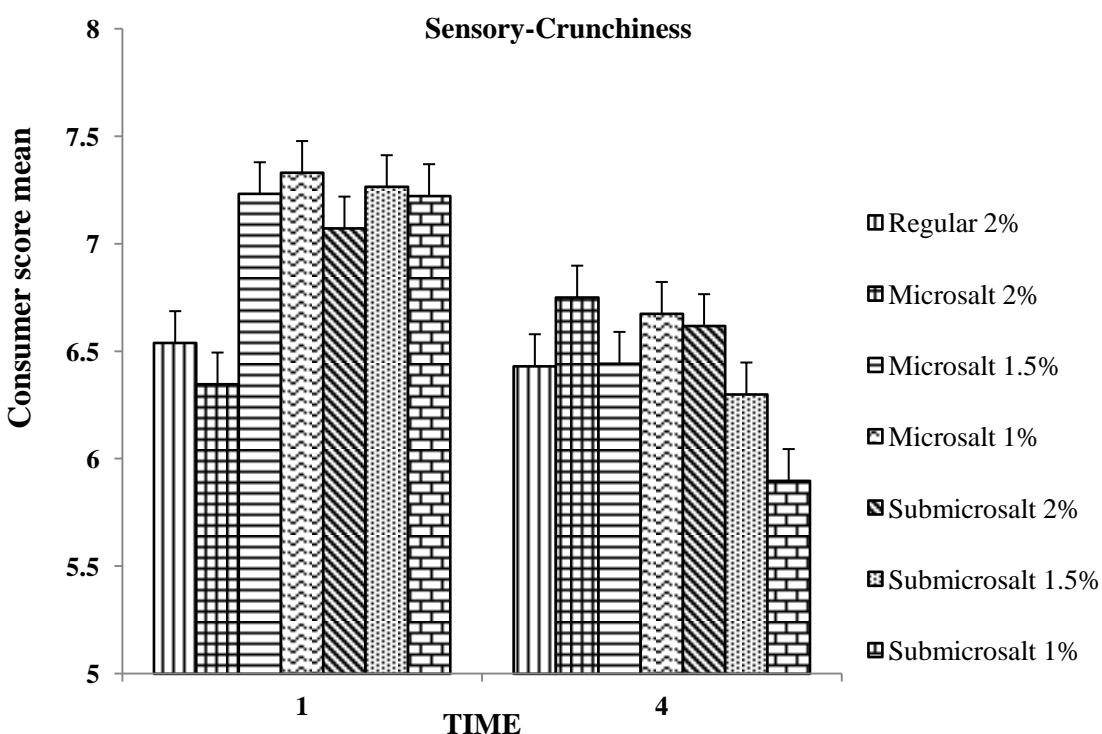


Figure 22. Sensory study for crunchiness of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 20. Probability > F of crunchiness of cheese cracker at different particle size treatments to control (Regular 2%)

Contrast	Pr > F
Submicrosalt 1% vs Regular 2%	0.5871
Submicrosalt 1.5% vs Regular 2%	0.0002
Submicrosalt 2% vs Regular 2%	0.0002
Micro 1% vs Regular 2%	0.5871
Micro 1.5% vs Regular 2%	0.0002
Micro 2% vs Regular 2%	0.0002
Micro vs Regular 2%	0.0059
Submicrosalt vs Regular 2%	0.0295
Micro vs Submicrosalt	0.3949

Table 21. Least Square Means for Crunchiness of surface salted cheese cracker as influenced by time of storage

EFFECT	Crunchiness	
	1 Wk	4 Mos
Regular 2%	6.5399 ^{CDE}	6.4314 ^{CDE}
Microsalt 2%	6.3473 ^{DE}	6.7506 ^{ABCD}
Microsalt 1.5%	7.2327 ^{AB}	6.4424 ^{CDE}
Microsalt 1%	7.3313 ^A	6.6747 ^{ABCD}
Submicrosalt 2%	7.0729 ^{ABC}	6.6179 ^{BCD}
Submicrosalt 1.5%	7.2654 ^{AB}	6.2999 ^{DE}
Submicrosalt 1%	7.2236 ^{AB}	5.8972 ^E

^{ABCDE} LSMeans with the different letter within the table are significantly different

3.10.6. Overall Liking

The overall liking attribute of surface salted cheese cracker as influenced by different salt particle sizes/concentrations is shown in Figure 23. There was a significant ($P < 0.05$) difference

for treatment * time interaction treatment effect and time effect (Table 11). The contrast for submicrosalt 1, 1.5 and 2% were significantly ($P < 0.05$) different compared to control (regular 2%) (Table 22). However, the contrasts of submicrosalt treatments were no significantly ($P = 0.7140$) different compared to microsalt treatments (Table 22).

At 1 week, submicrosalt (1, 1.5 and 2%) and microsalt (1, 1.5, and 2%) treatments had significantly ($P < 0.05$) more preferred scores in the overall liking attribute compared to control (regular 2%) (Table 23). At 4 months, microsalt (1, 1.5, and 2%) treatments had significantly ($P < 0.05$) more preferred score in the overall liking score compared to regular 2% (Table 23). There were no differences in submicrosalt 1 and 1.5% compared with control (regular 2%) for overall liking. This means that even for 25 and 50% reduction in salt content through lowered particle size the overall liking score remain the same compared to control (regular 2%).

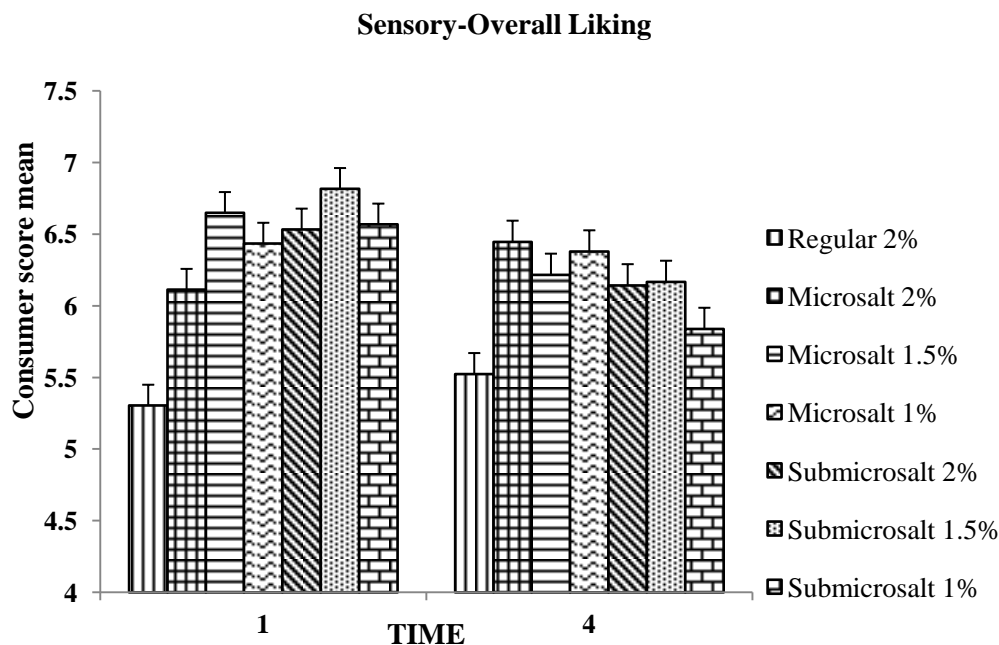


Figure 23. Sensory study for overall liking of surface salted cheese cracker as influenced by different salt particle sizes/concentrations

Table 22. Pr > F of overall liking of cheese cracker at different particle size treatments to control (Regular 2%)

Contrast	Pr > F
Submicrosalt 1% vs Regular 2%	<.0001
Submicrosalt 1.5% vs Regular 2%	<.0001
Submicrosalt 2% vs Regular 2%	<.0001
Micro 1% vs Regular 2%	<.0001
Micro 1.5% vs Regular 2%	<.0001
Micro 2% vs Regular 2%	<.0001
Micro vs Regular 2%	<.0001
Submicrosalt vs Regular 2%	<.0001
Micro vs Submicrosalt	0.7140

Table 23. Least Square Means for overall liking of surface salted cheese cracker as influenced by time of storage

EFFECT	Overall liking	
	1 Wk	4 Mos
Regular 2%	5.3041 ^D	5.5230 ^{CD}
Microsalt 2%	6.1127 ^{ABC}	6.4471 ^{AB}
Microsalt 1.5%	6.6489 ^A	6.2169 ^{AB}
Microsalt 1%	6.4346 ^{AB}	6.3800 ^{AB}
Submicrosalt 2%	6.5333 ^A	6.1436 ^{ABC}
Submicrosalt 1.5%	6.8167 ^A	6.1676 ^{ABC}
Submicrosalt 1%	6.5683 ^A	5.8392 ^{BCD}

^{ABCD} LSMeans with the different letter within the attribute are significantly different

3.10.7. Acceptability

The acceptability of surface salted cheese cracker as influenced by different salt particle sizes/concentrations had a significant ($P < 0.05$) effect for treatment * time interaction, treatment

effect, time effect (Table 11). The contrasts for the submicrosalt 1%, 1.5% and 2% were significantly ($P < 0.05$) different compared to control (regular 2%) (Table 24). However, the contrasts for submicrosalt treatments were not significantly ($P = 0.6073$) different compared to microsalt treatments (Table 24). At 1 week, submicrosalt (1, 1.5 and 2%) and microsalt (1, 1.5 and 2) treatments had significantly ($P < 0.05$) more preferred scores in the acceptability attribute compared to regular 2% (control) (Table 25). However, at 4 months there was no significant ($P > 0.05$) difference in acceptability preference scores between control (regular 2%) and submicrosalt treatments (Table 25). There were no differences in surface salted cheese cracker containing submicrosalt 1 and 1.5% compared with control (regular 2%) for acceptability. This means that even for 25 and 50% less salt content through lowered particle size the acceptability scores are kept the same compared to control (regular 2%).

Table 24. $Pr > F$ of acceptability of surface salted cheese cracker at different particle size treatments to control (Regular 2%)

Contrast	$Pr > F$
Submicrosalt 1% vs Regular 2%	<.0001
Submicrosalt 1.5% vs Regular 2%	<.0001
Submicrosalt 2% vs Regular 2%	<.0001
Micro 1% vs Regular 2%	<.0001
Micro 1.5% vs Regular 2%	<.0001
Micro 2% vs Regular 2%	<.0001
Micro vs Regular 2%	<.0001
Submicrosalt vs Regular 2%	<.0001
Micro vs Submicrosalt	0.6073

Table 25. Least Square Means for acceptability of surface salted cheese cracker as influenced by time of storage

EFFECT	Acceptability	
	1 Wk	4 Mos
Regular 2%	0.6476 ^D	0.6917 ^{CD}
Microsalt 2%	0.8252 ^{ABC}	0.8736 ^{AB}
Microsalt 1.5%	0.9280 ^A	0.8883 ^{AB}
Microsalt 1%	0.8842 ^{AB}	0.8310 ^{ABC}
Submicrosalt 2%	0.9377 ^A	0.8004 ^{ABCD}
Submicrosalt 1.5%	0.9561 ^A	0.8462 ^{ABC}
Submicrosalt 1%	0.8890 ^{AB}	0.7459 ^{BCD}

^{ABCD} LSMeans with the different letter within the attribute are significantly different

3.10.8. Purchase Intent

The Purchase intent's probabilities were estimated before and after the consumers were informed that the surface salted cheese cracker was low in sodium, proven health benefits. At 1 week, all the low sodium treatments had a significant ($P < 0.05$) purchase intent probability (Table 26 and Figure 24). The purchase intent (PI) of surface salted cheese cracker as influenced by different salt particle sizes/concentrations was measured using a McNemar's test and it is shown in the Figure 24 for 1 week and Figure 25 for 4 months.. At 4 months, microsalt 1 and 1.5% and submicrosalt 1.5% had shown a significant ($P < 0.05$) purchase intent probability (Table 27 and Figure 25). The purchase intent at 4 months for submicrosalt 1.5% and microsalt 1% and 1.5% and were 25.52, 19.64, and 29% respectively.

Table 26. Probability > F of low sodium treatments for purchase intent (PI) changes obtained from the sensory study before and after information about low sodium surface salted cheese cracker of 1 week

EFFECT	McNemar's Analysis	
	McNemar's Test	Pr > F
Microsalt 1.5%	6.05	0.0139
Microsalt 1%	10.89	0.0010
Submicrosalt 1.5%	18.75	<.0001
Submicrosalt 1%	5.22	0.0321

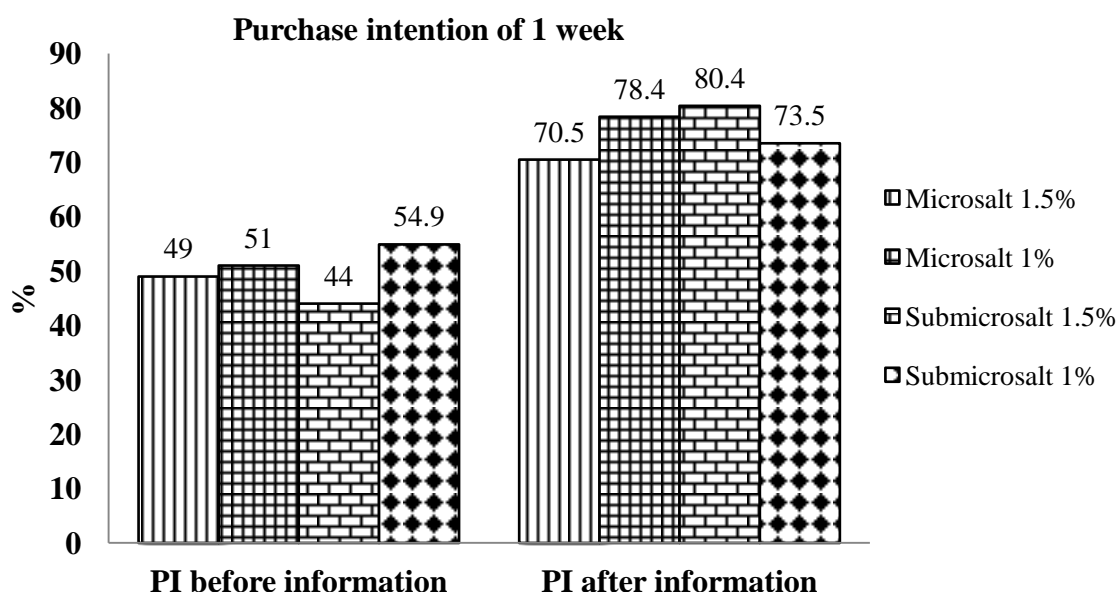


Figure 24. Purchase intent (PI) changes obtained from the sensory study before and after information about low sodium surface salted cheese cracker of 1 week

Table 27. Probability > F of low sodium treatment for purchase intent (PI) changes obtained from the sensory study before and after information about low sodium surface salted cheese cracker of 4 months

EFFECT	McNemar's Analysis	
	McNemar's Test	Pr > F

Microsalt 1.5%	11.21	0.0011
Microsalt 1%	5.41	0.0265
Submicrosalt 1.5%	10.24	0.0014
Submicrosalt 1%	1.0	0.3173

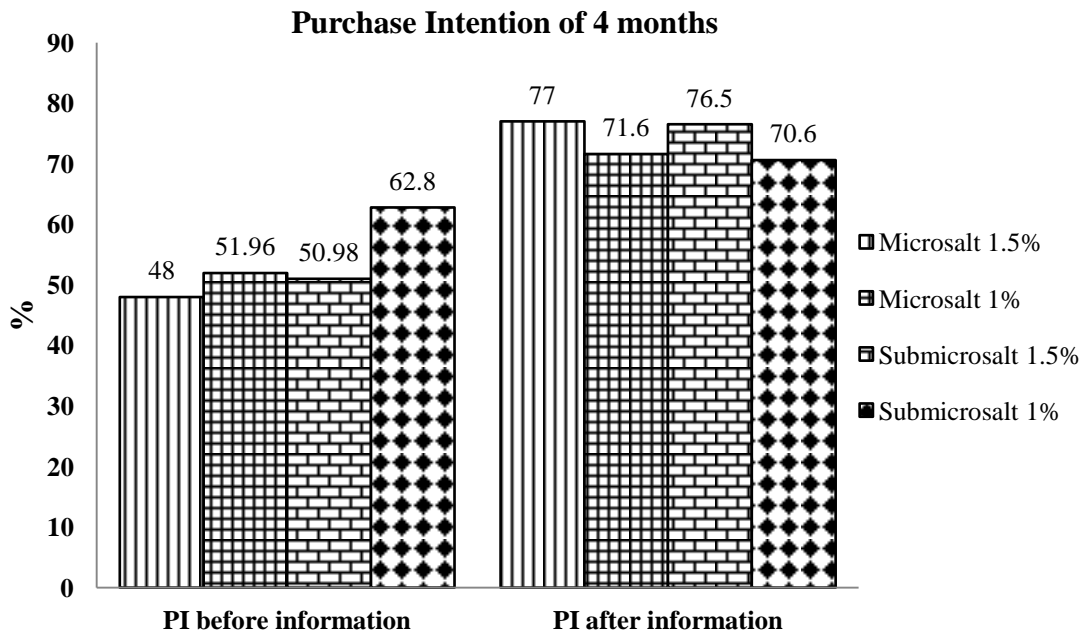


Figure 25. Purchase intent (PI) changes obtained from the sensory study before and after information about low sodium surface salted cheese cracker of 4 months

CHAPTER 4: CONCLUSIONS

The results found in this study demonstrated that the production of submicrosalt (sodium chloride) at 3% concentration by nanospray dryer B-90 had particle size distribution between 500 nm to 1900 nm. There were no significant ($P>0.05$) differences in salt content and sodium content within the same concentration (2% or 1.5 or 1%) of different particle sizes (submicrosalt, microsalt and regular). Water activity (A_w) and yeast counts in all treatments increased from 1 week to 4 months. Submicrosalt 2% and 1% had significantly ($P<0.05$) lower A_w compared to regular 2 and 1% at 4 months. Submicrosalt treatments (2, 1.5 and 1%) had positive effect in yeast reduction at 4 months compared to regular salt (2, 1.5 and 1%). There was no mold growth in all treatments at all times. The L^* , b^* , C^* and h values in all treatments increased significantly ($P<0.05$) from 1 week to 4 months. At 1 week and 4 months, submicrosalt 1.5% was significantly ($P<0.05$) higher in L^* (lightness) values compared to regular 1.5%. At 4 months, submicrosalt 2% had significantly ($P<0.05$) lower L^* (lightness) values compared to microsalt 2% and regular 2%. At 4 months, submicrosalt treatments (2 and 1.5%) had significantly ($P<0.05$) lower a^* values compared to the regular 2 and 1%. At 4 months, submicrosalt treatments (2, 1.5 and 1%) had significantly ($P<0.05$) lower b^* values compared to regular 2%. At 4 months, submicrosalt treatments (2, 1.5 and 1%) showed significantly ($P<0.05$) lower C^* (chroma) values compared to regular 2% and 1.5%. At 4 months there were no significant ($P>0.05$) differences in sensory color, aroma, crunchiness, overall liking and acceptability scores between all submicrosalt treatments (2, 1.5 and 1%) compared to control (regular 2%). At 4 months, submicrosalt treatments (2, 1.5 and 1%) resulted in having significantly ($P<0.05$) more

preferred saltiness scores compared to control (regular 2%). At 4 months, submicrosalt (1.5 and 2%) showed significantly ($P < 0.05$) more preferred just about right saltiness scores compared to control (regular 2%).

The consumers purchase intent increased by 25% in the submicrosalt 1.5% after they knew about the 25% reduction in sodium content of the cheese cracker. The reduction of 25 and 50% salt content in cheese cracker through use of micro and submicro particulated salt did not adversely influence mold counts, sensory color, aroma, crunchiness, overall liking and acceptability scores, which were the same compared to control (regular 2%). In other word sodium chloride micro and submicro particles enhanced saltiness in cheese cracker and at the same time maintained low counts in yeasts, no counts in molds and did not adversely influence other quality attributes. Salt particle size reduction to micro and submicro range can be recommended for use in surface salted cheese cracker for reduce sodium intake.

Conventional thinking is that reduction in particle size of salt increased surface area of salt, hence increase saltiness. In this study salt particle size was reduce 10 times and yet there was no increase in saltiness; which may be because instead of taking salt directly it was taken as a product (protein, fat and other biomolecules) which, masked the saltiness of the surface salted cheese cracker.

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APPENDIX A: CONSENT FORM

APPENDIX 1

RESEARCH CONSENT FORM

APPROVED BY
LSU AG CENTER
IRB AS HE 13-5
ON 6-24-2013

I, _____, agree to participate in the research entitled "Sensory characteristics of cheese crackers surface salted with micron to nano sized salt particles" which is being conducted by the School of Animal Sciences at Louisiana State University, phone number (225)-578-4411.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty of loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. A total of 100 consumers will participate in this research. For this particular research, about a 10 minute participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigators any allergies I may have.
2. The reason for the research is to gather information on consumer attitude and their acceptance of cheese crackers with (smaller) micron to nano sized salt particles. The benefits that I may expect from it are a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.
3. The procedures are as follows: Coded samples of cheese crackers will be placed in front of me and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risks: The only risk that can be envisioned is an allergic reaction to milk. However, because it is known to me beforehand what type of food to be tested, the situation can normally be avoided.
5. The results of this participation will be confidential and will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the investigators. In addition, I understand that research at Louisiana State University, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Philip Elzer, Assistant Vice Chancellor and Assistant Director of the LSU AgCenter 225 578 4182. I agree with the terms above and acknowledge I have been given a copy of the consent form.

Kayamush J. Anyane
Signature of Investigator

Signature of Participant

Date: _____

Witness: _____

APPENDIX B: QUESTIONNAIRE USED IN THE SENSORY ANALYSIS

Surface Salted Cheese Cracker:

Sample _____

1. Please evaluate the product and mark the score [v] that best reflects your feeling about the product.
2. Between the samples, you are required to drink water to clean your palate.

1. **OVERALL**, how would you rate the **color** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

2. **OVERALL**, how would you rate the **odor/aroma** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

3. **OVERALL**, how would you rate the **crunchiness** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

4. **OVERALL**, how would you rate the **Saltiness** this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

5. **OVERALL**, how would you rate the **Saltiness** of this product?

Not enough	Just right	Too much
[]	[]	[]

6. **OVERALL**, how do you **"LIKE"** this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

7. Is this product **ACCEPTABLE**?

YES [] NO []

8. Would you **BUY** this product if it were commercially available?

YES [] NO []

HEALTH BENEFIT:

Low Salt, Low Sodium = Proven Health Benefits

9. Would you **BUY** this product knowing it is low in sodium?

YES [] NO []

VITA

Marvin L. Moncada Reyes was born in Choluteca, Honduras, in 1984. In 2001 he graduated from Escuela Agricola Luis Landa high school in Nacaome Valle, Honduras. In fall 2005, he received his Bachelor of Science degree in agroindustry from Zamorano University in Honduras. Before becoming a graduate student in the School of Animal Science at Louisiana State University in spring 2009, he participated in an exchange program internship between the Zamorano University and the Agricultural Center at Louisiana State University in the summer 2008. During his internship, he had the opportunity to work on yogurt research projects. In May 2011, he received his Master's Degree in animal sciences under dairy foods technology concentration from Louisiana State University and Agricultural and Mechanical College. In May 2014, he is a candidate for a Doctor in Philosophy in Animal Sciences, with a concentration in dairy food technology from Louisiana State University and Agricultural and Mechanical College.