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Effect of germination with selenium in physical and chemical properties of nixtamalized maize (*Zea mays* L.) tortillas

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Dedication

A mis padres, que siempre me han motivado y apoyado en toda decisión y situación que ha acontecido en mi vida y en mi carrera profesional. Sembraron en mi el ímpetu de superarme en cada oportunidad, siempre con humildad, responsabilidad y empatía hacia los demás. El amor de un hijo hacia sus padres es difícil de expresar con palabras, así que no queda más que expresarlo con hechos y este posgrado es un hecho de ese amor y admiración que tengo hacia ustedes.

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Effect of germination with selenium in physical and chemical properties of nixtamalized maize (*Zea mays* L.) tortillas.

by
Juan Pablo Dávila Vega

Abstract

Maize is one of the most consumed cereals worldwide, especially in Mexico where staple foods like tortillas play a crucial role in the daily caloric intake, meaning in some cases up to the 40% of total caloric consumption. It has been demonstrated that germination of kernels offer an alternative for the improvement of the bioavailability of key nutrients and the intake of novel compounds like selenium (Se). Se plays a crucial role in human physiology by taking part in the antioxidant enzymatic mechanism, which is highly related to the prevention of diseases like cancer. Cancer, a multifactorial disease is generally treated with chemotherapy, although this induces negative responses and side effects in the organisms. By that, the search for preventive nutraceutical foods or complementary diets have gained attention in the last years. The main objective of the present research was to evaluate the effects of germination in the presence of Se and nixtamalization in maize tortillas in their physical, and chemical properties, and macronutrient composition. Results showed that germination can generate optimal masas and tortillas without further negative effects as chemical, physical or functional properties. This research clearly indicate the feasibility of processing sprouted grain kernels in the presence of Se via traditional nixtamalization for the production of table tortillas. The Se-enriched tortillas had excellent properties and contained high levels of Se, which is known to prevent oxidative stress and cancer.

Keywords: Maize, Nixtamal, Selenium, Germination, Texture.

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Abbreviations

Se: Selenium

Se-GT: Selenium Germinated Tortillas

GT: Germinated Tortillas

CT: Control Tortillas

GSH: Glutathione

GPx: Glutathione peroxidase

TrxR: Thioredoxin reductase

DIO: Iodothyronine deiodinases

Ca: Calcium

Fe: iron

HSe:hydrogen selenide

Na_2SeO_4:sodium selenate

Na_2SeO_3: sodium selenite

Se-2: selenide

Se2-2: diselenides

SeCN-: selenocyanate

Se-(C=O)-OH:selenoesters

CH_3Se-:methylselenol

SeMSC: Semethylselenocysteine

Sec:selenocysteine

SeCys: Selenocystine

SeMet:selenomethionine

SDG: selenodiglutathion

CH_3SeO_2H,MSeA:methylseleninic acid

Alb: albumin

Glo: Globulin

Glu: Glutelin

NaOH: sodium hydroxide

HCl: chloridic acid

NaCl: sodium chloride

DE: Digestible energy

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Chapter 1

1. Introduction

Maize *(Zea mays L.*) is one of the most produced and consumed cereals globally, with almost 3 million kg consumed daily for nourishment (FAO, 2021). In Mexico, it is the most significant and representative food from the gastronomic, social, political, and cultural viewpoints. It is also the center of origin, domestication, and diversification worldwide. The consumption per capita is around 195 kg per year, mainly in the form of nixtamalized tortillas (Rooney & Serna-Saldivar, 2015; Serna-Saldivar & Chuck-Hernandez, 2019).

Tortillas are the most common food in Mexico and are increasingly being consumed in Latin America and the United States (Bradley, 2019). For their elaboration, traditional lime-cooking (*nixtamalization*) is performed (Serna-Saldivar *et al*., 1990). In our country, it is estimated to provide 40% of total daily calories, representing in some rural communities up to 70% of the caloric consumption, making them the ideal staple food and vehicle for the nutritional and nutraceutical improvement (Serna-Saldivar & Chuck-Hernandez, 2019).

One of the nutraceutical agents that has been recently broadly investigated is selenium (Se). This essential micronutrient plays multifunctional roles in the physiology of humans, being the most relevant one its action as an antioxidant through the enzymes Glutathione (GSH), Glutathione peroxidase (GPx), Thioredoxin reductase (TrxR), and Iodothyronine deiodinases (DIO). Besides, optimal concentrations of Se (~55 µg/day) are essential for human physiology, while supranutrinional levels (100-350 µg/day) can lead to favorable effects to combat ailments like cancer. However, toxic concentrations (>350 µg/day) can generate liver and kidney injury, blood clotting, necrosis of heart and liver, hair and nail loss, nausea, and vomiting. Se deficiency (>20 µg/day) lead to muscle weakness and inflammation, fragile red blood cells, irregular skin coloration, heart muscle dysfunction, Kashin-Beck and Keshan, and higher susceptibility to diseases like cancer (Dos Reis *et al.,* 2017; Evans *et al.,* 2020).

Cancer, a multifactorial pathology with an uncontrollable cell proliferation developing in a tumor, is ranked as the second most common cause of death globally. In 2020 the World Health Organization (WHO) assessed 19 292 789 new cancer cases worldwide, with nearly 10 million deaths, representing in some cases a 60% of mortality for the patient (WHO, 2021).

Treatments include radiotherapy and chemotherapies, which have shown to be extremely cytotoxic, targeting both healthy and malignant cells, leading to disadvantageous consequences such as nausea, diarrhea, cephalalgias, alopecia, liver damage, amongst others (Islam *et al*., 2020). New and complementary adjuvants like functional foods have gained importance over the last years for reducing toxicity of previous treatments or as a dietary complement for patients (Nair *et al.,* 2021).

Nutraceuticals, such as Se-enriched foods, have been proposed, aiming for common and staple foods, such as cereals like maize. Given this, this investigation deals with innovative and complementary strategies for cancer adjuvants without or with low toxicity or side effects. One of the approaches is the development of Se-enriched nutraceutical products. Biofortification of tortillas has been achieved, however, the physical, chemical, and nutraceutical effects of nixtamalized tortillas made of Se-enriched maize kernels have not been addressed yet, making it an opportunity for the development of complementary diets for patients who are under chemotherapy.

1.1. Motivation

The importance of Se in human physiology has been broadly described due to its antioxidant properties and the involvement in redox enzymes, taking part in the prevention of oxidative stress diseases such as cancer. Additionally, the supplementation of Se into grains during seed germination offers a broad field of possibilities to improve the nutraceutical and nutritional effects of staple foods like nixtamalized tortillas which are widely consumed on a daily basis (Guardado-Félix *et al.,* 2019; Serna-Saldívar & Chuck-Hernandez, 2019)

Common maize tortillas contain an important macro- and micronutrients profile such as starch, protein, lipids, minerals and vitamins. However, Se deficiency is endemic in many developing countries due to the lack of this mineral in soils. Therefore, foods can be supplemented with selenium salts in order to protect humans against oxidative stress and cancer. To overcome this, the supplementation of grains offers an innovative resolution due to their high consumption, comparatively low cost, and their broad applications in common foods. Se can be incorporated to the food chain through soil and foliar fertilization, direct addition to cereal-based flours and during yeast or lactic acid bacteria fermentation (yeast-leavened breads) and through natural processes like sprouting or germination (Matilla, 2008; Perales-Sánchez *et al.,* 2014). The advantage of the last two systems is the conversion of inorganic Se into organic moieties that are known to be highly bioavailable and nutraceutical.   The enriched foods can complement and aid patients under chemotherapy (Evans *et al.,* 2020; Nair *et al.,* 2021;Radawiec, 2021). Still, the impact of germinated maize in the presence of Se has not been assessed in the elaboration of nixtamalized tortillas and their effects on physical and chemical properties.

1.2. Problem statement

The interest in Se-enriched foods has been recently proposed, due to their expected role as coadjutants and complementary agents for diverse ailments related to an irregularity in redox physiology (Guardado-Félix *et al.,* 2019). One of the methods for the elaboration of these products is through germination in the presence of inorganic Se, making an easy to implement large-scale and industrial process for the elaboration of foods like tortillas (Guardado-Félix *et al,* 2019; Radawiec *et al.,* 2021)

Tortillas are the most common food in Mexico and are ideal foods to provide a new way for consumers to improve their health (Serna-Saldivar, 2015; Bradley, 2019). Also, the widely used nixtamalization process offers an easy way to modify physical and chemical properties suited to produce good quality tortillas. Nutritionally wise, the lime-cooking enhances the concentration of some nutrients like calcium and the bioavailability of others like niacin (Serna-Saldivar, 2021). The nixtamalization of germinated or sprouted maize kernels provide additional benefits like higher bioavailability of phosphorus, iron, zinc, and protein nutrient digestibility (Serna-Saldivar, 2021).Nevertheless, the combination of germination and nixtamalization is poorly studied and understood. The analysis of the nutritional value of tortillas is complicated due to the nixtamalization process and the nutrient losses incurred during lime-cooking and tortilla baking (Valderrama *et al.,* 2021).

Given that, there is an attention to study the interaction between germination in the presence of Se and nixtamalization in the physical, chemical, and nutraceutical properties of tortillas. Grains, like soybean, have been successfully selenized and processed to final dietary products with optimal levels of Se and the enriched grains have demonstrated their effectiveness *in vivo* xenografted cancer models (Serrano-Sandoval *et al.,* 2019; Guardado-Félix *et al.,* 2019; Dai *et al.,* 2020; de Brito *et al.,* 2020).

1.3. Research question

The effects of germination with sodium selenite combined with the nixtamalization process in the elaboration of tortillas offers a whole new area of opportunity to develop functional and attainable foods for a vast percentage of the Mexican population. However, this dual process may induce different modifications on tortilla functional and nutritional characteristics. Therefore, this thesis was planned to determine if it is possible to generate high-quality maize tortillas after germination of maize kernels in the presence of selenium and the necessary processing changes needed to optimize quality of final tortillas. In addition, the physical and chemical, properties of the novel tortillas were assessed.

1.4. Solution overview

The germination of maize in the presence of Se and further traditional nixtamalization process were proposed as a solution for the improvement of chemical, physical, functional and nutraceutical properties of tortillas, with optimal concentration of Se based on the daily average recommended allowance. The experimental strategy was divided (Fig 1.) into two main phases:

1. Selection and cleaning of maize and the division into germinated and Se-germinated groups, taking a large-scale germination at the commercial plant "Alimentos Lee®" (Fig 1)

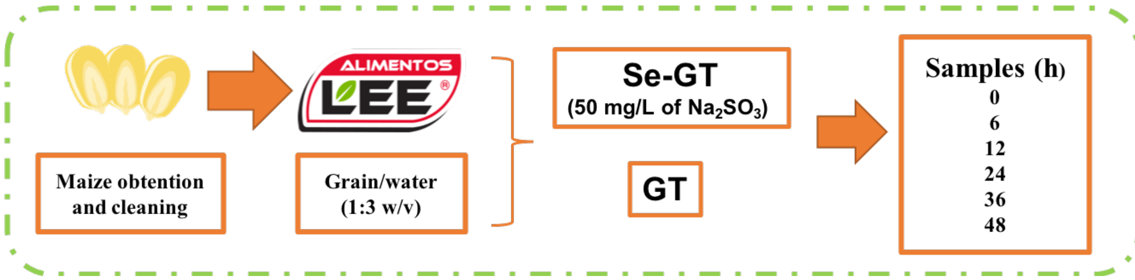

Fig 1.- Sequential steps used to produce germinated or sprouted maize kernels.

2. Tortilla production utilizing the regular nixtamalization process followed by the evaluation of the chemical, physical and functional properties of masa and tortillas stored at room temperature. Briefly; the masa and tortilla yields were calculated, followed by the determination of crude protein, starch, minerals, moisture, crude fat and α-amylase activity. Also, the protein fractionation and quantification of Se in each fraction were assessed. Furthermore, the physical and rheological analysis of masa and tortillas were determined with the Mixolab, Texturometer and colorimeter. Additionally, the tortillas were analyzed by an untrained sensorial panel to detect differences in flavor, color, texture and overall acceptability. Finally, the Se of all tortilla treatments were determined by ICP-MS (Fig. 2)

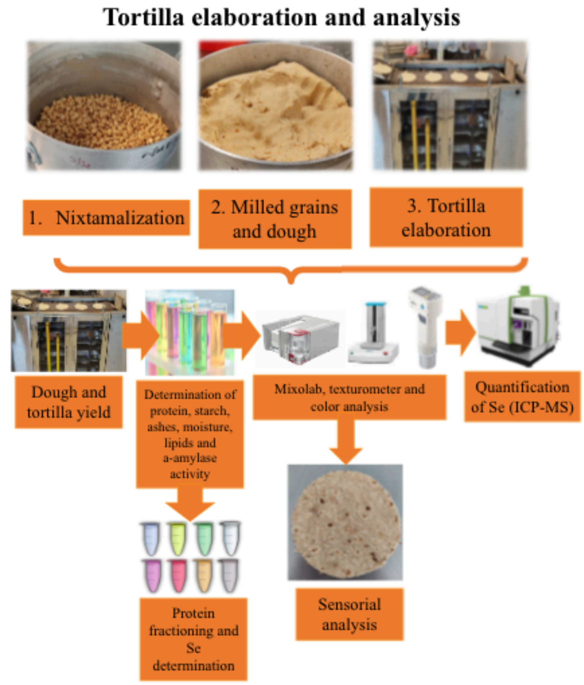

Fig 2.- Tortilla elaboration and analysis.

1.5. General objective

The aim of this study was to evaluate the dual effect of germination with selenium and nixtamalization for production of table tortillas and the effects of these treatments on physical and chemical properties of masa and tortillas.

1.6. Specific objectives

To accomplish the main objective the following specific objectives were established:

1. To evaluate the effects of germination with and without selenium on the nixtamalization properties of maize masa and tortillas.
2. To describe differences among treatments in terms of physical characteristics of sprouted kernels, masa and tortillas.
3. To determine the Se concentration in tortillas and its relationship with other biomolecules.

1.7 Hypothesis

The design is based on the following hypotheses:

H_0: Selenized, germinated and nixtamalized maize will not modify masas and tortilla physical, and chemical properties

H_A: Selenized, germinated and nixtamalized maize will modify the physical and chemical parameters, and enhance bioavailability of macronutrients in masas and tortillas.

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Chapter 2

Theoretical Framework

Maize (*Zea mays L*.) is the most significant and valuable food source in Mexico and it is transformed into diverse staple foods. The total consumption of maize in some cases represents up to 40% of the everyday caloric energy intake (Bradley, 2019; Serna-Saldivar, 2021). The chapter will focus on the relevance of maize for nourishment, its attributes, and the nixtamalization process, as well as the significance of Se in human physiology and nutrition.

2.1 Maize

Also known as "corn", *"centli", "choclo",* or *"elote",* maize is an annual grass (Poaceae) with a C4 metabolism, which started to be domesticated in Mexico and Central America about 8 000 years ago (Serna-Saldivar, 2015; Bradley, 2019). It is the most globally produced crop with almost 1100 million tonnes generated annually. The main uses of maize is for animal feeding, production of human foods, fuel bioethanol and other industrial uses like production of biodegradable or green plastics (Utrilla-Coello *et al*., 2009; Zilic *et al*., 2011; Ranum *et al.,* 2014; Covic *et al.,* 2018). In developing countries, maize is the most consumed daily ingredient, representing a considerable source of energy, especially in Mexico and Central America (Kljak *et al.,* 2018).

Table 1.- Taxonomic classification of *Zea mays* L (ITIS, 2021)

Kingdome	Plantae
Subdivision	Magnoliophyta
Clade	Liliopsida

Subclade	Commelinidae
Order	Poales
Family	Poaceae
Subfamily	Panicoideae
Tribe	Andropogoneae
Subtribe	Tripsacinae
Genus	Zea
Species	*Zea mays*

2.2 Maize as food source

In Mexico and Central America, maize represents the basis for the daily diet, with a *per capita* daily consumption of about 350 kcal. Foods derived from maize supply important macronutrients (starch, proteins and dietary fiber) and micronutrients (vitamins and minerals) (Serna-Saldivar, 2021).In Mexico, the average consumption of maize and related foods, especially table tortillas, range from 30-40% of the daily caloric intake; with a yearly intake ranges from 50 to 70 kg tortilla *per capita*. In some cases, the annual consumption in some rural areas exceeds 100 kg/year per capita (Serna-Saldivar &Chuck-Hernández, 2019). In addition, maize is also utilized for the production of animal feedstocks, flours, meals, beverages, oils, and bioethanol (Ranum *et al.,* 2014). The last usage is prohibited in Mexico because this cereal represents the most important source of food for the general population. In urban settlements, whole grain, cereals, tortillas, popcorn, energy bars, sweeteners or syrups and cornbread are among the most common ways of consuming maize (Lozano-Alejo *et al*., 2007; Uriarte-Aceves *et al*., 2018; Serna-Saldivar & Chuck-Hernandez, 2019; Serna-Saldívar, 2021)

From the nutritional point of view, maize contains approximately 70-75% of starch, 10% protein, 4% crude fat and is an important source of vitamin B, minerals and fiber (Gyori, 2017; Navarro *et al*., 201; Xu *et al.*, 2019). It is important to highlight that biofortification of tortillas with vitamins B12 and C, calcium (Ca), , iron (Fe), folates, and amino acids like lysine and tryptophan have been practiced in the last decades to generate more nutritious foods promoted by state and federal governments (Sumbo & Ikujenlola, 2014; Covic *et al.,* 2018).

Although maize and derived products are a considerable source of nutrients, the traditional methods for preparation are poorly scaled in the industry. The traditional nixtamalization process is known to partially or totally remove the bran, the aleurone layer and germ that store important nutrients. The new commercial processes are aimed to prolong the shelf life of packaged tortillas and supplement nutrients, which enhance the overall nutritional value of tortillas (Ranum *et al.,* 2014; Covic *et al.,* 2018).

2.3 Main anatomical and nutritional characterization of maize

The anatomy of the kernel often consists of a 10-15 mm long body, with an average 5 mm width and a weight of 1000 grains between 300-400 g. The grain is divided into four structures; endosperm (the main component of the grain comprising up to 80-85% of the total structure which is the main source of starch), germ (comprising from 10-12% rich in proteins and lipids), pericarp (comprising 5 to 7%, with high levels of cellulose, lignin, and phenolic pigments), and tip cap which constitutes only 1% of the kernel weight (Georgia Corn Comission, 2021).

The grain composition is mainly composed of starch followed by protein whereas the rest of the kernel contains lower a munts of dietary fiber, lipids, vitamins, minerals and pigments (Györi, 2017).

Starch is the most relevant component in the grain, with 70-75% of the whole seed. It is formed by two polysaccharides: amylose and amylopectin in a proportion of 1:3 in regular maize. The waxy genotypes contain about 95% amylopectin and only 5% amylose. Starch is the main agent for the texture of the seed, which can be divided according to the endosperm: floury (opaque in the inner of the grain), and vitreous (transparent or glassy in the periphery). On the other hand, floury endosperm has spherical starch granules with a spare protein matrix, while the vitreous endosperm has polygonal granules with a dense protein matrix (Xu *et al*., 2019).

Proteins are a critical element in maize, finding them mainly in the endosperm but also in the germ, with high concentrations of endosperm proteins zeins (prolamins) and glutelins and lower amounts of germ proteins albumins and globulins (Serna-Saldivar, 2016). Zeins are specific storage proteins and are subdivided into α-, β-, and γ-zeins, which have a direct correlation with endosperm texture (García-Lara *et al.,* 2019).

Triglycerides rich in polyunsaturated fatty acids, phospholipids and other minor lipids like phytosterols are mainly found in the germ. The oil is mainly constituted by linoleic (18:2 Δ9,12) and oleic (18:1 Δ9) acids followed by palmitic and stearic fatty acids (García-Lara *et al.,* 2019; Györi, 2017).

Among other biomolecules found in the pericarp of the kernel are cellulose, lignin, arabinoxylans and pigments of phenolic nature. In terms of minerals, phosphorus is the most abundant followed by potassium, magnesium, and sulfur. The raw kernel is practically devoid of calcium, which greatly increases after the nixtamalization process (Serna-Saldivar, 2016).

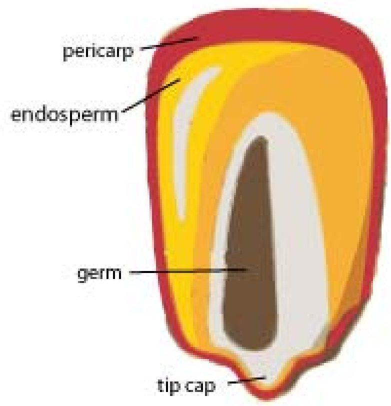


Figure 3. Anatomical structures of the maize kernel.

Source. Taken from the Georgia Corn Comission (2021)

2.4 Nixtamalization

Nixtamalization is a traditional lime-cooking with steeping time technique for the elaboration of an array of maize foods, chiefly tortillas. It is an ancient process dating back to Mesoamerican cultures and nowadays the same principles are used for the production of tortillas and related products with modern and massive scale-up processes. Most tortilla factories manufacture tortillas from fresh masa or alternatively from dry masa flour (Bello-Pérez *et al*., 2015, Serna-Saldivar and Chuck-Hernandez, 2019)

Description of the process

Briefly; mature and cleaned maize kernels are cooked with water (3:1 v/w) and food grade lime ($Ca(OH)_2$), which does not contain significant amounts of heavy metals and other toxic components. The grain is generally cooked with 3 parts water and 1% lime based on the original grain weight. The maize is normally cooked from 5 to 40 minutes, depending on the type, size, kernel hardness and variety. Immediately afterwards the lime-cooked grain is steeped for 8 to 15 hours. The waste-water or nejayote is drained and kernels hand-washed with water for the removal of the pericarp and residual lime. Finally, the cleaned nixtamal is ground with a stone-mill for the formation of the cohesive masa (Fig. 4) (Bello-Pérez *et al*., 2015; Camelo-Méndez *et al.,* 2017).

Every nixtamalization step is crucial for reaching the appropriate masa and tortilla characteristics. During cooking and steeping, kernels absorb up to 50% moisture, and important amounts of calcium ions which hydrolyzes hemicellulose bonds. This chemical reaction facilitates the removal of the pericarp tissue producing simpler moieties rich in hydrocolloids that contribute to produce softer tortillas (Serna-Saldívar *et al*., 1990; Rocha-Villarreal *et al.,* 2017). Lime-cooking is mainly aimed to partially gelatinize the starch and besides prevents microbial activity, favors flavor and aroma, facilitates water absorption and improves the bioavailability of niacin (Birt *et al.,* 2013). Steeping time is also crucial for the lixiviation of calcium and hydroxyl ions into the grain, promoting changes in starch and protein structure, especially zeins, which are partially hydrolyzed. These hydrolyzing reactions release part of the second limiting amino acid tryptophan and affects the leucine-isoleucine ratio. The $Ca(OH)_2$ free of toxic residues used for nixtamalization provides significant amounts of this major mineral. Most of the calcium hydroxide used for nixtamalization ends up in the nejayote, which is considered a high polluted wastewater with alkaline pH and corrosive properties (Arriaga, 2021).

More importantly, the nixtamalization process is known to diminish and degrade the phytochemical profile because the thermal alkaline treatment solubilizes important molecules that leach into the nejayote (Serna-Saldivar and Rooney, 2015; Serna-Saldivar and Chuck-Hernandez, 2019)

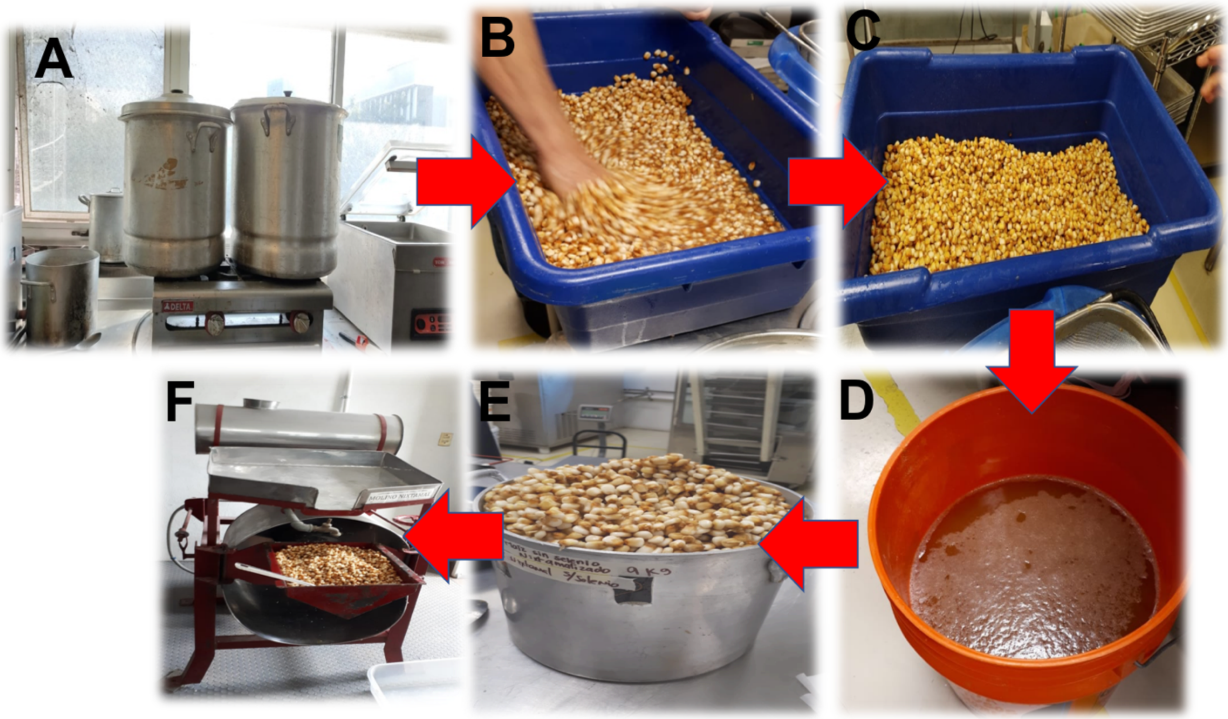


Figure 4. Nixtamalization process. The lime-cooking process is carried on from 5 to 40 minutes, following a steeping time (A), later the nixtamal is drained and hand-washed (B) to remove the nejayote (D). The clean and nixtamalized kernels (E) are later milled on a stone mill for the elaboration of masa (F). Schematized by the author.

2.6 Maize tortillas

Tortillas are the most common nixtamalized food produced in Mexico and constitute the base for many Mexican gastronomy dishes. They represent up to 40% of daily caloric intake and are the main source of proteins for the Mexican population. An average Mexican consumes 8-10 tortillas daily, representing an energy intake from 350 to 450 kcal. The annual per capita consumption is 80 kg although higher consumption rates are observed in rural areas of the country (Veloso *et al.,* 2011; Serna-Saldívar, 2015; Chávez-

Santoscoy *et al.,* 2016; Serna-Saldivar & Chuck-Hernández; 2019; Serna-Saldívar, 2021).

Besides serving as the main source of energy, tortillas contain significant amounts of resistant starch or prebiotics, which are known to stimulate the growth of the microbiota of the large intestine (Garcia-Lara *et al.,* 2012; Serna-Saldivar, 2015; Urias-Lugo *et al.,* 2015). However, nixtamalization reduces the concentration of minerals, vitamins, and nutraceutical phytochemicals like ferulic acid and carotenoids.

Elaboration

Tortillas are manufactured by two major methods: traditional nixtamalization that relies on fresh masa, and large-scale dehydrated dry masa flour. Nixtamalized masa for table tortillas should contain from 54-60% moisture with fine granulometry or particle size distribution. The fresh tortilla normally contains from crude protein between 6-10%, and low fat and mineral content (<5%) (Herrera-Sotero *et al.,* 2017; Rooney & Serna-Saldivar, 2015)

For the elaboration of traditional fresh tortillas, pieces of masa are hand patted or formed in preparation for baking on traditional hot griddles or “comales” . The masa discs are usually 25 cm of diameter and 27 g fresh weight and are baked for about 50 sec on each side at 250-300ºC. However, the search for industrially produced maize tortillas has sparked the use of instant or precooked dry masa flours. These tailored-made flours are mainly used in the international arena because they just need addition of water, blending and baking. The main advantage of usage of dry masa flours is the saving on space floor, energy and especially processing time (Serna-Saldívar, 2021). "Tortilladoras" *are* the most common way to form and bake tortilla in large-scale settings. These continuous machines found in most "tortillerías", usually manufactures tortillas of 15-25 cm diameter and 15-22 g weight. One of the main advantages of dry masa flours is that they can easily be biofortified with calcium, iron, zinc, B-vitamins and even proteins. There are basically two sorts of tortilla machines: in both systems the masa is placed in the masa hopper, which feeds the forming devices which presses and cut the thin sheets of masa via extruding through a metal mold or with a sheeting and cutting device. The resulting tortilla discs are conveyed into a three tier gas-fired oven set at 300-380ºC for a dwell or residence time of 45-60 sec. White maize is the preferred grain for table tortilla elaboration. However, yellow maize alone or mixed is also employed to produce tortillas with different organoleptic attributes. Large-scale production settings normally aim to produce tortillas with longer shelf life by adding antimycotic agents like organic acids and preservatives (Agarry *et al.,* 2014; Serna-Saldivar and Rooney, 2015; Serna-Saldivar and Chuck-Hernandez, 2019).

1.7. Physico-chemical modifications in nixtamalized tortillas

The lime-cooking process is strictly controlled to promote chemical and physical changes in the nixtamal especially in terms of starch (Campus-Baypoli *et al.,* 1999). These changes are described in the following sections:

- Starch: Undoubtedly, starch is the most functional chemical component. The uncooked starch is gelatinized during lime-cooking and further modified during the stone-grinding procedure. The main aim is to partially gelatinize the starch granules in order to bind water. Under and overgelatinization yields masas with lower and higher moisture contents. The corn starch normally gelatinizes at temperatures ranging from 68 to 76°C modifying the water absorption index and size of granules. The attrition generated during grinding disrupts starch granules releasing partially gelatinized amylose and amylopectin molecules, which form a colloidal matrix. Finally, a gel is generated with highly hydrated amylose chains. In the presence of $Ca(OH)_2$, calcium interacts directly with linear amylose chains, retarding the process. On the other hand, retrogradation is a phenomenon, which plays a significant role in the texture, machinability and overall functionality of masa. This phenomena mainly occurs among amylose chains which bind when the temperature of the masa decreases. This process is related directly to the amylose content, and it can arise in two ways: fast (a grain rich in amylose is overheated and then the temperature is quickly decreased, resulting in a rigid and reversible gel) and slow (after heating, the grain precipitates) (Waight *et al.,* 2000; Gaytán-Martínez *et al.,* 2006 ; Birt *et al.,* 2013; Ma *et al.,* 2020)

- Proteins: Lime-cooking enhances protein denaturation and solubility. The solubility of albumins and globulins decreases whereas glutelin and prolamins increases (Serna-Saldivar and Rooney, 2015; Békés & Wrigley, 2016; Kljak *et al.,* 2018)
- Fat: Nixtamalization generates a significant loss of lipids, although it is commonly seen as advantageous for the production of low-fat products. Lipids found in tortillas can bind with the internal helix of amylose chains. Also, due to basic pH levels, fatty acids may undergo saponification and oxidation, producing foaming and organoleptic properties of end-products (Preciado-Ortíz *et al.,* 2013).
- Vitamins and minerals: Commonly, tortillas lack optimal levels of essential minerals and vitamins; however, since the 1950's the national authorities in most countries

around the globe have enacted the enrichment of dry flours with thiamin, riboflavin, niacin, folic acid, iron and zinc in order to prevent deficiencies that lead to pellagra, beriberi, lack of growth and malformations. Nevertheless, these additions rheological properties and final tortilla texture (Zilic *et al.,* 2011; Ziegler *et al.,* 2018). In addition, the search for new enrichment methods of crucial micronutrients has gained importance over the last decade. Among new nutrients, selenium has gained relevance because of its beneficial effects against oxidative stress and cancer.

2.8 Selenium

Selenium (Se) is an essential, multifunctional micronutrient for human consumption. Mainly taking part as a crucial component for antioxidant enzymatic mechanisms (Glutathione (GSH), Glutathione peroxidase (GPx), Thioredoxin reductase (TrxR) and Iodothyronine deiodinases (DIO) pathways (Collery, 2018).

Chemical forms and seleno-compounds

Almost every sulfur-containing molecule has the potential to be substituted by Se in its structure. These organic molecules are known as seleno-compounds. Elemental Se, hydrogen selenide (HSe), sodium selenate (Na_2SeO_4), sodium selenite (Na_2SeO_3), selenide (Se-2), diselenides (Se2-2) and selenocyanate (SeCN-) are inorganic forms, On the other hand, selenoesters (Se-(C=O)-OH), ethaselen ((1,2-[bis(1,2-benzisoselenazolone-3(2H)-ketone)]ethane or 1,2-BBSKE), methylselenol (CH_3Se-), Se-aromatic containing molecules, and selenoaminoacids like Se-methylselenocysteine (SeMSC), selenocysteine (Sec) selenocystine (SeCys), selenomethionine (SeMet), selenodiglutathion (SDG), and methylseleninic acid (CH_3SeO_2H, MSeA) are organic compounds (Gandin *et al.,* 2018). Furthermore, Se can be a component of proteins or enzymes like Glutathione peroxidase (GPx) Thioredoxin reductase (TrxR), deiodinases (Dio), and all Selenoproteins (H,I,K,M,N,O,P,R,S,T,V, and W) (Avery & Fontman, 2018; Fontangne-Dicharry *et al.,* 2020).

Selenium in human health

Optimal concentrations of Se (~55 µg/day) are crucial to combat inflammation processes, and regulate thyroid hormones, fertility and the immune system (Garousi *et al.,* 2017). Se deficiency (>50 µg/day) can lead to several pathologies such as muscle weakness and inflammation, fragile red blood cells, irregular skin coloration, heart muscle dysfunction, red blood cells, susceptibility to cancer, Kashin-Beck and Keshan diseases. On the other hand, supranutrinional levels (100-350 µg/day) and toxic concentrations (>350 µg/day) can generate liver and kidney damage, blood clotting, necrosis of heart and liver, hair and nail loss, nausea and vomiting. (Dos Reis *et al.,* 2021).

The organic selenium forms of selenoproteins have been associated positively with inhibitory results in tumors of lung, bladder, colorectal, breast, and prostate (Abedi *et al.,* 2018; Bertz *et al., 2018*; Saeed *et al.,* 2019; Sonkusre, 2020; Wang *et al.,* 2020*;* Evans *et al*., 2020). Moreover, it has been proved that consumption of 200 µg of selenium per day can reduce considerably the risk of developing of any cancer (Guo *et al.,* 2021). Additionally, there is data concerning that HSe, CH_3Se, SDG, SeMet, and SeCys supplementation in different *in vitro* and *in vivo* models decreased cancer risk, cancer cell proliferation, adenoma, carcinoma growth, and even angiogenesis (Bertz *et al.,* 2018; Evans *et al.,* 2020 Saeed *et al.,* 2019; Sonkusre, 2020; Wang *et al.,* 2020).

The consumption of Se has been associated with anticancer activity, mainly due to the connection between Se levels and antioxidant activity (Lavu *et al.,* 2016). The potential anticancer mechanism of Se has been extensively explained in several scientific works with specific ways of action: activation of apoptotic pathways (Abedi *et al.,* 2018; Guardado-Félix *et al.,* 2019), endoplasmic reticulum stress (Lobb *et al.,* 2018; Evans *et al.,* 2020), reduced angiogenesis (Abedi *et al.,* 2018; Saeed *et al.,* 2019; Rajkumar *et al.,* 2020), selective cytotoxicity (Li *et al.,* 2017; Barbanente *et al*., 2020), improvement of DNA repair (Yildiz *et al.,* 2019; Safarzad *et al.,* 2019), epigenetic regulation (Hughes *et al.,* 2018), Se nanoparticles target-effects (Jabłońska, & Reszka, 2017; Zhao *et al.,* 2018; Hughes *et al.,* 2018; Rajkumar *et al.,* 2020; Sonkusre, 2020; Barbanente *et al.,* 2020), synergism with other trace elements (Safarzad *et al.,* 2019; Ranjbary et al., 2020), and antioxidant modulation (Hu *et al.,* 2019; Brzacki *et al.,* 2019; Marciel & Hoffman, 2019; Bertz *et al.,* 2018 Wang *et al.,* 2020).

2.9 Se in food sources

Humans absorb Se in both inorganic and organic forms; nevertheless, the net absorption is more effective with organic Se forms associated to meat, seafood, grains, and yeasts (Bizerea *et al.,* 2018). Se-food sources can be categorized into two groups:

- <u>Natural Se-rich sources</u>*:* traditional dietary products like Brazilian nuts, chicken eggs, cow milk, red meats, seafood, wheat, among others (Tamires *et al.,* 2020).
- <u>Biofortified:</u> The lack of Se is principally due to the low bioavailability in farm soils and poor fortification of everyday foods (FAO, 2021). Biofortified *Brassica*

oleracea L. var*. gongylodes (*Golob *et al.,* 2020), *Spirulina* (Fontagne-Dicharry *et al*., 2020), and yeast (Mouasie, 2021) have been successfully used to satisfy the demands of people who had Se deficiencies (Tamires *et al.,* 2020; Fontagne-Dicharry *et al.,* 2020; Mouasie, 2021). Recently, Se biofortification of grains like chickpea (Guardado-Félix *et al.,* 2017; Serrano-Sandoval *et al.,* 2019), rice (Ei *et al.,* 2020), soybean (Dai *et al.,* 2020), wheat (Islam *et al.,* 2020), and even coffee beans (de Brito *et al.,* 2020) have been used to upgrade the Se status of deficient populations (Ligowe *et al.,* 2020).

To accomplish the last, it is important to highlight that Se absorption in plants relies essentially on the system of biotransformation from inorganic to organic forms. The process relies on Se availability in the soil, geographical circumstances, and plant general requirements. Moving on into the trophic chain, Se is incorporated in red meats, seafood, milk, eggs, yeast and related processed foods (Kieliszek, 2019). For that reason, the quest for new Se-enriched foods has been recently a topic of concern (Fernandes & Gandin, 2015; Mouasie, 2021).

2.10 Germination with Selenium

The consumption of germinated maize products has gained attention due to the improvements in the bioavailability of both macro and micronutrients. Briefly, grains remain in quiescence until favorable conditions are reached (19ºC, 80% humidity), then the embryo starts to absorb water, and seed tegmentum is disrupted; finally, the radicle emerges between 20-24 h (Audersik *et al.,* 2018).

The consumption of germinated grain products has gained attention due to the improvements in macro and micronutrients and the bioavailability of so. Briefly, grains remain in quiescence until favorable conditions are reached (19+1 ºC, 80% humidity), then the embryo starts to absorb water, and the seed tegmentum is disrupted. Thereafter, the radicles or rootlets emerge between 20-24 h. During this physiological process, different types of enzymes are sequentially synthesized, especially α-amylase. This particular enzyme catalyzes the hydrolysis of α-glycosidic bonds of starch. It is important to highlight that seeds will absorb water and by then, all the micronutrients become dissolved in water. Some of these nutrients are crucial for correct germination and seedling growth (Kikafunda *et al.,* 2006; Hubner & Arendt, 2013; Kide *et al.,* 2015; Singh *et al.,* 2017).

In terms of plant physiology, Se acts as an analog of sulfur in many metabolism-related processes. It has been demonstrated that diverse grains can effectively uptake dissolved Se, such as chickpea (Guardado-Félix *et al.,* 2019; Serrano-Sandoval *et al.,* 2019; Guardado-Félix *et al.,* 2020), soybean (Lazo-Velez *et al.,* 2018), huazontle (Lazo-Velez *et al.,* 2016), wheat (Lazo-Velez *et al.,* 2016), cowpea (Lapaz *et al.,* 2019), and rice (D'Amato *et al.,* 2018).

Chapter 3

Materials and Methods

3.1 Acquisition of maize

Maize *(Zea mays* L.*)* kernels with intermediate endosperm texture suited for tortilla production were purchased from the Guadalupe Central Supply Market (Nuevo León, Mexico) in March, 2021. The maize was first cleaned with a sieve and then hand-cleaned in order to remove chaff, foreign material and off-colored, broken and damaged kernels. The cleaned kernels were kept in airtight plastic sealable bags at room temperature until usage.

3.2 Disinfection and determination of grain moisture

Grains (100 g) were washed for 3 minutes with 200 mL of a 0.2% sodium hypochlorite solution. Subsequently, they were rinsed for 30 seconds with distilled water, vigorously shaking the grains. The determination of grain moisture was based on the loss of weight during drying in a convection oven set at 100°C (Rocha-Villarreal *et al.,* 2018).

3.3 Maize germination test

Before large-scale germination, a laboratory test was carried out with 100 kernels to observe viability. The kernels were disinfected as described above and subsequently rinsed for 30 seconds with distilled water. Grains were hydrated in 150 mL of distilled water for 8 hours with constant stirring. Thereafter, soaked kernels were placed on a 30x25x15 cm plastic tray with approximately 2 cm of separation between kernels. Wet absorbent paper was placed on top of the grain bed to prevent moisture loss. The tray with the kernels was placed in a germination chamber set at 20ºC for 48 hours. Finally, the germination percentage was calculated based on the following formula:

(Germinated grains* x 100) / (Total number of grains to germinate).

**Sprouted or germinated kernels were considered kernels which developed rootlets and/or acrospires after two-days germination.*

3.3 Large-scale germination

For the experimental phase, the previously cleaned kernels were separated into two groups: Germinated Tortilla (GT) and Se-Germinated Tortilla(Se-GT) treatments; 15 kg were weighed on a scale (Toro Rey EQB 50/100) for each group, and germination was carried out at large scale in Alimentos Lee® (Apodaca, Nuevo León, México). The disinfection and clarification of the seed were carried out under the protocols of the company. The kernels were first hydrated with distilled water (3: 1 v / p) for 8 hours, shaking the grains every 30 minutes. For the case of selenized maize, 50 mg of sodium selenite (Na_2SeO_3, Sigma Aldrich, St. Louis, MO, USA) were dissolved for every 1 L. The process lasted 48 hours. During this period, hydrated kernels were sampled at 0, 6, 12, 24, 36 and 48 hr. Germination was performed under complete darkness using the conditions established by the company (Guardado-Félix *et al.,* 2019). Finally, germinated maize kernels were placed in plastic bags, transported to the tortilla pilot plant and immediately nixtamalized or lime-cooked.

3.4 Nixtamalization and tortilla elaboration

The germinated kernels were weighed and the dry weight was calculated based on the grain moisture content. Before nixtamalization, water (3: 1 w/v) was placed in galvanized metal pots with a volume capacity of 45 L. When the water reached 95°C food-grade lime ($Ca(OH)_2$) was added at the rate of 10 g per each kg of dry grain. The resulting lime-cooking solution was heated to 93° to 95°C before addition of the sprouted grains. The cooking time established for the sprouted kernels was 4 minutes whereas for the control maize 30 min. After completing the cooking time, the heat was discontinued and contents allowed to steep for 14 hours. The whole cooking and steeping steps were performed in containers covered with a lid. The following day, the lid was removed and the nejayote discarded. Then, the nixtamal was washed twice with tap water in order to remove leftover pericarp tissue and excess lime. The clean nixtamalized grains were stone-milled (Nixtamal mill,1HP with stones of 5”) into masa. During the milling process, small quantities of water were added to avoid overheating and increase the masa content to its optimum level (56-58% moisture). Finally, the masa was shetted and cut into round tortillas using a commercial tortilla machine (VillamexModel / V25 CB, Aidex, Mexico).The equipment was adjusted to generate 2 mm thick tortillas of 16 g (±1) with a diameter of 10.5 cm. The round masa pieces were continuously baked into tortillas in a three-tier baking oven set at 178ºC -180° C. The average dwell or residence baking time was 59 sec. After baking, tortillas were left to equilibrate or cool down to room temperature (23ºC) for 30 minutes. Tortillas were divided into different batches: batch 1 was left to dry at 50ºC in a convection oven (Europan Unox) and weighed until reaching a constant weight. The dehydrated tortillas were ground for the preparation of experimental diets for animal studies. The second batch of tortillas was packaged in sealed plastic bags for 5 days at room temperature (22ºC) whereas the third batch stored at 3-4ºC in sealed plastic bags for further texturometer and sensorial analyses. Furthermore, tortillas belonging to batch 3 were placed at -20ºC for chemical characterization (Espinosa-Ramírez *et al*., 2020).

3.5 Tortilla characterization and rheological properties of masa and tortillas

Masa texture was evaluated using the TVT 6700 Texture Analyzer (Perten Instruments) employing the compression-tension test reported by Espinosa-Ramírez *et al*. (2020), with modifications. Samples were compressed to 65% of their original height with a cylinder probe (75 mm diameter, aluminum) at initial/retract speed of 1 mm/s, test speed 0.1 mm/s, and trigger force of 10 g. Five replicates of each masa were performed. The parameters evaluated were hardness (maximum force during the compression phase) and adhesiveness (area under the negative curve) (Espinosa-Ramírez *et al*., 2020).

3.6 Color determination

Color was measured employing a colorimeter (Konica Minolta CR-400, Japan). The L*, a* and b* objective color values were assessed on three random places of the tortillas. The color scores (E) were calculated by the following equation:

$$\textbf{Color score (E)} = (L^2+a^2+b^2)^{1/2}$$

3.7 Mixolab masa analysis

Fresh masas were characterized with Mixolab 2 equipment (Chopin Technologies, France). A protocol for tortilla was developed by Espinosa-Ramírez *et al.* (2020) using Chopin+ protocol as a reference. Briefly, 85 g of masa was used, with three temperature stages (30, 95, and 50ºC) and the parameters adjusted as described in Table 2:

Table 2.- Protocol parameters used to assess the rheological properties of masa with the Mixolab.

Mixolab Tortilla 85 protocol	
Mixing speed	80 rpm
Target torque (for C1)	0.678

Dough weight	85 g
Tank temperature	30ºC
Temperature 1st step	30ºC
1st gradient	At C1-x%
Value of x	10%
Temperature 2nd step	90ºC
1st temperature gradient	6ºC/min
Duration 2nd step	5 min
2nd temperature gradient	6ºC/min
Temperature 3rd step	50ºC
Duration 3rd step	5 min

Parameters measured from the Mixolab curve were: water absorption percentage (WA%) or percentage of water required for the masa to reach the target torque, C1 (Nm) or the maximum consistency at phase 1 (30°C), masa development time (min) or time to reach C1, stability (min) or time during which torque was C1-10%, C2 (Nm) or minimum torque during phase 2 (30–60°C), C3 (Nm) of peak torque at phase 3 (60–90°C), the difference among C3 and C4 (Nm) or minimum torque through the heating phase (phase 4, holding at 90°C), and C5 (Nm) or maximum torque reached in phase 5 (cooling from 90°C to 50°C) (Espinosa-Ramírez *et al.,* 2020).

3.8 Tortilla flour production

The dehydrated tortillas from Batch 1 were milled in an electric coffee grinder (KRUPS GX4100, Germany) and passed through a mesh sieve no. 50.

3.9 Total Se determination

Microwave digestion of samples (500 mg) was performed with 10 mL of HNO_3 (77%, metal trace), with a Mars 5 CEM equipment (Matthews, NC, USA) with the next assay parameters: 15 min rising from room temperature to 180°C, then a step of temperature hold for 10 min and finally a gradual temperature drop to 50°C in 20 min. Following, the samples were adjusted to 25 mL with double deionized water and transferred to vials. The concentration of selenium was performed with an X Series2 inductively coupled plasma mass spectrometer (ICP-MS E-448, Agilent Technologies, USA) with a Type C glass concentric nebulizer (Meinhard, MA, USA). The running parameters were the following: Se standard (78Se) was employed and Ar with 7% hydrogen was used as the reaction gas, ion intensity m/z 77 (77Se) was controlled using time-resolved analysis software, purge time of 50s, stabilization time 20 s, peak patron of 3 point with 100 cycles, by triplicate, and acquisition time 18.5 s. Concentrations were displayed as µg of Se/g (Guardado-Félix *et al.,* 2017; Serrano-Sandoval *et al.,* 2019)

3.10 Physicochemical evaluation

The moisture, crude protein, ash and crude fat content were determined according to the methods established by the AACC: 44-15.02, 46-13.01, 08-01.01 and 16-032 (AACC International, 2000).

3.11 Protein fractionation

The fractions for albumin (Alb), globulin (Glo), and glutelins (Glu) were obtained by acid-alkali precipitation reported by Chang *et al*. (2011) and modified by Serrano-Sandoval *et al*. (2019). Briefly, tortilla flours were defatted with hexane (1:4w/v) for 48 h, at 50ºC, and with agitation (200 rpm), with the replacement of hexane every 12 h. Later, 60 g of each flour was mixed with 800 mL of distilled water, for a 2 h stirring. The solution was centrifuged at 10, 000 g for 20 min (Thermo Scientific SL 16R, Germany), and the pellet (remainder A) was separated, while the supernatant was placed in a glass container and precipitated with HCl (1 M) adjusted to pH 4.1. The albumin fraction was obtained after a 10,000 g, 20 min centrifugation. Remainder A was resuspended in 800 mL of NaCl (5%) and mixed for 2 h for succeeding centrifugation; the pellet (remainder B) was resuspended in 800 mL of NaOH (0.1 M), stirred, and centrifuged for later precipitation with HCl (1 M) at pH of 4.8. Following centrifugation (10, 000 g, 20 min) the leftover pellet was recovered representing the glutelin fraction. For supernatant B, it was precipitated with HCl (1 M) until pH 4.3 was achieved, centrifugation (10, 000 g, 20 min) was followed and the pellet represented the globulin fraction. All fractions were kept at least 48 h, at -80ºC in preparation for freeze-drying.

3.12 Determination of total soluble protein content

Soluble proteins were quantified with Coomassie dye (Coomassie Plus Reagent, Thermo Scientific, St. Louis, MO, USA). Summarily, BCA kit (Sigma-Aldrich, USA) was employed for the elaboration of a standard curve (50–1500 µg/mL). Protein stock was prepared and 10µL of the solution was mixed with 300 µL of the dye in a 96-well plate. Absorbance was measured at 595 nm.

3.13 Determination of total starch and α-amylase

Enzymatic kits acquired from Megazyme (Sydney, Australia) were employed for the determination of Total Starch (T-TSTA with a pre-washed ethanol (80%). For α-amylase activity, the Ceralpha method was assayed according to the Ceralpha method (303, Megazyme, 2014), by using non-reducing end blocked ρ-nitrophenyl maltoheptaoside (BPNPG7) as a substrate. Test was carried by triplicate.

3.14 Sensorial analysis

For each treatment, sensorial analysis were performed based on a hedonic scale with the aim of evaluating the overall acceptance with untrained panelists of the experimental tortillas compared to nixtamalized common counterparts. Tests were based on 7 points of a hedonic scale. In addition, panelists were asked three questions regarding consumer perception in terms of texture, flavor and aroma. Tests were performed by 50 people between 25-65 years (Scazzina *et al.,* 2008).

3.15 Estimation of total digestible energy

The digestible energy (DE) of each tortilla was calculated based on their proximal content of starch (S), crude protein (P) and crude fat (F), employing the next formula:

$$D.E= (\%S*4Kcal/g)+(\%P*4kcal/g)+(\%F*9kcal/g)$$

3.16 Statistical analysis

Analysis of variance (ANOVA) was used for the proper analysis of the results. Means were compared using Tukey's test to detect significant differences ($p< 0.05$). All statistical analyses were performed using Minitab® 18 software with a 95% confidence.

Chapter 4

Results

4.1 Germination performance and Se concentration.

Experiments consisted in the comparison of two experimental tortillas elaborated: from Germinated kernels (GT) and Se- enriched germinated kernels (Se-GT) compared to a regular control tortillas (CT) produced from unprocessed grains. Germination process it was achieved after 48 h and the resulting samples at times 0, 6, 12, 24, 36, and 48 after steeping were compared as seen in Fig 5. Finals sprouts for Se-GT had a length of 0.83 (±0.08) mm while GT presented 0.9 (±0.05) mm, with a significant difference between them ($p<0.05$). Nixtamalized kernels are also presented as a reference.

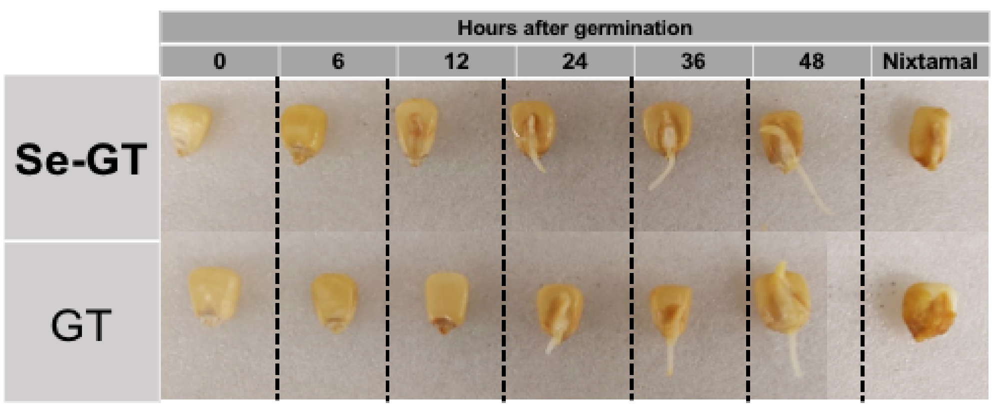


Fig 5.- Maize grains after 0,6,12,24,36, and 48 h of germination, also with the nixtamalized grains. The average sprout length was 0.83 (±0.08) mm for kernels used for Selenium Germinated Tortillas (Se-GT) and 0.90 (±0.05) mm for Germinated Tortillas (GT). Grains after nixtamalization are also presented as a reference.

4.2 Masa and tortilla yield

The yield of masas and tortillas were estimated based on the original grain weight. For Se-GT, there was an increment in 31.3% of biomass from dry kernels to germinated grains, with another 26% increment of weight after sprouted kernels were nixtamalized. The moisture uptake during lime cooking is mainly attributed to the gelatinization of the starch granules. The final moisture content of nixtamal produced from sprouted kernels was around 49% moisture which is within the optimum range for table tortilla production. The use of water during the stone grinding process to produce masas increased the masa moisture content to levels between 57-60% for all groups. All treatments presented no significant difference in terms of masa moisture ($p>0.05$).

Table 3.- Maize before nixtamal (kg), lime cooking time (min) and yield of the treatments after the nixtamalization process. Moisture had no significant difference between treatments with a $p>0.05$ (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas. Sane letters in the same column indicate no significant difference between them with an $\alpha=0.05$)

	DW of maize (kg)	Germinated maize (kg)	Moisture of sprouted maize (%)	Lime-cooking time (min)	Steeping time (h)	Nixtamalized maize (kg)	Moisture Nixtamal (%)	Masa (kg)	Moisture of masas (%)
Se-GT	12.93(±0.32)^{a}	19.7	34.36(±.1)^{a}	4	14	24.88	48.98(±0.12)^{a}	32.1	59.71(±.14)^{b}
GT	13.23(±0.29)^{a}	20.1	34.179(±.33)^{a}	4	14	25.94	49.12(±0.1)^{a}	31.2	57.6(±.0.8)^{b}
CT	1.7(±0.4)^{b}	2.0^{ng}	15.02(±.01)^{b}	4	14	3.34	49.09(±0.2)^{a}	4.3	60.38(±.19)^{a}

For the elaboration of tortillas, Se-GT presented an average masa disc weight of 20.5 (±0.6) with final tortilla weight of 8.27 (±.2), with a final 48.46 % moisture. For GT, masa disc weight as of 22.04g (±0.8) with a final tortilla weight of 9.25g (±.2), with 43.92% of moisture. Finally, CT presented 25.1 g (±1) of masa disc weight with a final 8.81g (±0.44) tortilla weight, presenting a 45.80% moisture content. Finally, yield of tortillas per kg of original corn was estimated, with values of 1.69 for Se-GT, 1.72 for GT, and 1.66 for CT, corresponding with optimal yields. Statistical analysis pointed out that all treatments were significant different between them for masa disc weight ($p>0.05$), while no significant difference was shown with fresh tortilla values ($p<0.05$). For dry weight tortillas, GT was no significant different compared with the other two groups which are significant different among them ($p<0.05$). Finally, CT all groups presented significant difference from each other for moisture content ($p>0.05$) (Table 4).

Table 4.- Masa disc weight and tortilla average weights after nixtamalization process, and yield of tortillas per kg of original corn, with their standard deviation (SD). (DW: dry weight after 24 h, 50ºC. YT: yield of tortillas. Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas. Same letters in the same column indicate no significant difference with an $\alpha=0.05$ and a p value<0.05).

	Masa disc weight (g)	Tortilla "as basis" (g)	Tortilla DW (g)	Moisture of tortillas (%)	YT/kg original corn.
Se-GT	20.5(±0.6)^{c}	15.91(±0.7)^{a}	8.27(±.2)^{b}	48.46(±.3)^{a}	1.69(±.1)^{a}
GT	22.04(±0.8)^{b}	15.72(±1.1)^{a}	9.25(±.2)^{a}	43.92(±.2)^{c}	1.72(±.08)^{a}
CT	25.1(±1)^{a}	15.55(±1.43)^{a}	8.81(±0.44)^{ab}	45.80(±.07)^{b}	1.66(±.04)^{a}

4.3 Chemical composition

The proximate content in Se-GT, GT and CT is presented in Table 5. Briefly; ashes content in dry tortillas had no significant difference between the groups ($p<0.05$). Crude protein was no significant difference between Se-GT and GT treatments ($p<0.05$), but significant different in CT ($p>0.05$). Crude fat and starch was significant different between all treatments ($p>0.05$).

Table 5.- Proximal chemical composition (%) of ashes, crude protein, crude fat and starch for Se-GT, GT and CT values are reported with their standard deviation (SD) (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas. Same letters in the same column indicate no significant difference with an $\alpha=0.05$ and a p value<0.05).

	Ash (%)	Crude Protein (%)	Crude fat (%)	Starch (%)
Se-GT	1.37(±0.17)^{a}	6.50(±0.46)^{b}	2.37(±0.01)^{a}	66.33(±0.3)^{b}

GT	1.22(±0.05)[a]	6.99(±1.08)[b]	1.45(±0.01)[c]	61.45 (±0.04)[c]
CT	1.25(±0.1)[a]	9.15 (±0.65)[a]	1.55(±0.03)[b]	74.48 (±0.01)[a]

During germination time, a concentration of 50 mg/L of Na_2SO_3 was dissolved in steeping water for Se-GT treatment. By ICP-MS determination, a final 0.219 µg of Se pear g of tortilla was calculated; leading to an approximate 3.5 µg of Se for each fresh tortilla. For non-selenized treatment (GT) basal levels of Se were achieved (0.4 µg of Se for each tortilla). In contrast, for reaching the supranutrinional upper limit of Se levels, 100 tortillas should be consumed within 10 days, of the Se-GT(Table 6).

Table 6.- Sodium selenite concentrations during germination process, and Se final concentration in tortillas (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla).

Treatment	Na_2SO_3 (mg/L)	Selenium (µg/g of tortilla)	Se(µg) pear tortilla "as basis"
Se-GT	50	0.219	3.504
GT	0	0.026	0.416

For the quantification of soluble protein fractions, the BCA kit protocol was performed, with a calibration curve (r^2=0.9977), resulting in a value of 27.37% for Albumins, 5.7% of globulins and 31.5% of glutelin, for Se-GT. On the other hand, albumins represented a 33.04%, globulins a 3.41% and 40.93% for glutelin in GT. In addition, each fraction was analyzed for the determination of their Se concentration. Selenium was only detected in Se-GT treatment with a higher concentration in glutelin fraction (17.19 µg), followed by albumin (10.1895 µg) and 1.381 µg for globulins (Table 7). Fractions among treatments were significant different among them ($p>0.05$).

Table 7.- Se-GT and GT concentration (µg/mL) of Alb, Glo, and Glu fractions with their corresponding percentage (%). With a corresponding Calibration curve and interpolate values for Alb, Glo, and Glu fractions of both GT and

Se-GT, with the equation and the r^2 value (0.9977). Quantification of Se for both Se-GT and GT protein fractions were determined; for Se-GT Alb had 10.18 of Se, Glo 1.38 µg and Glu 17.19 µg of Se. For GT fractions, selenium was not detected (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. Alb: Albumin. Glo: Globulin. Glu: Glutelin. Pro: prolamins. Same letters in the same column indicate no significant difference with an $\alpha=0.05$ and a p value<0.05).

	µg/mL	Fraction (%)	µg of Se/g of fraction
Se-GT			
Alb	183.38[b]	27.37[b]	10.1895[b]
Glo	38.55[c]	5.75[c]	1.381[c]
Glu	211.17[a]	31.52[a]	17.193[a]
GT			
Alb	221.39[b]	33.04[b]	Not detected
Glo	22.85[c]	3.41[c]	Not detected
Glu	274.20[a]	40.93[a]	Not detected

The enzymatic activity in sprouted kernels for α-amylase was determined in Units of (U/g) was determined, by the understanding that one (1) unit of activity is the quantity of enzyme required to release 1 µM of p-nitrophenol from BPNPG7 under 60s, by defined conditions specified in the kit. Both GT and Se-GT presented ascended values according to germination time reaching 1.11 and 1.12 U/g respectively (Figure 6)

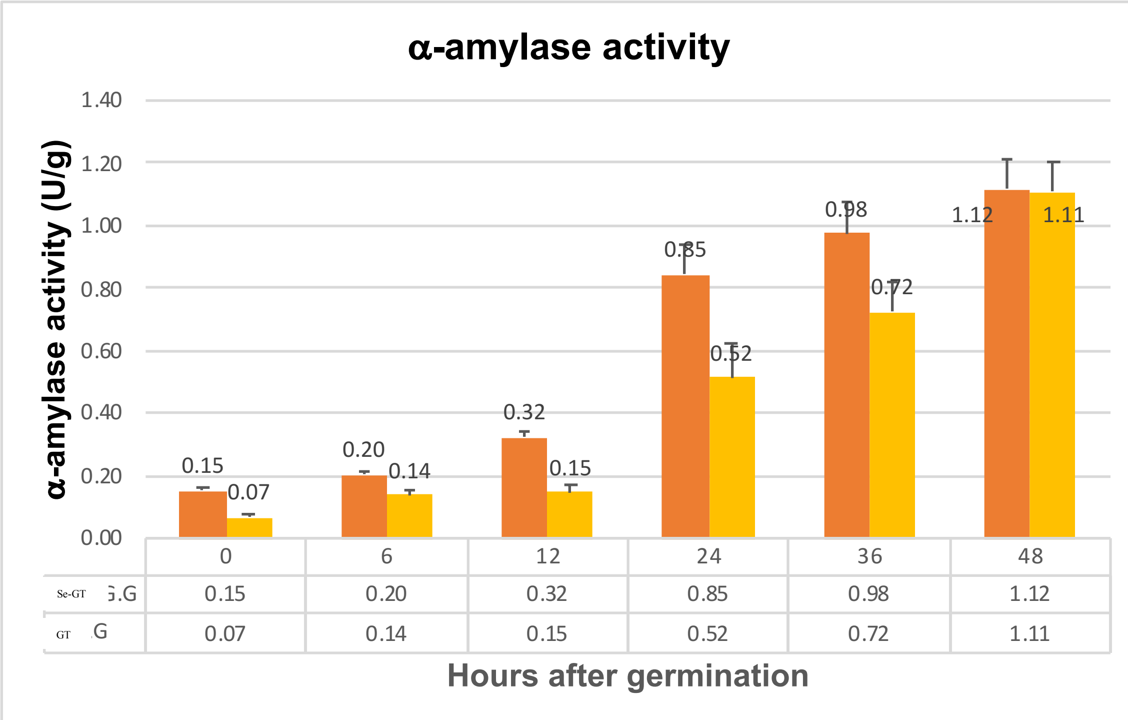


Fig 6.- α-amylase activity (U/g) activity for Se-GT and GT (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla)

4.4 Physical and rheological properties

A complete Mixolab (Chopin Technologies) analysis was performed for each masa treatment. WA corresponded for water absorption (%), or the amount of water required to obtain C1 value, giving an idea for the quantity of water that every masa needs to obtain a given consistency, at stable temperature. C2 measures the protein weakening and its correlation with mechanical effort and temperature. C3 corresponds for starch gelatinization, while C4 measures hot gel stability (Fig 7). Finally, C5 is a direct parameter for starch retrogradation in cooling phase. A Pearson correlation test ($p<0.05$) was executed, same letters in a column correspond for a non-significant difference among treatments (Table 8)

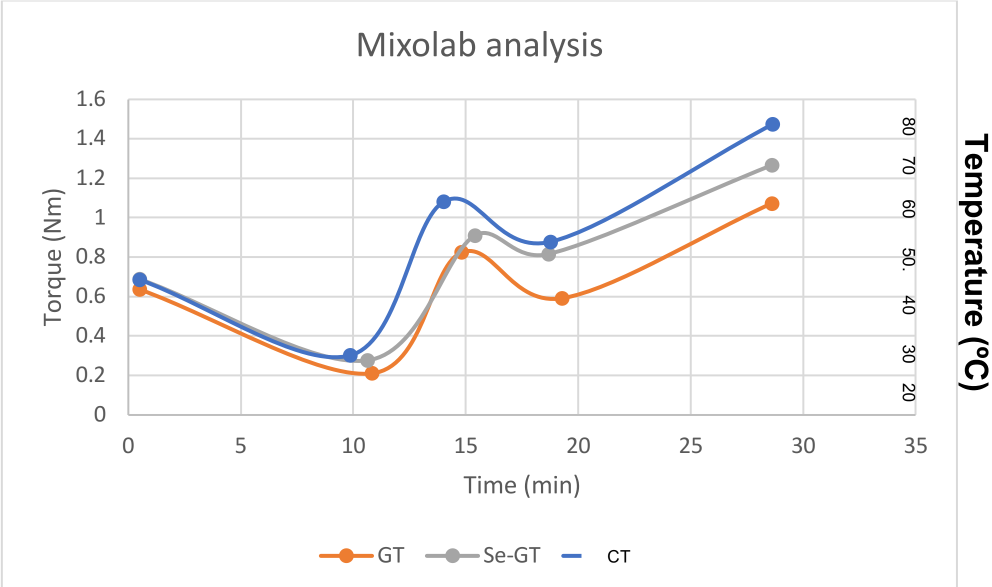


Fig 7.- Mixolab analysis for GT, Se-GT and CT masa. Every point in the graphic represents a torque (from C1 to C5). Similar behavior was shown in all treatments. (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT:Control Maize

Table 8.- Mixolab parameters for Se-GT, GT. Different letters for every mean value represent significant difference among treatments (Se-GT: Selenium Germinated Nixtamalized Maize Tortillas. GT: Germinated Nixtamalized Maize Tortilla. CT: Control Maize. Same letters in the same column indicate no significant difference with an $\alpha=0.05$ and a p value>0.05)

	Minimun torque (C2) (Nm)	Peak torque (C3) (Nm)	C3-C4 (Nm)	C5(Nm)
Se-GT	0.275^{b}	0.915^{b}	0.816^{b}	1.2665^{b}
GT	0.218^{c}	0.825^{c}	0.5915^{c}	1.0725^{c}
CT	0.303^{a}	1.075^{a}	0.8775^{a}	1.4725^{a}

Additionally, texturometer analysis were performed. Firstly for masas, reporting relative Hardness (N), Adhesiveness (N*cm) and subjective machinability (Table 9), with similar behavior with no significant difference between treatments ($p<0.05$) (Fig 8).

Table 9.- Texturometer analysis for masas of each treatment reported with their standard deviation (SD) (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas. Same letters in the same column indicate no significant difference with an $\alpha=0.05$ and a p value<0.05).

Sample	Hardness (N)	Adhesiveness (N*cm)	Subjective machinability
Se-GT	1662.5 (±1.9)[b]	1.09(±0.21)[b]	Good
GT	1451.25 (±0.99)[c]	2.12(±0.45)[a]	Good
CT	1974.062 (±0.39)[a]	0.64(±0.12)[c]	Good

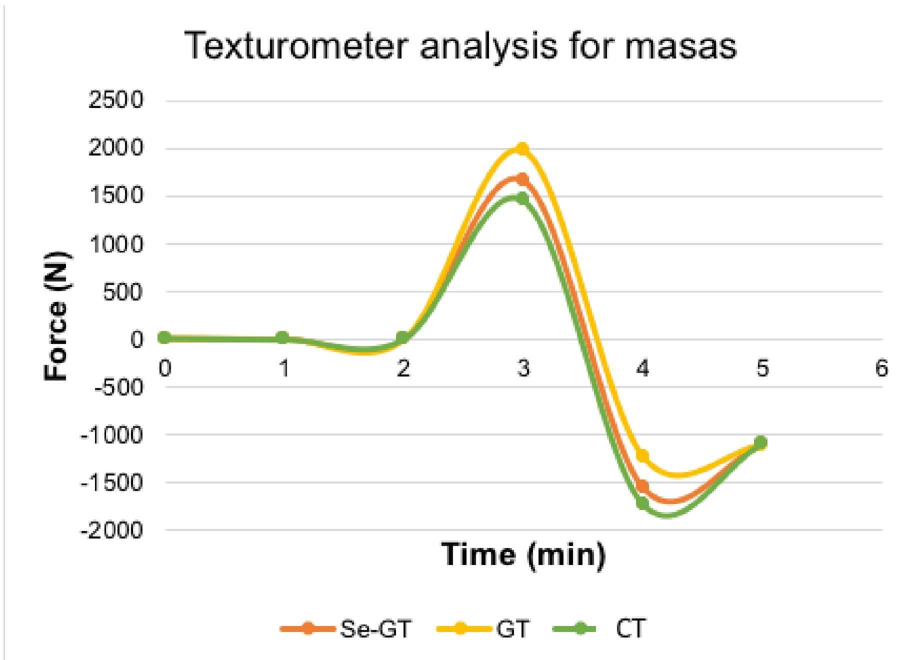


Fig 8.- Texturometer behavior for the masas of each treatment. No significant difference was shown between them ($p<0.05$). (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas)

Later for tortillas, the force break (N) and extensibility (mm) at day 0, 1 and 3 for each treatment was reported. Data was arranged by significant difference among the treatments ($p>0.05$) (Table 10)

Table 10 .- Texturometer analysis for each sample for 3 days, reporting moisture (%), force to break (N) and extensibility (mm) reported with their standard deviation (SD) (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas. Same letters in the same column indicate no significant difference with an $\alpha=0.05$ and a p value<0.05).

	Day 0		Day 1		Day 3	
Sample	Force to break (N)	Extensibility (mm)	Force to break (N)	Extensibility (mm)	Force to break (N)	Extensibility (mm)
Se-GT	395.5(±5.5)[c]	10.036(±1.1)[a]	382.4(±3.2)[a]	6.7(±.65)[b]	321.166(±3.6)[b]	10.835 (±0.57)[a]
GT	551.8 (±7.5)[a]	10.47125(±1.2)[a]	368(±4.0)[b]	6.938(±.69)[b]	292.6(±2.3)[c]	7.397 (±0.37)[c]
CT	424.17(±1.99)[b]	9.318 (±.0.5)[a]	361(±2.9)[b]	7.5675(±1.8)[a]	468.833(±4.3)[a]	8.3825(±1.96)[b]

Color was determined in the space L*, a* and b*; where L* represents luminosity (0= black, 100=white), a* goes from green to red spectra (- to +), and b* from blue to yellow (- to +). Color score (E) was also calculated. All treatments presented a significant difference between them for final color score(p>0.05) (Table 11) (Fig 10)

Table 11.- Color values for Se-GT, GT and CT reported with their standard deviation (SD) (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas. Same letters in the same column indicate no significant difference with an $\alpha=0.05$ and a p value<0.05).

Treatment	L value	a value	b value	Color score (E)
Se-GT	65.69(±0.09)^{c}	3.2175 (±0.35)^{ab}	25.465 (±0.11)^{a}	70.5265(±0.45)^{c}
GT	67.095(±0.10)^{b}	3.555(±0.36)^{a}	25.4225(±0.75)^{a}	71.8378(±0.33)^{b}
CT	70.45(±1.13)^{a}	2.477 (±0.92)^{b}	25.18 (±2.8)^{a}	74.8556(±0.43)^{a}

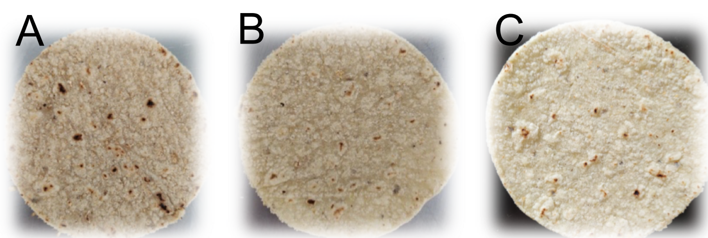


Fig 9.- Tortillas from each treatment: Se-GT (A), GT (B) and CT (C) (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortillas. CT: Control Tortillas).

4.5 Sensorial analysis

As seen above, textural and chemical characteristics are an important factor for human acceptance, based on the previous sensorial analysis based on an hedonistic scale were performed for a complete evaluation of flavor, odor and texture. A group of 30 random non-trained participants with ages between 25-66 were recruited to evaluate each batch of tortillas from all groups. For Se-GT, the 63.33% of participants reported that they like the tortilla, 20% of them slightly dislike it and 10% were indifferent with their characteristics. For GT, 73.3% slightly like the tortillas, with both 13.3% corresponding for

"I like it" and indifferent. As for control tortillas, CT had a 20% of total acceptance, 16.67% like them, 23.33% slightly like them and 10% were indifferent.

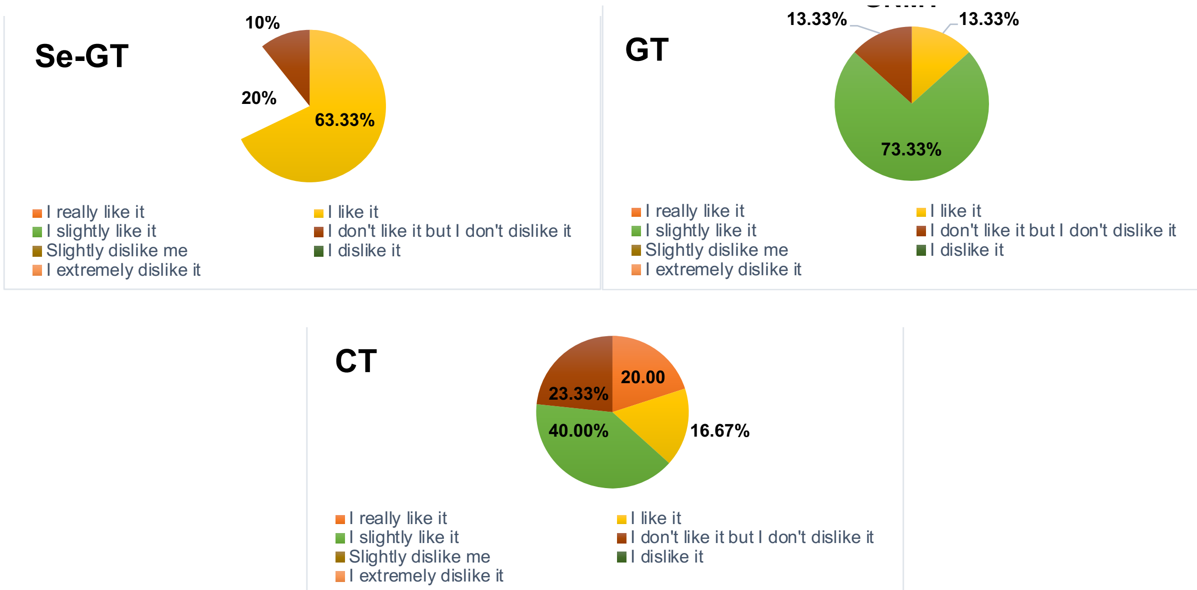
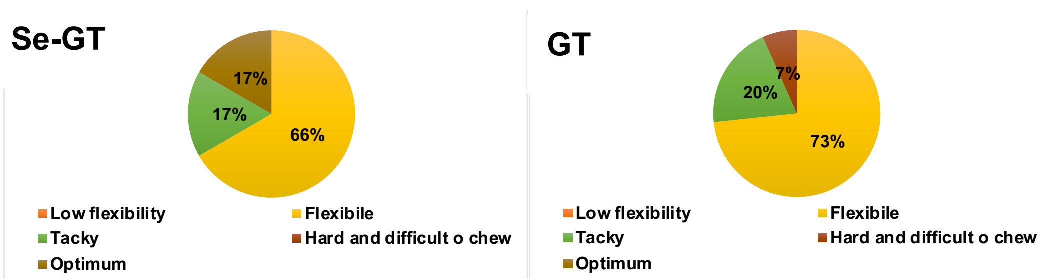


Fig 11.- Sensorial analysis for 4 treatments of tortillas: 2 experimental (Se-GT and GT) and control (CT) , with their corresponding percentage of acceptance (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Maize).

Specifically for texture, participants were asked to describe tortillas on a previously elaborated scale. For Se-GT tortillas, 66% of participants described them as flexible, while 17% for both tacky and optimum texture. For GT 73% described them as flexible, 20% as tacky and 7% as hard and difficult to chew. For CT, 73% reported them as flexible, 17% as optimum and 10% as hard and difficult to chew (Fig. 12)



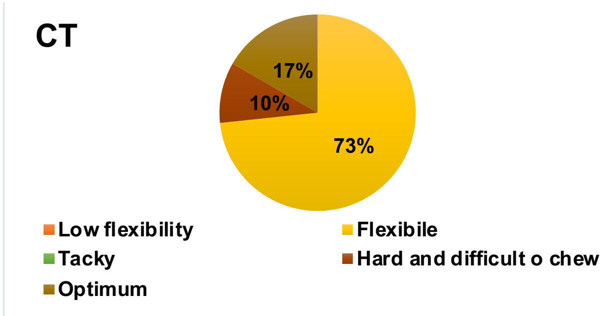


Fig 12.- textural description for each tortilla treatment, with a scale ranged from "hard and difficult to chew" up to "optimum" texture. Reporting the percentage acceptance (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT:Control Tortillas).

4.6 *Digestible energy*

The digestible energy (DE) was calculated according to the formula mentioned above . Calculations indicate that Se-GT diets had total 314.7 kcal/100 g, GT had 286.29 kcal/ 100 g, and CT with 348.47 Kcal/100 g (Table 12).

Table 12.- Digestible energy (DE) for each treatment and in total diet reported with their standard deviation (SD) (Se-GT: Selenium Germinated Tortillas. GT: Germinated Tortilla. CT: Control Tortillas. Same letters in the same column indicate no significant difference with an α=0.05 and a p value<0.05).

	Kcal/100 g
Se-GT	314.7(2.31)^{b}
GT	286.29 (1.89)^{c}
CT	348.47 (1.2)^{a}

Chapter 5

1. Discussion

Maize was chosen due to its importance as the main staple for the nourishment of most of the Mexican population, as well as the broad variety of related foods that can be produced from it, especially tortillas (Bradley, 2019).

According to Audersik *et al*. (2018), the germination process should start in the presence of water within the germ, causing the synthesis of gibberellic acid and finalizes with the radicule elongation, giving origin for a plantule (Audersik *et al*., 2018). In maize, and based on the conditions established by Alimentos Lee®, sprouting started after 8 h of soaking, with water (1:3 w/v), and temperature of 20ºC (1ºC). These conditions triggered the physiology of germination so most kernels had viability with the growth of rootlets and acrospires. Based on the kernel common germination process, 48 h after germination was considered as the optimal time for its further processing. Longer germination times are not recommended because of the over synthesis of amylases that are known to affect the masa rheology and quality of tortillas. There was no significant difference between treatments in terms of sprout length (Fig 5). Selenium was effectively incorporated into the grains during their sprouting, and by then, assimilating inorganic forms of Se and turning it into organic Se (probably Se-Met and Se-Cys) by employing it as a sulfur substitute (Guardado-Félix *et al*., 2019). On sprout length, it has been reported that if the sprout is too large, several factors, including α-amylase activity and protein degradation can occur, generating an undesired and difficult-to-manipulate masa, and by then, poor quality tortillas (Suhendro et al., 1999). As seen in Fig. 6 and Table 3 there was a significant difference in terms of sprout length, where the Se GT showed shorter radicles. Benincasa et al. (2020) recently assessed the sprouting of maize in the presence of Se. These authors reported that Se did not affect germination. Likely the concentration used was optimal and did not affect the osmotic mechanism leading to an elevated cellular turgor and development that translated into optimal sprouting. A noticeable sprout was observed at 24 h after soaking. Previous studies highlight that the concentrations of Na_2SO_3 (100, 150, and 200 mg/L) did not affect grain viability, however, other parameters such as temperature and moisture affect the speed of germination (Agarry et al., 2014; Lazo-Velez et al., 2016). On the other hand, the emergence of the radicle is strongly related to water absorption. Therefore the soaking step was considered as the most crucial to activate the physiology of germination and therefore the incorporation of Se and synthesis of enzymes that in turn affected masa and tortilla characteristics.

The moisture of masas should be not exceed 60% to avoid sticky texture. This particular defect negatively affects machinability because the masa tends to stick to the forming rolls and the discharge of tortilla discs into the moving belt that feeds the oven. On the other hand, moistures below 55% may generate granular and non-cohesive texture increasing the susceptibility of the tortilla discs to beak during conveying and baking, as previously reported by Bothelo et al. (2013) and Espinosa-Ramírez et al. (2020). For tortillas, reports showed that they must contain about 42-48% of moisture in order to enhance their flexibility, softness and reheatability. Interestingly, the tortillas produced with sprouted kernels contained high levels of moisture and adequate textures despite that they were only lime-cooked for few minutes. The utilization of hydrated sprouted kernels containing significant levels of α-amylase significantly reduced the cooking time compared to regular corn. In addition, the tortilla baking temperature was high (350ºF), promoting further starch gelatinization and deactivation of all enzymes. As expected the baking procedure significantly reduced the moisture of tortillas.

Tortillas as the principal staple food and intake caloric source for the national population, so proximate analysis is required in every new tortilla formulation.

The mineral content of nixtamal is affected by the nixtamalization process in two distinctive ways. First, the inclusion of lime during the cooking process enhances the absorption of highly bioavailable calcium whereas the thermal alkaline cooking process hydrolyzes cell walls of pericarp tissue that detaches from the nixtamal during the nixtamal washing procedure. It is well known that most of the minerals are associated to the pericarp and the final concentration greatly depends of the amount removed during lime cooking, steeping and washing steps. (Serna-Saldivar & Chuck- Hernandez, 2019). Also, it is ought to consider that the mineral profile is essential for the optimal nutritional value of any staple food. The content among treatments did not differ significantly, even though the Se-GT contained a slightly higher concentration, presumably by the uptake of both calcium hydroxide and sodium selenite during germination and nixtamalization.

The crude protein concentration is crucial, due to its key role as storage molecules during grain germination, being the main source of amino acids, nitrogen, and sulfur for the synthesis of other important metabolites. The degradation of proteins by the proteases synthesized during germination has been documented for both cereals and legumes like rice, soybean and chickpeas (Liu, et al., 2011; Serrano-Sandoval et al., 2019). Additionally, it is inquired that Se accumulates mainly in the form of proteins due to germination processes, It is known that the inorganic Se absorbed by the germinating seed is used as substrate for the production of Se-proteins by substitute sulfur sites with Se. This translocation yields Se-amino acids like SeMet and SeCys (Hu et al., 2014; Liu et al., 2015; Bianga et al., 2013; Zhang et al., 2019; Serrano-Sandoval et al., 2019; Guardado-Félix et al., 2019). Results herein indicated that there was a significant difference ($p>0.05$) among protein contents being the CT treatment the one containing the highest level. The reduction in protein levels in the experimental treatments can be attributed to the leaching of simpler moieties into the *nejayote* due to the enzymatic activity of proteases.

The crude fat content of maize and tortillas is generally in low concentrations. The nixtamalization process is known to slightly reduce the fat content due to the loss of germ. In general terms, commercial tortillas contain between around 3% fat (dwb). The slight lower fat content is due to losses of seed coat, tip cap, germ, and aleurone layer into the nejayote. The Se-GT and GT slightly differed ($p<0.05$) in terms of crude fat concentration. The lower crude fat levels are attributed to the lime-cooking process, the activation of lipases that generated hydrosoluble fatty acids and the saponification reaction that occurred due to the reaction between Ca ions with carboxyl groups of fatty acids, as reported by Preciado-Ortíz et al (2018) and Chávez-Santoscoy et al. (2016).

In terms of starch, the principal organic compound present in cereals, the different tortillas contained significant amounts of this complex carbohydrate. The lime-cooking process is known to partially gelatinize the starch granules although the calcium hydroxide is known to delays this phenomena due to interactions between Ca ions and amylose as reported by Serna-Saldívar (2021). Also, during the stone milling process, components of the kernel that are bonded with a the protein matrix, are liberated and by that, partially gelatinized by the mechanical attrition. Throughout the stepping step, the starch also starts to create complexes with fat and amylose chains tend to associate due to retrogradation (Bressani 1990). On the other hand, overcooked nixtamal confers a higher water absorption index due to a higher extent of starch gelatinization, producing sticky masas with high adhesivity, ending in final tortillas with low flexibility. During tortilla elaboration, retrogradation is essential because it is known to increase concentrations of type-3 resistant starch. The resistant starch acts as prebiotic because this undigestible type reaches the hindgut where it is readily fermented by the microbiota. O'Callaghan et al. (2012) reported that resistant starch is essential for an optimal colon status, due to the generation of short-chain fatty acids by the effect of fermentation of microbiota which serves as the principal source of energy for colonocytes. The resistant starch is also known to prevents diseases like colorectal cancer and improves the overall health status of individuals (O'Callaghan et al., 2012).

On the other hand, the content of starch is highly related to a-amylase activity, a parameter that should be assessed for cereal-derived products (Aljabi, 2016). It hydrolyzes the 1-4 α glycosidic bonds, mainly in starch, breaking it down to amylopectin and amylose subunits or dextrins that serve as an important source of energy (Waigh et al., 2000; Aljabi, 2016). Like all enzymes, the amylase activity is strongly affected by temperature and the metabolic stage that grains undergo. In the present research, the amylase activity was highly related to germination time. The activity gradually incremented during germination of the Se supplemented and unsuplemented kernels . The supplementation of sodium selenite did not affect this parameter. The lime cooking process inactivated the enzymes while due to denaturation and alkaline pH (Aljabi, 2016). Kernels with high amylase activities are detrimental to the tortilla process because the enzymes change the rheology of the masa and more importantly its machinability. Therefore, the key for the production of tortillas from sprouted kernels is to limit the germination so to prevent excess synthesis of amylases. The other important parameter affected by amylases is the cooking time and the generation of reducing sugars that react with proteins through Maillard reactions. All these effects should be taken into consideration for the production of high-quality Se-enriched tortillas.

As expected, the Se quantification of both experimental tortillas indicated that the Se-GT contained higher amounts of this essential mineral. The supplementation of sodium selenite during the soaking and germination process should be carefully controlled because this mineral will be partially lost during lime cooking, stepping and nixtamal washing. According to the national average consumption of tortillas, the Se-GT should be consumed for 10 consecutive days to reach supranutrinional levels of Se. However, it is ought to consider that daily diets are not only based on a tortilla, and thus, these staple foods can be considered as supplements or adjuvants in some cases. For protein fractionation, the results showed similar concentrations between Se-GT and GT, with Glu at higher rates. Between treatments, Glu fractions have a higher amount of Se, which agrees with a similar finding in chickpea sprouts by Serrano-Sandoval et al (2019) and rice by Fang et al (2010). Following, Alb fractions were the second most Se-concentrated fraction and finally Glo, which showed the lowest relative concentrations as previously reported by Serrano-Sandoval et al (2019) and Fang et al (2010). It is important to point out that several studies focused on Se-enriched crops indicate that Se is mostly found in Glu fractions in the form of SeMet. This organic selenium form is known to be highly bioavailable and less toxic compared to inorganic selenium salts. Results are encouraging because the tortilla technology proposed herein is viable to generate optimal levels of organic Se in tortillas and other foods (Gong et al., 2018; Oliveira et al., 2017; (Liu et al., 2011).

The Mixolab is a dough rheology instrument mainly designed for wheat. However, Espinosa Ramirez et al (2020) recently developed assays to study the rheology of fresh masa and dry masa flours. The assay assesses the rheology changes mainly affected by gluten and starch throughout mixing at different temperatures (Dubat, 2010). The Mixolab protocol applied to nixtamalized masa can effectively detect differences attributed to cooking and particle size distribution even tough maize lacks of viscoelastic gluten. , The first stage of the Mixolab evaluates nixtamalized masa components mainly related to prolamins, and glutelins whereas the second stage to pre-gelatinized starch subjected to high temperature. More specifically, the Mixolab assay evaluated masa properties through five phases: water required for proper dough formation (C1), mixing properties, dough development time, and weakening of proteins by mechanical work and temperature (C2), starch gelatinization (C3), hot gel stability (C4), and starch retrogradation by cooling temperature (C5) (Rosell, Collar, & Haros, 2007). Hydratation was performed until the C1 torque was reached, with a higher WA index associated mainly with dough moisture (41-43%); for all treatments, the WA (%) was the same in order to reduce the experimental error. For C2 parameters, Espinosa-Ramírez et al. (2020) reported values ranging from 0.540 to 0.590 Nm, pointing out a decrease in tortilla consistency due to protein degradation incurred during nixtamalization. In the present study, Se-GT and GT values were even lower with a range from 0.218-0.275, existing a significant difference between them. These values can be explained due to the protease and a-amylase activities observed during the sprouting process, in combination with nixtamalization and thermal treatment for tortilla elaboration. Also, the decrement in granular starch stability plays an important aspect in the stabilization of the protein matrix (Paljwal et al., 2018). As to starch gelatinization, C3 pointed out that there was a significant difference between GT, and CT, with no significant difference when compared to the Se-GT. Again, the nixtamalization process is the main cause for starch gelatinization, with similar values found by Espinosa-Ramírez et al. (2020). Milling is one of the main facts for starch gelatinization in tortilla production due to the partial gelatinization of remanents starch-protein complexes, especially prolamins which are highly bonded with the starch granules. In terms of C3-C4 values, there were significant differences among all treatments, with CT being the lowest with similar values reported for commercial maize flours (Espinosa-Ramirez et al., 2020). Higher values indicate lower viscosity drop, meaning more stability at higher temperatures, which is essential for tortilla production (Almeida-Domínguez et al., 1997; Rosell et al., 2007). Finally, the C5 values related to starch retrogradation and degradation were different among the different doughs, with no significant difference. Enzymatic activity during sprouting, specially α-amylase is the main factor that influence on starch degradation into amylopectine and amylose subunits, given that, lower values of Se-GT and GT can be attributed to starch degradation due to sprouting. Also, lower values for C5 may indicate a high concentration of hydrocolloids in the mixture, forming a matrix between starch granules and proteins, holding water, and by that, delaying starch degradation (Alvarez-Ramirez et al., 2018).

The texture of masas is among the most important features to validate in tortilla production, mainly for large-scale malleability and machinability, and by that, establishing optimum consumer access. Table tortillas should be soft, flexible and easy-to-break especially after storage and reheating (Suhendro et al., 1999). Texture analyses were highly related with tortilla moisture. Chel-Guerrero et al. (2014) reported that moisture is the principal factor tied to masa adhesiveness and machinability. Adhesiveness increased with higher moisture levels and more degradation of starch. Machinability was evaluated as "good" in all treatments indicating that the adjustment in the lime cooking of sprouted kernels was effective. On the other hand, hardness increments with lower moisture, probably related to size particle in doughs and poor cohesiveness between masa components. If an optimal masa is desired, a more extensive lime cooking, nixtamal washing and milling process should be considered in order to generate an adequate starch gelatinization.

The texture of tortillas was analyzed on day 0 (after elaboration), 1, and 3. In addition, the visible growth of molds was observed after day 3. The first estimated value was force to break which was evaluated as hardness of tortillas after ripping strips apart (also related to chewing resistance). As the days passed, a lower break force was reported for all treatments, however, at day 0 higher force was reported in contrast with the ones reported as optimal for tortillas. This was attributed to the degree of starch gelatinization and particle size distribution after milling (Suhendro et al., 1999). Commonly tortillas tend to increment their hardness over the days, however, germination incremented dextrins and glucose, making them more prone to mold contamination. Extensibility, on the other hand, is associated with tortilla rollability and flexibility. It is reported that the extensibility of tortillas increment at room temperature may be caused by the degradation of starch by microbes, while refrigerated tortillas may decrease their extensibility due to staling or retrogradation. Longer lime-cooking and heating times are associated with the damaged structure of starch granules. Gelatinized starch should be thermodynamically unstable and tend to take its original structure under the optimal temperature range (4-20ºC) as reported by Espinosa-Ramírez et al. (2020). Also, retrogradation is a common phenomenon that influences directly textural values. Besides, moisture loss at room temperature enhanced starch retrogradation, mainly between amylose chains. For this reason, hydrocolloids can be added to masa in order to retard retrogradation and bind more strongly water. However, the over supplementation of hydrocolloids may result in an undesired sticky masa texture (Almeida-Domínguez et al., 1997; Suhendro, 1997). Finally, it is relevant to highlight that as a common characteristic for eating tortillas in urban areas, people tend to refrigerate the staple, promoting the development of resistant starch and by that, varying texture, so further studies aiming to assess these freeze-heating effects on tortillas are required.

Color is one of the most important factors for food acceptance by the consumer. For the specific case of tortillas color is the result of the type of maize variety, residual lime, pH and baking time and temperature. Lightness (L* value) significantly differed among tortillas with CT being the lighter. Industrially, lighter colors are desired because is regularly more accepted by the consumer. The tortillas produced from sprouted kernels independently of the usage of sodium selenite had lower lightness values because the proteases and amylases generated peptides and soluble sugars that upon baking generate Maillard compounds. Both Se-GT and GT tortillas had similar a* values, while the control tortillas had a significant difference with values closer to green. Finally, there was no significant difference between b* values, meaning that the three tortillas presented lighter-yellowish colors. To generate optimal colors, prolongation of steeping time and an exhaustive wash after nixtamalization are recommended to improve colors in nixtamal, masa and tortillas (Corrales-Bañuelos et al., 2016; Lozano-Alejo et al., 2017).

The sensorial analysis is usually one of the most critical parts if a product is desired for large-scale production and distribution. Most of the tortillas presented high acceptance organoleptic scores. It is important to point out that while general acceptance in flavor is a favorable response to the incorporation of Se and sprouts into the population, texture scores reported more discrepancy among panelists. Some panelists reported a total disagreement with the chewing properties of tortillas. Further analysis are recommended with tortillas stored at both refrigeration and room temperature in order to better determine differences in color, texture, and overall organoleptic acceptance (Vázquez, 2013).

The digestible energy of tortilla expressed in kcal/100g (Table 14) indicated that the control and Se-GT contained slightly higher amounts of calories compared to the GT counterpart. Values ranged from 286.29-348 kcal/ 100 g of fresh tortillas, which might seem elevated but it would fit in fewer tortillas the average caloric requirements of the Mexican population.

It is undoubtedly that germination in the presence of Se and further nixtamalization process generates changes in physical and chemical properties of maize tortillas, however, these differences did not seem to be of high impact for the development of an optimal and plausible nutraceutical staple food. The tortillas produced from sprouted kernels containing supplemented Se should be more thoroughly studied from the nutrition and nutracetical viewpoints. These new types of tortillas will probably prevent oxidative stress and cancer without losing their caloric and protein densities.

Chapter 6

4. Conclusions and perspectives

a. Conclusions

a. Germinated maize kernels were suitable for tortilla production due to their optimal levels of moisture, proteins, fat, ashes and starch. Results herein clearly indicated that there were not significant differences between Se-GT and GT.

b. Se intake during sprouting could induce the transformation into seleno-proteins and more specifically, into seleno-aminoacids like SeMet and SeCys, crucial molecules for antioxidant protection. The most abundant protein fraction was Glu, followed by Alb and Glo.

c. Se did not seem to affect functional parameters or major properties of the tortilla with the exception of concentration of Se in protein fractions, especially Glu.

d. As expected, amylase activity incremented with sprouting time until reaching 1.12 U/g at 48 h

e. There were significant differences between Se-GT and GT in terms of protein and starch.

f. For masas, there were significant differences in Mixolab parameters C2, C3, C3-C4 and C5 among all treatments. The objective texture of tortillas assessed with the texturometer indicated that the Se-GT was similar to the control CT at day 0.

g. Texture of tortillas were optimal for human consumption, although modifications especially in terms of extent of lime cooking were required to obtain similar tortillas.

h. Tortilla color of both treatments were significant different in color scores (E), although a more yellowish or whitish colorations are desired. Steeping time and extent of nixtamal washing should be performed in order to improve this parameter.

i. Sensorial analyses indicated that both Se-GT and GT tortillas had high acceptance scores independently of the type of treatment.

j. Digestible energy content was significant different between all treatments.

k. Undoubtedly, the Se-enriched tortillas elaborated from germinated maize are promising because they could help to prevent oxidative stress and related ailments, like cancer.

b. Perspectives

i. The standardization for Se concentration during germination is still a crucial factor to the elaboration of enriched tortillas. The level of selenium should be controlled according to the national tortilla consumption in order to avoid over-dosification.

ii. Higher concentrations of Se during sprouting should be studied in order to meet the upper limits for supranutritional Se levels.

iii. The production of nixtamalized instant flours enriched with selenium should be tested in order to offer more options to the consumers.

iv. Perform further studies with an *in vivo* xenografted model in terms of:

1. Evaluation of diet enriched with Se-GT and GT as a weight factor.
2. Assessment of the kinetic growth of xenografted tumors.
3. Assess biochemistry parameters of blood employing a COBAS C-111 equipment (Roche, Germany) especially in terms of glucose, triglycerides, cholesterol , HDL, and LDL.
4. Evaluate GPx and TrxR activities in mice livers in order to find out correlations between Se-intake and enzymatic activity.
5. Finally, study apoptotic metabolic events related to Se metabolic genes aimed to correlate the intrinsic relation of Se with a plausible anticancer activity.

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Published papers

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Curriculum Vitae

Juan Pablo Dávila Vega was born on May 4th 1996, in Mexico City. He earned his bachelor degree in Biology at the Universidad Nacional Autónoma de México on October 2018. Later, he worked as a science teacher and in July 2019, he was admitted into the postgraduate program of Master of Science in Biotechnology at the Instituto Tecnológico y de Estudios Superiores de Monterrey. He joined NutriOmics research group, led by Dr. Sergio Serna Saldívar. Over the last 2 years, his work has been focused on nutraceutical foods, especially with Se-enriched grains and their validation with *in vivo* protocols. In February 2021 he participated at the 50th Research and Development Congress of ITESM, Monterrey Campus.

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