**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Effect of ultrasound technology on structure, physical
properties and lycopene *in vitro* accessibility in guava juice**

Stephanie Suarez Campoli

Dissertation presented to obtain the degree of
Master in Science. Area: Food Science and
Technology

**Piracicaba
2018**

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**Effect of ultrasound technology on structure, physical properties and
lycopene *in vitro* accessibility in guava juice**

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RESUMO

Efeito da tecnologia de ultrassom na estrutura, propriedades físicas e acessibilidade *in vitro* de licopeno em suco de goiaba.

O consumo de suco de goiaba é interessante não apenas devido às suas características sensoriais agradáveis, mas também devido ao seu valor nutricional, em especial às altas concentrações de licopeno. A tecnologia de ultrassom é um método não convencional de processamento de alimentos com grande potencial na industrialização de sucos. A presente dissertação avaliou o efeito do processamento por ultrassom na estrutura e propriedades do suco de goiaba. O suco de goiaba foi processado por ultrassom de alta potência em diferentes condições de processamento, sendo avaliado o efeito na estrutura, concentração e acessibilidade *in vitro* do licopeno e propriedades físicas (sedimentação da polpa, turbidez e cor). Os resultados deste estudo demonstram que o processamento por ultrassom promoveu rompimento nas células de goiaba, liberando seu conteúdo e alterando suas propriedades. Embora o processamento tenha diminuído a quantidade de licopeno no suco de goiaba, a maior exposição aumentou a acessibilidade *in vitro* deste carotenoide. Ainda, a redução do tamanho das partículas dispersas aumentou a estabilidade física do suco, evitando a precipitação de polpa durante o armazenamento, porém sem alteração significativa na coloração. Em conclusão, o presente trabalho demonstrou que a tecnologia de ultrassom pode ser uma alternativa interessante para melhorar as propriedades físicas e nutricionais de sucos frutas, indicando sua importância tanto acadêmica quanto industrial.

Palavras-chave: Suco de goiaba; Acessibilidade *in vitro*; Licopeno; Propriedades físicas; Ultrassom

ABSTRACT

Effect of ultrasound technology on structure, physical properties and lycopene *in vitro* accessibility in guava juice

The consumption of guava juice is not only because of its pleasant sensory characteristics, but also due to its nutritional value, especially the high concentration of lycopene. The ultrasound technology is a non-conventional method of food processing with great potential in the industrialization of juices. The present Dissertation evaluated the effect of ultrasonic processing on the structure and properties of guava juice. Guava juice was processed by high power ultrasound in different processing conditions. The effect on the structure, concentration and *in vitro* accessibility of lycopene and physical properties (pulp sedimentation, turbidity and color) was evaluated. The results of this study demonstrate that the ultrasonic processing disrupts the guava cells, releasing their content and altering their properties. Although this processing decreases the amount of lycopene in guava juice, the consequent higher exposure increased the *in vitro* accessibility of this carotenoid. Furthermore, the reduction of the dispersed particles size increased the physical stability of the juice, avoiding pulp precipitation during storage, but without significant modification in its colour. In conclusion, the present work demonstrated that ultrasound technology can be an interesting alternative to improve the physical and nutritional properties of fruit juices, indicating its importance both academic and industrial.

Keywords: Guava juice; *in vitro* accessibility; Lycopene; Physical proprieties; Ultrasound treatment

RESUMEN

Efecto de la tecnología de ultrasonido en la estructura, propiedades físicas y accesibilidad *in vitro* del licopeno en jugo de guayaba

El consumo de jugo de guayaba es interesante no sólo debido a sus características sensoriales agradables, sino también debido a su valor nutricional, en especial debido a las altas concentraciones de licopeno. La tecnología de ultrasonido es un método no convencional de procesamiento de alimentos con gran potencial en la industrialización de jugos. La presente disertación evaluó el efecto del procesamiento por ultrasonido en la estructura y propiedades del jugo de guayaba. El jugo de goiaba fue procesado por ultrasonido de alta potencia en diferentes condiciones, siendo evaluado el efecto en la estructura, concentración y accesibilidad *in vitro* del licopeno y propiedades físicas (sedimentación de la pulpa, turbidez y color). Los resultados de este estudio demuestran que el procesamiento por ultrasonido promovió un rompimiento en las células de guayaba, liberando su contenido y alterando sus propiedades. Aunque el procesamiento disminuye la cantidad de licopeno en el jugo de guayaba, la mayor exposición aumentó la accesibilidad *in vitro* de este carotenoide. La reducción del tamaño de las partículas dispersas aumentó la estabilidad física del jugo, evitando la precipitación de pulpa durante el almacenamiento, pero sin cambios significativos en la coloración. En conclusión, el presente trabajo demostró que la tecnología de ultrasonido puede ser una alternativa interesante para mejorar las propiedades físicas y nutricionales de los jugos de frutas, indicando su importancia tanto académica como industrial.

Palabras claves: Jugo de guayaba; Accesibilidad *in vitro*; Licopeno; Propiedades físicas; Procesamiento de ultrasonido

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ABBREVIATION LIST

ANOVA	Analysis of variance
BSS	basal salt solution
CDR	completely randomised design
Eq.	Equation
HPLC	High performance liquid chromatography
HSD	honest significant difference
IS	Sedimentation index
LOD	limit of detection
LOQ	limit of quantification
MTBE	Tert-Butyl Methyl Ether
US0min	Juice sample processed by ultrasound for 0 minutes
US3min	Juice sample processed by ultrasound for 3 minutes
US6min	Juice sample processed by ultrasound for 6 minutes
US9min	Juice sample processed by ultrasound for 9 minutes

1. INTRODUCTION

The consumer view in relation to the food consumption is changing. Food is not seen just for eating anymore, but also has an important role to the health, mainly because of the bioactive components present in it (Kussmann *et al.*, 2007; Espin *et al.*, 2007).

Bioactive compounds are natural substances present in small quantity in the food matrix that can bring an biological effect activity (Kitts, 1994). The carotenoids are one example of these bioactive compounds. Besides being natural and fat-soluble pigments, carotenoids have biological activities. It can increase the immune response by the organism, reduce the risk of degenerative diseases; macular degeneration, cataract and cardiovascular diseases for example Astorg, (1997), Matioli and Rodriguez-Amaya, (2003).

The carotenoids can be found in fruits and vegetables with different colours, from yellow to red. Some of the carotenoids most found in foods are α -carotene, β -carotene, γ -carotene, β -cryptoxanthin, lutein, lycopene and xanthophyll. Guava (*Psidium guajava*) is a tropical fruit with high amount of carotenoids, whose interest is growing. According to Rodriguez-Amaya, (1996), the main carotenoids present in guava are lycopene and β -carotene. Consequently, the guava products can be used as natural sources of carotenoids.

The focus on carotenoids properties and benefits for the health and its stability and changes during processing has gaining importance as showed in studies done by several studies such as Anese *et al.*, (2013); Berni *et al.*, (2015); Chandrika *et al.*, (2009); Chitchumroonchokchai *et al.*, (2004). Therefore, the development of different studies regarding the stability and accessibility of these nutrients for the human body is still necessary.

The absorption of substances from food is influenced by many factors, from the human being physiology aspects until the own food matrix; as well as processes properties and conditions. Consequently, different methodologies are being developed to measure the quality of foods in relation to the source of carotenoids. Examples of these are the evaluation of *in vitro* accessibility of each component. The *in vitro* accessibility can be understood as the sum of digestibility and possible assimilation of a compound through *in vitro* simulation (Schulz, 2016). In other words, it is defined as a sequence of steps that mimics the digestion by the

transformation of food into components that can be absorbed by the body into the intestinal epithelium (Fernández-García *et al.*, 2009).

In this context, the absorption of a nutrient must be studied in order to provide knowledge about the food consumption and the improvement of quality of life by the population. Furthermore, it is important to study the effect of processing the food material on its bioactive compounds, in special considering the emerging, or non-conventional technologies.

The ultrasound technology is a non-conventional technology with big potential for fruit products processing. The high power ultrasound is disruptive and induces effects on the physical, mechanical or chemical properties of food (Awad *et al.*, 2012). For example, it is being used to promote desirably changes on structure and physical properties of juices; activate, inactivate or thermal sensitize fruit enzymes; as well as enhances the microbial inactivation (Rojas *et al.*, 2017). Although the number of studies using this technology in food processing is increasing, the effect of ultrasound on bioactive compounds, in special regarding its accessibility and the relation with structural modifications still needs investigation.

For instance, Rojas *et al.*, (2016) studied the effect of the ultrasound technology on the structure, physical properties and stability of peach juice. The authors observed that this technology causes the disruption of the suspended cells following a complex pattern: It is initiated with the breakage of intracellular constituents (organelles), followed by cell damage and release of intracellular content, resulting in disruption of the whole cells and particle size reduction, the dispersion of constituents.

Based on Rojas *et al.*, (2016) results, our hypothesis were built:

- the different steps of cell disruption during ultrasound processing of juices (in special the breakage of organelles, resulting on the dispersion of constituents inside the cells, and the cell damage, releasing the intracellular content to the serum) can affect both the concentration and accessibility of bioactive compounds;
- the releasing of carotenoids to the serum and the decrease of the particle size can affect the carotenoids in two ways: by releasing and exposing the nutrients, the process can facilitate its degradation and/or improving its bioaccessibility. The balance between these two mechanisms will determine the final result.

The present dissertation was based in this context.

2. OBJECTIVES

General objectives:

The objectives of the present work were to evaluate the effect of ultrasound processing on the structure, physical properties and lycopene *in vitro* accessibility in guava juice.

Specific objectives:

The specific objectives were:

- Processing guava juice using the ultrasound technology, considering different amount of added energy (time of processing).
- Evaluating the *in vitro* accessibility of the main guava carotenoids (lycopene through *in vitro* digestion (micelles) during ultrasound processing.
- Evaluating the changes on the juice microstructure during ultrasound processing.
- Evaluating the main physical properties of guava juice during ultrasound processing.
- Correlating the structural modifications with the changes on the different properties.

3. LITERATURE REVIEW

3.1 Guava

Guava (*Psidium guajava*) comes from the *Myrtaceae* family, original from tropical and subtropical regions of America (Manica *et al.*, 2000). Guava is one of the most important tropical fruits, not only because of its aroma and flavor but also because of its nutritional value. Brazil is the largest producer of red guavas and India is in the first place in the production of white guavas (Sebrae, 2016). According to the Brazilian Institute Statistics and Geography, the production of guava in Brazil was approximately 425 tons in 2015 (IBGE, 2015).

This fruit is recognized by a high nutritive value. It is known to be one of the best sources of vitamin C, as well as a high content of sugar, vitamin A and vitamins of complex B and minerals, as well a high amount of fibers (Manica *et al.*, 2000). Furthermore, it contains a high amount of carotenoids, whose interest is growing. According to Chandrika *et al.*, (2009); and Rodriguez-Amaya, (1996), the main carotenoids present in guava are lycopene and β -carotene. Consequently, the guava products can be used as natural sources of carotenoids.

In fact, further then being consumed directly *in natura*, the guava fruit can be industrialized, mainly in the form of pulp, jellies, pasta, fruit in syrup, puree, baby food, base for drinks, soft drinks, juices and syrups.

Therefore, the importance of the juice consumption and the nutritional characteristics of the guava make the study of guava juice with non-conventional processing applicable for the society.

3.2 Carotenoids

Carotenoids are known as a group of fat-soluble and polyunsaturated pigments which have their colors ranging from yellow to dark red, being fruits and vegetables the main sources of this component (Germano and Canniatti-Brazaca, 2004; Krinsky, 2005; Rodriguez-Amaya, 1996). In the nature there are approximately 700 identified carotenoids, of which 10% are present in the human diet (Fernández-García *et al.*, 2012; Krinsky, 2005; Rodriguez-Amaya, 2001). Carotene, α -carotene, γ -carotene, β -cryptoxanthin, lutein, lycopene and xanthophylls are the most common carotenoids in foods (Kohlmeier, 2015;

Rodriguez-Amaya *et al.*, 2008).

Carotenoids can be classified as carotenes and xanthophylls. Carotenoids containing hydrocarbon such as β -carotene and lycopene, for example, are called carotenes. Xanthophylls are those that have oxygenated chemical functions. Among the oxygen groups, the most common are the hydroxyl, keto, aldehyde and epoxide groups, resulting in β -cryptoxanthin, canthaxanthin, β -cyclocurcin and violaxanthin, respectively. In general, they are present in the all *trans* form, which is the most stable, and may appear some *cis* isomers (Rodriguez-Amaya *et al.*, 2008; Rodriguez-Amaya, 1996).

The carotenoids are synthesized only by plants, algae, fungi, yeasts and bacteria, since the animals are unable to synthesize them. Consequently, the animals depend on the food to obtain these substances. However, the carotenoid absorption is selective, and can be converted to vitamin A or only form carotenoids typical of animals (Rodriguez-Amaya *et al.*, 2008).

According to Rodriguez-Amaya *et al.*, (2008), these substances have an important sensorial property, since colour is the attribute that most influences the acceptance of food consumers. However, and more relevant than that, these compounds are considered important in the prevention of a range of diseases (Krinsky 2005; Rodriguez-Amaya, 1996).

This characteristic is attributed to the relevant biological functions and actions, what makes this component among the most important foods constituents, even though they are micronutrient and present in very low levels (Rodriguez-Amaya, 1996; Rodriguez-Amaya *et al.*, 2008; Schulz, 2016). Some of the carotenoids are precursors of vitamin A, others have antioxidant properties strengthening the immune system, reducing the risk of degenerative diseases such as cancer, cardiovascular disease, macular degeneration and cataracts (Rodriguez-Amaya *et al.*, 2008; Rodriguez-Amaya, 1996; Schulz, 2016).

The carotenoids that are found in humans come from their own diet, possessing different functions essential to the maintenance of their physiology. They are, for example, tetraterpenoids (C₄₀) constructed from 5 carbon bond (C₅, isoprene). Because they have a basic, symmetrical and linear structure, they can undergo other reactions, such as cyclization at their tips, diversifying their structure, and consequently originating various carotenoids found in nature (Rodriguez-Amaya, 1999). These compounds are responsible for the colours of red, orange and

yellow tones of the great majority of foods, especially fruits as punctuated previously (Rodriguez-Amaya, 2001; Rodriguez-Amaya *et al.*, 2008).

The content of carotenoids in the fruits is higher if compared to the vegetables (Rodriguez-Amaya *et al.*, 2008)

Due to its high occurrence in food and its bioactivity, β -carotene is the most important provitamin A. It is composed essentially by half molecule of β -carotene with a water molecule at the other end of the chain, for this reason, β -carotene is a potent pro-vitamin A, responsible for 100% of its activity ,besides that, has antioxidant action and it can be found in a widely types of foods (Rodriguez-Amaya, 1996). In fact, some β -carotenes can be converted to vitamin A, performing the function of preventing vitamin A deficiency syndrome, which may lead to decreased incidence of xerophthalmia and also growth disorders in early childhood (Krinsky and Johnson 2005; Ramalho *et al.*, 2001).

Lycopene is an unsaturated, efficient antioxidant carotenoid, and its consumption can prevent both aging and cardiovascular diseases (Agarwal *et al.*, 2000, Böhm 2002; Gärtner *et al.*, 1997). It is responsible for the red colour in the fruits and vegetables, it can be find in high amounts in tomatoes, watermelon and guava for example (Gammone *et al.*, 2015).This pigment does not have pro-vitamin A activity, but its benefices to the human health are mainly in the direct protection against free radicals (Krinsky and Johnson, 2005). It is considered a cellular layer protector by reacting to the peroxides radicals, and mostly, with the molecular oxygen (Moritz and Tramonte, 2006). Summarizing, the proprieties of this pigment are in the prevention of chronic diseases, such as, cardiovascular and in different types of cancer; digestive tract, uterine, breast, skin, bladder and prostate, besides that this pigment can prevent the osteoporosis, hypertension, neurodegenerative diseases, male infertility until transmission of acquired immunodeficiency syndrome from mothers to infants (Giovannucci,1995; Rodriguez-Amaya, 2001; Rodriguez-Amaya *et. al.*, 2008).

The effects of lycopene are attributed to its ability in act as an antioxidant and deactivate the singlet oxygen. This carotenoid protects the human organism against attacks of pathogen responsible for a large number of diseases (Roldán-Gutiérrez and de Castro, 2007). Therefore, it can eliminate free radicals and inhibit oxidation of lipids, it also strengthens the immune system (Gammone *et al.*, 2015). It is an

unsaturated, efficient antioxidant carotenoid, and its consumption can prevent both aging and cardiovascular diseases (Gammone *et al.*, 2015; Schulz, 2016).

According to the studies of Chandrika *et al.* (2009), Padula and Rodriguez-Amaya 1986, Rodriguez-Amaya, (1996) , lycopene is one of the major carotenoid present in guava. Chandrika *et al.* (2009) described the quantity of lycopene for guava as $45.3 \pm 8.0 \mu\text{g/g}$ and β -carotene was around $2.0 \pm 0.2 \mu\text{g/g}$, similar results of Padula and Rodriguez-Amaya, (1986). For this reason, the present study was focused on lycopene.

3.3 Bioaccessibility of nutrients

It is not all the present amount of a nutrient in a food product that the human body can absorb. The proportion of a particular amount of a substance that is absorbed by the intestine and utilized by the body is defined as the bioavailability of that particular substance (Jackson, 1997). The substance should be converted into a form which performs a biochemical function; for this the cellular uptake is required and the substance becomes 'accessible' and it can be metabolized within the cell, available for excretion or simply stored for the future (Mourão *et al.*, 2005).

Therefore, the content of a particular substance present in the food and the bioaccessibility of a nutrient are different parameters. For example, Ball, 1998 explained that processing can reduce the content of a labile substance in foods, but increasing the bioaccessibility of the remaining amount. Therefore, the stability of a substance and bioaccessibility are totally different parameters (Mourão *et al.*, 2005).

In this context, the absorption of a substance is influenced by different factors, which are linked with the individual physiology of the human being until factors related to the food itself (Yonekura and Nagao, 2007). Examples of this are the gastrointestinal disorders or others diseases that cause poor absorption of these compounds, or different processes that can better expose the nutrients, enhancing their bioaccessibility (Ball, 1998; Willcox *et al.*, 2003).

The carotenoids bioaccessibility can be influenced according to the source, amount ingested, combination with another carotenoid, isomeric form, food matrix where the carotenoids are incorporated, the culinary preparation, lipids content, fibers amount in the meal, food processing, among others Garrett *et al.*, (1999), Moritz and Tramonte (2006), Gaino and Silva (2012). For example, cooking can help the release of a certain carotenoid from the matrix, resulting in better

absorption by the body or until its oxidative destruction (Brasil, 2007). Another factor that may affect the accessibility is the presence of some other carotenoids: for example, lutein and β -carotene may decrease the bioaccessibility of lycopene by competition during intestinal absorption of lycopene (Bramley, 2000).

The composition of the diet is a very important factor, the ingestion of other substances such as alcohol and drugs, can affect the absorption. Thus, it is noted that there are a large number of parameters that can influence the bioavailability of a particular compound, with great variation (Ball, 1998).

In this context, processing plays an important role in carotenoids bioaccessibility.

The increased absorption of carotenoids in body tissues may occur through thermal treatment and mechanical homogenization. On the other hand, cooking may adversely affect some beneficial components such as flavonoids, vitamin C and vitamin E. The presence of lipids in the diet and heat-induced isomerization forming more cis isomers and the presence of other carotenoids such as β -carotene may improve the bioaccessibility (Gärtner, 1997; Willcox, 2003).

Therefore, the studying of how the different unit operations and processes influences the carotenoids bioaccessibility is of high importance for Food Science and Technology. Different approaches can be used to evaluate and/or estimate the carotenoids bioaccessibility.

The digestion of carotenoids is a procedure similar to the retinyl esters and fat-soluble compounds, being divided in parts (Failla and Chitchumronchokchai, 2005).

The first step happens when the carotenoids are released from the food matrix by the action of chewing, digestive enzymes and peristalsis from the mouth to the stomach. The physical action of the chewing, the chemistry of the hydrochloric acid from the stomach and the enzymatic action of the amylase, pepsin and lipase are essentials. After the releasing of the carotenoids, they form an emulsion with the lipidic phase (Chitchumroonchokchai *et al.*, 2004; Failla and Chitchumronchokchai, 2005; Garrett *et al.*, 1999).

The following step is the small intestine, where the pancreatic enzymes and the bile act chemically on bolus. Lipid droplets, that increase the enzyme action, are produced by the lumen lipase. The free carotenoids can be formed from the polar

esterified carotenoids by the action of enzymes such as cholesterol esterase and lipase triglyceride. The lipid micelles, those acts in the carotenoids adaptation to the absorption by the absorptive epithelial tissue cells, are formed by the bile salts. After formed, the micelles are dispersed by the chyme soluble phase until the apical surface from the intestinal mucosa. The enterocytes absorb the micelles by passive diffusion. There is no evidence about an existence of a protein existence in the cellular membrane with a specific affinity for carotenoids (Failla and Chitchumronchokchai, 2005).

In the enterocytes is where the conversion of carotenoids into retinol occurs. In the case carotenoids such as β -carotene, it suffers a cleavage within the molecule, with the β -carotene monooxygenase 1-BCMO1 help, becoming two retinol molecules. The 15, 15 monooxygenase and 9,10 dioxygenase can act in other pro vitamin A carotenoids also, by the cleavage out of the molecule center. Next, the lecithin: retinol enzyme forms chylomicrons in the Golgi complex. Those chylomicrons, when entering in the blood plasma, act as vitamin A carries to the liver, where are storage and again secreted into the bloodstream (Failla and Chitchumronchokchai, 2005).

In order to quantify the absorption of carotenoids, different methodologies are being developed, considering both *in vivo* and *in vitro* methods.

Considering the *In vivo* methods, there are the balance techniques such as metabolic, ileostomy, gastrointestinal lavage, plasma response (changes in carotenoids concentration in plasma, appearance-disappearance of carotenoids in plasma), sampling from gastrointestinal lumen after carotenoids ingestion and intestinal perfusion techniques for example (Failla and Chitchumronchokchai, 2005).

Although the *in vivo* methods can describe better the process, they are expensive, complex and time consume. Therefore, different *in vitro* models can be used to estimate the carotenoid uptake.

The *in vitro* digestion models simulate the digestive process in the mouth, stomach and small intestine. Digestion simulation processes, isolated intestinal cells and intestinal segments, microvilli and basolateral membrane vesicles also represent some of these *in vitro* models used for the study of specific characteristics and regulation of complex processes associated with digestion and absorption. These models were used to investigate the effects of chemical speciation, food matrix and processing, and stability of dietary components, accessibility and intestinal transport,

and carotenoid metabolism of foods and supplements (Failla and Chitchumronchokchai 2005).

The *in vitro* methodology can estimate the effect of food matrices on the bioaccessibility of particular compounds. The simulation of the chemical composition of the fluids, pH and incubation periods in each compartment are the principles of this method (Versantvoort *et al.*, 2005). The *in vitro* simulation model can then be understood as a succession of biological reactions that simulate the environment in each of the compartments of the digestive tract where food follows its flow. The conditions of pH, temperature, ionic strength and enzymatic concentrations are simulated and adjusted in each of these structures: mouth, stomach and duodenum. While it is an easy and a simple method to use, it may not be physiologically realistic because it is extremely difficult and complicated to simulate all the complexity of gastrointestinal tract conditions. Some of these physiological factors are for example, the transient chemical conditions, the enzymes used that are not of mammalian origin in most cases, the enzymatic and ionic concentrations, the flow of food through the different compartments and microbiota existing in the body. Thus, the development of *in vitro* digestion models with greater reliability has been studied (Failla and Chitchumronchokchai, 2005; Ferreira, 2004).

For the development of the present work, the *in vitro* digestion was the methodology used and the estimative of the accessibility is described through the *in vitro* accessibility.

3.4 Ultrasound Technology: Principles and Juice Processing

In order to minimize the food undesirable changes due to processing, maximize quality and ensure the safety of food products, the use of the ultrasound technology has been widely studied in food processing. It is an emerging technology defined as sound waves with frequency higher than 20 kHz, which exceeds the human ear. It can be used to food processing, analysis and quality control, depending on the frequency range and power intensity (Rastogi, 2011; Knorr *et al.*, 2011).

Ultrasound is divided into high and low energy (Mason, 1998).

The first one is utilized to disruptive cell, tissues and even molecules, and can affect the physical, mechanical or chemical/biochemical proprieties of foods.

The ranges of energy density used in this case are higher than 1 W/cm^2 and frequencies since 20 kHz until 500 kHz. These results are effective in the food processing, preservation and safety (Knorr *et al.*, 2011). Even its is been used on the microbiological inactivation, it is not efficient by itself, and the combination with other thermal processing is required (Agusto *et al.*, 2018). In fact, the high power ultrasound was proposed to improve the juice consistency (apparent viscosity), cloudy stability and sensory acceptance, as well as enhances the microbial and enzymatic stability and the biocompounds stability (Rojas *et al.*, 2017).

The low power ultrasound is used for non-invasive analysis and monitoring of food compounds, to evaluation of food composition and quality control of fruits and vegetables, for example. The energy density used in this case ranges until 1 W/cm^2 . This emerging technology has been used for controlling microstructure and modifying textural characteristics of fat products, emulsification, extraction of bioactive compounds in food and others, as an alternative to conventional processing (Knorr *et al.*, 2011).

The application of ultrasound in fruit juices is dependent from extrinsic and intrinsic parameters as described by Augusto *et al.*, (2018). They include external factors as environmental conditions and equipment specifications (temperature, US frequency, amplitude and power and time of process). The intrinsic parameters are identified according to the juice matrix and composition. The modifications caused by the ultrasound application could be particle surface erosion, cell breakage, reduction on the particle size and changes on molecular conformation (Augusto *et al.*, 2018).

The advantages of this new technology, according Gallego-Juarez, (2010), is that it is versatile and profitable to the food industry, and it can support large scale operations to the food industry and can be used to different food processes.

As introduced the sonication application has been widely used to promote desirable changes on the physical proprieties of fruits products, as described by Aguilar *et al.*, 2017; Anese *et al.*, 2015; Anese *et al.*, 2013; Bhat, 2016; Bora, 2017; Cassani, 2017; Cheng *et al.*, 2007; Costa *et al.*, 2011; del Socorro Cruz-Cansino *et al.*, 2015; Fonteles *et al.*, 2016; Gomes *et al.*, 2017; Liu *et al.*, 2017; Ordóñez-Santos *et al.*, 2017; Rojas *et al.*, 2016; Saeeduddin *et al.*, 2017; Silva *et al.*, 2010; Tomadoni *et al.*, 2015. It represents very interesting results that are able to help the industry in the sensorial evaluation of physical proprieties of fruit juices.

For example, Rojas *et al.*, (2016), studied the effect of the ultrasound technology on the structure, physical properties and stability of peach juice. The authors observed that this technology causes the disruption of the suspended cells following a complex pattern, which is initiated with the breakage of intracellular constituents (organelles), finally resulting in the disruption of whole cells. Consequently, the juice properties showed a complex behavior: for example, the product consistency was increased, decreased and then increased in relation to the processing time. The authors concluded that the ultrasound technology can be used to improve the physical properties of peach juice, avoiding pulp sedimentation. In fact, Šimunek *et al.* (2013) demonstrated that juices processed with ultrasound had better sensory acceptability than thermally pasteurized juices.

Costa *et al.*, (2011), showed that the US processing reduced the pineapple juice viscosity at the same time that enhanced the juice colour and stabilization along 42 days of storage. Aadil *et al.*, (2015) studied the ultrasound processing of grapefruit juice, detecting that this technology did not impact the product pH, °Brix and acidity. At the same time, it resulted in changes on colour, cloudiness and antioxidant capacity due to bioactive compounds.

Anese *et al.*, (2013) described sensorial benefits of ultrasound processing of tomato juice, as improvement of gel-like proprieties, for example. Furthermore, Anese *et al.*, (2015) did not observe any degradation of lycopene after ultrasound processing of tomato juice.

In fact, Zinoviadou *et al.*, (2015) and Aguilar *et al.*, (2017) described that many published works reported an increase in juice bioactive content after ultrasound processing. As these molecules are not synthesized during processing, this effect was attributed by Aguilar *et al.*, (2017) to the disruption of juice cells, releasing intracellular components, increasing its extractability and, thus, the value obtained in the assays.

Therefore, by changing the juice structure, the ultrasound can affect the bioactive compounds in two ways: by releasing and exposing the nutrients, the process can facilitate its degradation and/or improving its bioaccessibility – highlighting the importance of the present study.

4. MATERIAL AND METHODS

4.1 Sample preparation

The guava juice was obtained from industrial pasteurized guava pulp diluted in distilled water (15% of pulp w/w). The juice characteristics were pH 3.99 ± 0.01 and 2.5 ± 0.2 °Brix. To avoid microbial growth during storage, potassium sorbate ($1000 \text{ mg}\cdot\text{kg}^{-1}$) was added to the juice for the analysis of sedimentation, turbidity and colour along the storage time, as described by Kubo *et al.*, (2013) and Rojas *et al.*, (2016).

4.2 Ultrasonic processing

The ultrasound processing was carried out as described by Rojas *et al.*, (2016). The reactors containing 200 mL of juice were placed in the ultrasound probe (ECO-SONIC, QR1000 Model, Brazil) with a maximum nominal power of 1000 W, frequency of 20 kHz and using a 1.26 cm^2 titanium tip. The applied intensity and volumetric power were $15 \text{ W}/\text{cm}^2$ and $121 \text{ W}/\text{L}$, respectively. The temperature was controlled at 25°C . Based on pre-tests, the ultrasonic processing was conducted until 9 min, obtaining the following samples: US0 (control); US3 (3 minutes of processing); US6 (6 minutes of processing) and US9 (9 minutes of processing). After processing, the samples were stored for 14 days at 25°C for the evaluation of physical properties. The treatments were carried out in triplicate.

4.3 Structure and physical properties evaluation

In order to evaluate the effect of ultrasonic processing on the physical properties of guava juice, analysis of microstructure, pulp sedimentation, turbidity and colour were performed, based on the works of Anese *et al.*, (2015); Kubo *et al.*, (2013); and Rojas *et al.*, (2016).

4.3.1 Microstructure

The juice microstructure was evaluated by optical microscopy (model L100, Bioval, Curitiba Brazil), using a 10x objective and a digital camera (Q-Color 3 OLYMPUS America INC, including the SQ Capture 2.90.1 Ver. 2.0.6 Software, Canada). The samples were diluted ten times and dispersed on a glass slide with a

coverslip. The images were captured for each sample after assuring being representative.

4.3.2 Pulp sedimentation

The pulp sedimentation was evaluated over storage at 25°C (BOD TE- 391, Tecnal, Brazil) during 14 days. The guava juice was placed in 25 mL graduated cylinders and observations were done each hour in the first 6 hours after processing, each 12 hours for the following 7 days and each 24 hours between the 7th day and the 14th day of storage. The sedimentation index (IS in %) was calculated as described by Silva *et. al.*, (2010), according to Equation 1, where S(t) is the sediment volume measured along the storage time (t) and V is the total volume of the sample.

$$IS\% = \frac{S(t)}{V} \cdot 100 \quad (\text{Eq. 1})$$

4.3.3 Turbidity (Serum cloudiness)

The turbidity (serum cloudiness) was evaluated during storage. Samples (6 mL) were placed in 15 mL falcon tubes and centrifuged during 5 minutes at 1330 g at 5°C (ROTINA 420R refrigerated centrifuge, Hettich, England). The supernatant was placed into 3 mL cuvettes and analyzed by the UV-visible spectrophotometer (FEMTO spectrophotometer 600s, Brazil) under 660 nm, as describe by Rojas *et al.*, (2016). The evaluation was conducted daily for the first 3 days, and after 7 and 14 days of storage.

4.3.4 Instrumental colour

The colour was measured once a day in the first three days and after 7 and 14 days of storage at 25°C. The colorimeter (Konica Minolta, Chroma Meter, CR-400, Mahwah, NJ, USA) was used with a measurement of area of 64 mm², observation angle of 10° using the D65° illuminant and applying the CIELab technique (where, L* corresponding to lightness, a* corresponding to the redness (green to red) and b* corresponding to yellowness (blue to yellow)). An aluminium cylinder container (6.5 cm of height and 3.7 cm of diameter) was filled with 5 mL of guava juice and three readings were performed for each sample, after calibration with standard white porcelain. The total colour change (ΔE) over the storage time was evaluated according to Equation 2.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (\text{Eq. 2})$$

4.4 Carotenoid in vitro accessibility

4.4.1 Carotenoid extraction

The methodology described by Tibäck *et al.*, 2009, with adaptations, was used. Standards of β -apo-8'-carotenal and lycopene (purity ≥ 95) was purchased from Sigma® (St Louis, MO, USA). The reagents used were chromatographic grade solvents: n-hexane 95% (Tedia Company, Inc. Fairfield, OH, USA), ethanol (Quimica moderna, Brazil), acetone (LS Chemicals, Brazil), Methanol (Tedia Company, Inc. Fairfield, OH, USA) and tert-butyl methyl ether (Loba Chemie Pvt, Ltda, Colaba, Mumbai).

The samples were immediately frozen after ultrasonic processing and stored at -26°C until carotenoids quantification analysis and at -80°C until *in vitro* digestion simulation followed by carotenoids quantification in the different phases. The samples were then thawed in a water bath (Cientec, Model Ct 265, Piracicaba, Brazil) at 25°C just before extraction.

The carotenoid extraction was conducted in 50 mL centrifuge tubes, containing the sample (aliquots ranged from 0.2 to 0.5 grams of juice, 3-5mL of digesta and 5-9mL of accessible phase), solvent (15 mL of hexane:ethanol:acetone, 2:1:1 v:v, with 0.1% of butylated hydroxytoluene - BHT) and salt solution (5 mL of saturated NaCl solution: 36 g NaCl / 100mL of distilled water). The tubes were agitated in the vortex for 30 seconds (NOVA instrumento, Model NI 1055, Piracicaba, Brazil), then placed during 10 min in an ultrasound bath (Unique- Ultra Cleaner 1400, Frequency 40kHz; $135\text{W}/\text{cm}^2$; 80VA, Brazil) to guarantee the guava cells disintegration to improve the extraction, and then back to the vortex for 1 min. After that, the tubes were centrifuged for 5 minutes at 800 g and 4°C . The supernatant was collected with a pipette, placed in 20 mL glass flasks and dried under a stream of nitrogen gas. This procedure was conducted until the sample become colorless, indicating the complete extraction of carotenoids.

The dried extract were dissolved in methanol and MTBE (1:1, v:v), whose total volume depend on the carotenoid concentration (0.2 – 4 mL); it was filtered in 0.22

μm pore size (Millex[®] PTFE) and add to vials. Finally, the vials were placed in the HPLC-DAD system for injection.

4.4.2 Identification and quantification of carotenoids by high performance liquid chromatography (HPLC)

High performance liquid chromatography analysis was conducted using a Shimadzu 20A system (SHIMADZU, Japan), equipped with a pumping system (model LC-20AT), automatic injector (SIL-20AHTU), column oven (CTO-20A), communicator (CBM-20A) and diode-array detector (DAD). The column used was the polymeric YMC[™] C₃₀ (150mm x 4.6 mm, 5 μm particle size) maintained at a constant temperature of 30 °C. The mobile phase were 90% methanol and 10% of tert-butyl methyl ether, with flux equal to 0,8 mL.minute⁻¹. The volume of injection ranged between 20 μL to 80 μL . The wave lengths used were 450 nm (for β -carotene detection) and 470 nm (for lycopene detection) as describe in the literature Rodriguez-Amaya and Kimura, (2004). The chromatograms were analysed using Lab solution LCGC, Shimadizu, Japan software.

The identification of the major carotenoids was carried out according to Rodriguez-Amaya, (2001), using standards of β -carotene and lycopene, evaluating the absorption spectrums, the maximum absorption wave length (λ_{max}) and its fine structure (%III/II).

The calculation of the limits of detection and quantification (LOD and LOQ) were carried out according to Aragão *et al.* (2009) and Melo *et al.* (2015). The LOD value were 0,36 $\mu\text{g.g}$ and LOQ value were 1,09 $\mu\text{g.g}$.

4.4.3 Carotenoid in vitro accessibility

The lycopene *in vitro* accessibility was evaluated after simulated oral, gastric and small intestinal digestions, as described by Berni *et al.*, 2015 and represented in the Figure 1. The enzymes were purchased from Sigma (St Louis, MO, USA) and each enzyme specifications are described as follows.

Figure 1: Schematic representation of the in vitro digestion assay.

The samples were weighted (2.5 g) and homogenized with 10 mL of basal solution (BSS: NaCl 120 mol/L, CaCl_2 6 mmol/L and KCl 5 mmol/L) in 50 mL falcon tubes. The same BSS was used to volume adjustment, when required.

The oral digestion was conducted according to Oomen *et al.*, (2003), by adding 1.11 units/mL of artificial saliva containing 106 units/mL of α -amylase (Sigma[®] A3176- Type VI-B), and placed inside of an incubator (Laboven, model SLLB033, USA) at 37°C for 10 min in a shaker with orbital agitation (120 rpm).

After this, the pH was adjusted to 2.5 with HCl 1 M and 10 units/mL of pepsin solution (Sigma[®] P7000; 50000 units/mL in HCl 100 mM) was added to simulate the gastric digestion. The volume was adjusted to 40 mL with BSS and the tubes were placed in the incubator for 1 h at 37°C and 120 rpm. The gastric digestion was interrupted by raising the pH until 6.0 with NaHCO_3 1 M, in order to conduct the small intestinal digestion.

The intestinal digestion was then performed by adding 1.6 units/mL of porcine bile extract solution (Sigma[®] B8631; in 100 mM NaHCO_3), 40 units/mL of pancreatin (Sigma[®] P1750; in 100 mM NaHCO_3) and 5 units/mL of lipase (Sigma[®] L3126; in 100 mM NaHCO_3). The pH was adjusted to 6.5 and volume to 50 mL, before incubation for 2h at 37°C.

Finally, the digested fraction (also called digesta, D) was obtained, that corresponds to the final suspension after digestion, comprising the carotenoids released from the food matrix with the enzymes used in the *in vitro* digestion.

Then, aliquots of the digested fraction (15 mL) were homogenized (on a shaker) and then centrifuged at 5000 g for 60 min at 4°C. The supernatant was collected and passed to a new centrifuge tube (15 mL), resulting in the accessible fraction, which corresponds to the carotenoids available for absorption. The samples in the 15 mL falcon tubes were covered with nitrogen gas, sealed with parafilm® and stored at -80°C until its extraction and HPLC analysis.

The *in vitro* accessibility index (%) was calculated by Equation 3, where the concentration of carotenoids in the accessible fraction is divided by the concentration of carotenoids in the initial sample, expressed in %.

$$in\ vitro\ accessibility\% = \frac{C_{accessible\ fraction}}{C_{initial\ sample}} \times 100 \quad (Eq. 3)$$

4.5 Experimental design and statistical analysis

A completely randomised design (CRD) was used. The analysis of variance (ANOVA) was conducted using a significant probability level of 95% ($p < 0.05$) and the Tukey's HSD (honest significant difference) post-hoc pairwise comparison test determined significant differences between mean values. The software IBM SPSS Statistics 23 (IBM SPSS, USA) was used in order to analyse the data. A non-linear regression and the Software STATISTICA 7 (StatSoft, USA) were used when required.

5. RESULTS AND DISCUSSION

5.1. Juice structure

Figure 2 shows the microstructure of guava juice after 0, 3, 6 and 9 min of ultrasound processing. These pictures are representative of each process, being selected after evaluation of a range of 100 pictures of each treatment and juices replicates.

The juice is composed by the pulp and the serum phases. The pulp is the dispersed phase, composed by remained whole cells and its fragments due to tissue and cell disruption during processing. The serum phase contains water and the intracellular soluble components – such as sugars, minerals, acids, etc. As described by Gil, (2002), the lycopene is localised within the cell in the plastids, such as chromoplasts, and it can be identified in Figure 2 due to its typical colour.

The control juice (US0) still exhibit whole guava cells with intact walls. The carotenoids can be seen within the structure, inside of the chromoplasts. After 3 minutes of ultrasound processing, similar structures to the control juice can be seen. Even that, the intracellular compound is slightly released to the serum. As described by Rojas *et al.*, (2016), the first effect of fruit cell disruption during sonication is the movement of the intracellular components. It can explain the first release of carotenoids after 3 minutes of ultrasound application (Figure 2). Figure 2 show the guava juice after 6 and 9 min of ultrasound processing, respectively. Both figures revealed a clear difference in comparison to the control juice. After 6 min of sonication, the cell rupture is evident, releasing carotenoids to the serum. Even so, it still shows some intact cells. On the other hand, the guava juice after 9 minutes of processing present only empty cells and their fragments, with the intracellular compounds dispersed in the serum. Several authors observed Anese *et al.*, (2015); Anese *et al.*, (2013); Rojas *et al.*, (2016) similar patterns for peach juice and tomato paste.

These results suppose that the releasing of carotenoids to the serum and the decrease of the particle size can affect the carotenoids in two ways: by releasing and exposing the nutrients, the process can facilitate its degradation and/or improving its bioaccessibility – highlighting the present study.

For example, considering others non-conventional technologies with similar principles (cavitation and high shear), some comparisons can be carried out with the high pressure homogenization process (Rojas *et al.*, 2016). Another study done by Silva *et al.*, (2010) applied the homogenisation process (0, 300 and 600 bar) in pineapple pulp. The untreated pulp presented larger particles suspended in the pulp in comparison to the pulp after being processed. The process broken down the particles, reducing the size and increasing the numbers of small particles, resulting in a new structure. This structure contained fibrous particles, cells, cells wall, fragments and polymer. Similar results were observed by other authors and products. Further, Kubo *et al.*, (2013) reported the lycopene release after cell disruption by the high pressure homogenization (HPH) processing of tomato juice, which exposed this carotenoid, resulting in its degradation by the dissolved oxygen. On the other hand, by exposing the lycopene, its bioaccessibility can be increased – especially considering the ultrasound technology, which promote the sample degassing.

In fact, Anese *et al.*, (2015) observed a decrease on the lycopene *in vitro* accessibility due to the oil addition either in the untreated and ultrasonic treatment (probe, 24 kHz, 105 W/cm² up to 60 min) of tomato pulp. The authors attributed this behaviour to the increase in the product consistency, which was claimed to be a result of a new network formed by new interactions among the pectin molecules. However, it is important to highlight that each food matrix may respond in a different and unique way to the ultrasound processing, due to changes and interactions among process conditions and food structure and composition Rojas *et al.* (2016). For example, Rojas *et al.*, (2016) demonstrated a complex behaviour for peach juice during ultrasonic processing, with consecutive steps of consistency increase and decrease due to the different steps of structural modifications. Further, Soria and Villamiel, (2010), discussed that the consistency of a food product can be changed permanently or temporarily, either increasing or decreasing, depending on the ultrasonic condition. Consequently, the behaviour for the guava juice must be evaluated.

Figure 2: Optical microstructure of guava juice: control sample (US0min) and those processed with ultrasound by 3 (US3min), 6 (US6min) and 9 (US9min) minutes. The scale bar represents 100 μm .

5.2 Serum cloudiness

Figure 3 shows the effect of the ultrasonic processing on the serum cloudiness of guava juice throughout 14 days of storage at 25 °C. The ultrasound processing increased the serum cloudiness, while the storage time slightly reduced it, with the same behaviour for all the samples.

As described by Rojas *et al.*, (2016) an increase in the serum cloudiness indicates a higher amount of small particles in the serum, which did not precipitated during centrifugation. It is in accordance with the structural results here presented, as the ultrasound technology disrupted the suspended particles, reducing its size. On the other hand, the reduction in the serum cloudiness during storage indicates the

formation of particle aggregates due to non-hydrodynamic forces (electrostatic, Van der Waals), which precipitate during centrifugation (Kubo *et al.*, 2013).

Cheng *et al.*, (2007) studied the guava juice processing by using ultrasound and carbonation, observing a small serum cloudiness after processing, which was attributed to the higher number of fine particles retained in the supernatant.

Rojas *et al.*, (2016) studied the effect of ultrasound processing on the structure and properties of peach juice. The authors described a complex behaviour of serum cloudiness during ultrasound processing, which were attributed to the different reported steps of cell disruption. Therefore, the obtained results are in accordance with the literature.

Figure 3: Effect of the ultrasonic processing on the serum cloudiness of guava juice throughout 14 days of storage at 25 °C: control sample (US0min, x) and those processed with ultrasound by 3 (US3min, ■), 6 (US6min, ▲) and 9 (US9min, ◆) minutes. Vertical bars are the standard deviation for each value.

5.3 Pulp Sedimentation

The pulp sedimentation of guava juice processed with ultrasound throughout 14 days of storage at 25 °C is shown on Figure 4.

While the control juice presented a sharply precipitation of pulp even in the firsts hours, reaching a ~50% of precipitation after 2-4 days of storage, the juice processed with ultrasound for 9 min did not present any pulp precipitation even after 14 days of storage.

The juices processed with ultrasound at intermediate conditions (3 and 6 min) presented intermediate results. But it is highlighted that even the juice processed for 3 min presented only ~15% of pulp precipitation even after 14 days of storage.

The IS index was modelled as a function of storage time (at 25°C) using an exponential decay function as showed in Table 1 (the sample processed for 9 min did not show pulp precipitation and therefore it was not evaluated). It can be noticed that the ultrasound technology reduced not only the amount of precipitated pulp, but also its rate. While the precipitation kinetic parameter (K) was 10.47 days⁻¹ for the control juice, it dropped to 1.78 days⁻¹ for juice processed for 3 min and 0.93 days⁻¹ for the juice processed for 6 min.

Rojas *et al.*, (2016) studied the effect of the ultrasound on the physical properties of the peach juice. They compared the pulp sedimentation among ultrasonic processed juices for 0, 3, 6, 9 and 12 min. The pulp sedimentation was avoided during the storage time (21 days) for juices starting from 6 min of ultrasound application. It is interesting to notice that the pulp precipitation kinetic parameter (K) of the peach juice was 1.38 days⁻¹ for the control juice, which is only ~10% the value for the guava juice here reported. Therefore, the guava juice presented a tendency to pulp precipitation much higher than the peach juice reported by Rojas *et al.*, (2016). Even so, the ultrasound technology was efficient to eliminate this undesirable reaction during storage, demonstrating how interesting this technology is to avoid fruit pulp precipitation.

Kubo *et al.*, (2013) presented similar results when studying the effect of high pressure homogenization (HPH) on tomato juice. The non-processed samples showed a quick pulp sedimentation than samples that passed by the equipment at 0 MPa (gauge pressure), which was attributed to the possible disruption of the large particle-aggregates. For juices processed at higher pressures, any pulp sedimentation was observed.

The obtained results, a consequence of the disruption of large particles caused by the processing, reinforces the possibility of using the ultrasound technology to enhance the pulp stability in fruit juices. In fact, as described by Rojas *et al.*, (2016), the ultrasound technology can replace the use of hydrocolloids, which is desirable for many reasons, such as meeting the consumer demand (to reduce the use of additives), simplifying the process (as the hydrocolloid dissolution is a complex

step during processing) and reducing the number of ingredients (with both logistic and economic gains).

Figure 4: Effect of ultrasonic processing on the pulp sedimentation of guava juice throughout 14 days of storage at 25 °C: control sample (US0min, ♦) and those processed with ultrasound by 3 (US3min, ♦), 6 (US6min, ♦) and 9 (US9min, ♦) minutes. The dots are the mean values, the vertical bars are the standard deviation and the dashed curves are the models described in Table 1.

Table 1: Mathematical modelling of guava juice pulp sedimentation during storage (14 days at 25°C): control samples (US0min) and processed by ultrasound for 3 min (US3 min) and US6 min (US6min). Mean value $\pm$ standard deviation is presented (IS in % and t in days mean values $\pm$ standard deviation).

Model: $IS = IS_{equilibrium} + (IS_{initial} - IS_{equilibrium}) * e^{-K*t}$			
Treatment	US 0min	US3min	US6min
IS equilibrium (%)	47.92 $\pm$ 1.84	85.03 $\pm$ 1.01	91.90 $\pm$ 0.02
IS initial (%)	97.95 $\pm$ 0.37	100.36 $\pm$ 0.64	100.83 $\pm$ 0.51
K (1/days)	9.94 $\pm$ 2.57	1.76 $\pm$ 0.36	0.98 $\pm$ 0.45
R ²	> 0.95	>0.96	>0.97

5.4 Carotenoids identification and quantification through ultrasound processing

Figure 5 shows the chromatogram of the carotenoids present in guava juice. The two main peaks confirm that the major carotenoids present in the guava are β -carotene and lycopene, as described by Chandrika *et al.*, (2009), Rodriguez-Amaya *et al.*, (2008). Even so, the LOD and LOQ for β -carotene in the present work were higher than the observed peaks. For this reason, the measurement of this carotenoid was not carried out and only the lycopene was the focus of this study.

Figure 5: Chromatogram of guava juice before ultrasound processing (US0 min). The β -carotene was detected at 450 nm and the lycopene at 470 nm.

Figure 6 presents the lycopene content in the original guava juice (US0min, control), as well as after ultrasound processing for 3 min (US3), 6 min (US6) and 9 min (US9). During the ultrasound processing, the content of lycopene decrease ($p < 0.05$) from $29,4 \mu\text{g/g} \pm 3,64$ (US0) to $15,18 \mu\text{g/g} \pm 3,58$ after 9 min of processing (US9).

The lycopene degradation by the ultrasound can be due to different reasons, all of them related with the cavitation phenomenon: the high temperatures reached at the hot spots (regions of bubble implosion during cavitation) (Rastogi, 2011), the consequent formation of free radicals (Ulloa, 2015), mechanical effects due to high shear during cavitation (Rastogi, 2011) and/or oxidation due to the oxygen release during the disruption of intracellular structures (Aguilar *et al.*, 2017, Rojas *et al.*, 2016)

Anese *et al.*, (2015), studied the effect of ultrasound processing (probe, 24 kHz, intensity of 105 W/cm^2 up to 60 min) on lycopene contents of tomato paste, and reported no significant changes in the lycopene content. On the other hand, Sun *et al.*, (2017) observed the degradation of anthocyanins from red raspberry after ultrasound processing (probe, 25 kHz, up to 120 W/mL and 60 min), which was attributed to the formation of reactive oxygen species ($\bullet\text{OH}$). Rawson *et al.*, (2011) observed a decrease on the lycopene concentration of watermelon juice after ultrasound processing, listing different possibilities for this degradation, such as heat and oxidation, which can result in fragment products like acetone, methyl-heptenone, laevulinic aldehyde and glyoxal. Oliveira *et al.*, (2015) described that the ultrasound had no direct effect on the stability of pure lycopene in solution. On the other hand, the authors observed a reduction on this carotenoid retention when tomato puree was processed by ultrasound, which were attributed to the formation of hydrogen peroxide. Once again, we highlight that differences can be observed for each food matrix due to changes and interactions among process conditions and food structure and composition.

Considering others processes, Knockaert *et al.*, (2012), observed a decrease of lycopene content in tomatoes puree due to isomerisation during thermal sterilization. On the other hand, several other studies reported any degradation on lycopene due to thermal processing For example, Pérez-Conesa, (2009); Nguyen, (2001); Nguyen *et al.*, (2012), highlighting the differences due to the product-process interactions.

In our study, the processing temperature did not overpassed 35°C. Therefore, the lycopene degradation must be attributed to possible reactions with molecules formed during cavitation as described by Oliveira *et al.*, (2015); Sun *et al.*, (2017), Rawson *et al.*, (2011) and/or by the air released from the vacuole during cellular degradation by the ultrasound (Aguilar *et al.*, 2017; Rojas *et al.*, 2016). In fact, it highlights the complexity of understand the effect of ultrasound processing on each food matrix (structure, composition), as well as in the consequent changes on its properties. Furthermore, not only the lycopene concentration must be evaluated, but also its accessibility.

Figure 6: Lycopene content in guava juice: control sample (US0min) and those processed with ultrasound by 3 (US3min), 6 (US6min) and 9 (US9min) minutes. Vertical bars represent the standard deviation in each value; different letters indicate significant differences ($p < 0.05$).

5.5 Lycopene *in vitro* accessibility

As explained in 4.4.3, in this work *in vitro* accessibility is defined as the lycopene micellization. Figure 7 shows the efficiency of carotenoids micellization, that have influence at accessibility of lycopene after *in vitro* digestion in guava juice processed with the ultrasound technology. Despite the high data variability, the ultrasound processing increased the lycopene *in vitro* accessibility ($p < 0.05$). Therefore, even considering the partial degradation of lycopene during ultrasound processing (Figure 6), the releasing of this carotenoid from the food matrix was increased, exposing this nutrient, which can be responsible for the increase in its

accessibility. This behaviour is in agreement with the literature, being highly desirable.

Anese *et al.*, (2015), studied the effect of the ultrasound treatment, oil addition and storage time on lycopene stability and *in vitro* accessibility of tomato pulp. They observed that the ultrasound increased the lycopene *in vitro* accessibility only when 5% of sunflower oil was added to the system. Any statistical difference was observed in the pulp processed by ultrasound without oil addition. Further, the lycopene *in vitro* accessibility was reduced with the storage time, demonstrating the drawback of better exposing this molecule. As the authors have not described the atmosphere during storage, the described reduction can be related with degradation by oxygen. Consequently, we highlight the need for an adequate packaging system, considering the storage atmosphere, after ultrasound processing.

Once again, some comparisons can be carried out with the homogenization processes, due to the similar structural changes in vegetable products. Tibäck *et al.*, (2009) observed that the application of rotor-stator homogenisation in tomato did not increase the *in vitro* accessibility of lycopene. Svelander *et al.*, (2010) observed an increase in α and β -carotenes *in vitro* accessibility in emulsions after the application of high pressure homogenisation. On the other hand, the *in vitro* accessibility of lycopene emulsions has not changed, probably because the network formed entrapped the lycopene. On the other hand, Kubo *et al.*, (2013) observed lycopene degradation during the storage of tomato juice processed by high pressure homogenization (HPH), which was attributed to its release after cell disruption, become exposed. In fact, Stahl and Sies, (1992) reported that the lycopene degradation due to processing and storage can be due to isomerisation and oxidation reactions.

In fact, there are many variables that can influence the lycopene accessibility, such as the type of processing, increase or decrease in consistency/texture, presence of lipids or fibers, food matrix, among others. Therefore, our results are in agreement with previous studies, taking in consideration the type of processing, where there was lycopene degradation but an increasing on its accessibility due to structural modification. Our results, therefore, demonstrate that the ultrasound technology can be used in guava juice processing in order to increase the lycopene *in vitro* accessibility.

Figure 7: Lycopene in vitro accessibility in guava juice: control sample (US0min) and those processed with ultrasound by 3 (US3min), 6 (US6min) and 9 (US9min) minutes. Vertical bars represent the standard deviation in each value; different letters indicate significant differences ($p < 0.05$).

5.6 Colour

A similar behaviour in relation to the sample colour during storage was observed for both treated and untreated samples in all the colour parameters. Figure 8 shows the colour parameters as a function of processing time and storage time. A small variation in each parameter can be seen, with a negligible effect of ultrasound processing: all the samples showed a bigger decrease on L^* , a^* and b^* in the first day of storage, being then slightly reduced. Similar results were described in previous works.

Cheng *et al.*, (2007) studied the effect of sonication and carbonation on guava juice quality. They observed no significant changes for the L^* and b^* parameters to the samples treated with sonication if compared to the control juice, and only negligible changes on the a^* parameter.

Rojas *et al.*, (2016) studied the ultrasound processing on the peach juice. The colour was measured by Cielab m results for colour as L^* indicate an increase with treatments longer than 3 minutes, becoming the samples clearer with the ultrasound processing. No significant values were observed to the parameters a^* and b^* in the

same work, considering both time of processing and storage. Even that, the slight saturation in the parameter b^* were observed during storage, which could be related with a possible carotenoid degradation on the peach juice.

Anese *et al.*, (2015) studied the effect of ultrasound treatment, oil addition and storage time on the tomato pulp. After 15 days of storage, the processed and unprocessed samples with oil addition presented a redness loss if compared to the untreated and non-addition of oil, evidences of occurred lycopene degradation during the storage time.

Tiwari *et al.*, (2009) described that the all the parameters L^* , a^* , b^* of orange juice processed by ultrasound increased during storage period, attributing to the cavitation that induces to physical, chemical or biological reactions, increasing diffusion rates helping to disperse the aggregates.

The obtained results indicate a negligible effect of the ultrasound processing on guava juice colour, even considering the partial degradation of lycopene. This result is interesting from an industrial point of view, since it is expected a negligible impact of this technology on the product appearance, which is highly desired in the consumer sensorial evaluation.

Figure 8: Effect of the ultrasonic processing on the colour of guava juice throughout 14 days of storage at 25 °C: control sample (US0min, $\blacklozenge$) and those processed with ultrasound by 3 (US3min, $\blacksquare$), 6 (US6min, $\blacktriangle$) and 9 (US9min, $\times$) minutes. Vertical bars are the standard deviation for each value. The curves are only to facilitate the interpretation.

5.7 General discussion

The present master dissertation concludes that the application of ultrasound processing improves the lycopene *in vitro* accessibility in guava juice, as well as enhance its physical proprieties.

Firstly, it was observed that the ultrasound technology disrupts the suspended guava cells and its fragments. According to the microstructure results, the carotenoids are then released within the guava cells, which changes both the

lycopene content and accessibility. Furthermore, the dispersed particles get smaller, affecting the juice physical properties.

The ultrasound processing reduced the lycopene content on guava juice, probably due to the lycopene degradation by exposing it to the oxygen. On the other hand, the lycopene *in vitro* accessibility was increased, probably as its releasing from the guava cells is easier with the disruptive effect of ultrasound. Consequently, this carotenoid became more exposed. In fact, the *in vitro* accessibility of the untreated juice was 10% (US0min), being increased to 15% (US3min), 24% (US6min) and 20% (US9min) with the ultrasound processing.

The ultrasound reduced the suspended particles size, which could be verified by both optical microscopy and an increase on serum cloudiness. However, part of these small particles form aggregates during storage, which was demonstrated by a further reduction on the serum cloudiness. Even so, it does not affect the juice colour.

Furthermore, the ultrasound processing enhanced the physical stability of guava juice, which did not show any sedimentation during the 14 days of observation (9 min of ultrasound processing), while the control juice showed sedimentation in the first hour of observation.

As conclusions of this work, we demonstrated that the ultrasound processing improves the guava juice stability and increases the lycopene *in vitro* accessibility. Consequently, the ultrasound technology shows benefices on the nutritional and physical proprieties of guava juice and its applicability in the industry is suggested.

However, further studies are needed to confirm the effect of this technology on the lycopene bioaccessibility and bioavailability, using both *in vitro* and *in vivo* tools, confirming the possible nutritional improvement.

6. CONCLUSIONS

The conclusion of the present dissertation work is that the application of a non-conventional treatment such as ultrasound improves both the *in vitro* accessibility of lycopene and the physical proprieties of the guava juice. The application of ultrasound breaks the guava cells releasing the lycopene making it more accessible for the absorption. Despite of it, the reduction on the particles size brought a high stability to the physical proprieties of guava juice. Consequently, the ultrasound technology shows possible benefices on both nutritional and physical proprieties of guava juice and, consequently, its applicability in the industry is suggested.

7. SUGGESTIONS FOR FUTURE WORKS

This work demonstrated interesting results of using the ultrasound technology for juice processing. Even so, further studies are needed. Future works would be helpful to understand the next step of the bioavailability of the carotenoids in the human body.

For future works, it would be very interesting to measure the absorption of the released carotenoids due to the ultrasound application. The ways of this measurement can be using *in vitro* or *in vivo* tools, such as Caco-2 cells absorption or animal/human trials.

Despite of this, other juices/matrix/sources of carotenoids should be studied, as well as other bioactive compounds.

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