AMANDA UMBELINA DE SOUZA

ULTRASOUND APPLICATION IN ISOMALTULOSE IMPREGNATION FOLLOWED BY CONVECTIVE DRYING IN APPLE SLICES

LAVRAS-MG
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Thesis submitted to the Federal University of Lavras, as part of the requirements of the Graduate Program in Food Science, area of concentration in Food Science, to earn the Doctorate degree.

Prof. Dr. Jefferson Luiz Gomes Corrêa
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RESUMO GERAL

A isomaltulose, também conhecida como palatinose, é um carboidrato funcional que apresenta propriedades como digestão lenta, baixo índice glicêmico e liberação prolongada de energia. Ela é comercializada na forma de pó e tem sido comumente acrescentada em bebidas utilizada por esportistas e diabéticos. Sua incorporação em um fruto seco poderia ser uma alternativa prática para seu consumo. O presente trabalho teve como objetivo a utilização da técnica de duplo estágio (D3S) para incorporação de isomaltulose e secagem de fatias de maçã. A etapa de D3S priorizou uma condição que conduzisse, principalmente, à incorporação de isomaltulose e à perda de água. No primeiro estágio do processo D3S, os açúcares nativos foram parcialmente removidos da fruta. No segundo, a isomaltulose foi incorporada na fruta. Ambos os processos ocorreram à 25 e 35 °C. A influência do uso de ultrassom também foi testada. A incorporação de isomaltulose foi medida pelo ganho de sólidos. Outros parâmetros como perda de água, relacionados à desidratação dos frutos, atividade de água (a_w), teor de sólidos solúveis (°Brix), parâmetros de cor e encolhimento volumétrico também foram avaliados. Após o D3S, a secagem foi realizada em secador de túnel a 50 e 70 °C e velocidade do ar de 1,5 m/s até o teor de umidade de 0,12 ± 0,01 kg H_2O/kg matéria seca. O efeito do pré-tratamento D3S sobre o produto final foi avaliado quanto aos parâmetros teor de umidade, a_w, teor de fenólicos totais, atividade antioxidante, encolhimento volumétrico e parâmetros de cor. O processo que promoveu a maior incorporação de isomaltulose e maior perda de água consistiu na imersão por 25 min no primeiro estágio e impregnação assistida por ultrassom por 40 min no segundo estágio. Foi observada uma redução na a_w, mudança nos parâmetros de cor e diminuição no encolhimento nesta condição. Entretanto, o tempo de secagem aumentou e houve perdas significativas dos compostos fenólicos totais, redução da atividade antioxidante e capacidade de reidratação. Além disto, foi observada maior diferença de cor em comparação com as amostras não tratadas. Em suma, o processo de D3S seguido de secagem proporcionou um produto incorporado de isomaltulose, mas com alterações de propriedades nutricionais e físicas.

Palavras-chave: Impregnação. Desidratação Osmótica. Método fosfomolibidênio. Método Abts. Processo D3S. Substituição de açúcar. Palatinose.

GENERAL ABSTRACT

Isomaltulose, also known as palatinose, is a functional carbohydrate that has properties such as slow digestion, low glycemic index, and prolonged release of energy. It is marketed as a powder and has been commonly added to beverages used by sportsmen and diabetics. Its incorporation in dried fruit could be a practical alternative for its consumption. The present work aimed to use the double stage technique (D3S) for isomaltulose incorporation and drying of apple slices. The D3S stage prioritized a condition leading mainly to isomaltulose incorporation and water loss. In the first stage of the D3S process, native sugars were partially removed from the fruit. In the second, isomaltulose was incorporated into the fruit. Both processes took place at 25 and 35 °C. The influence of the use of ultrasound was also tested. The incorporation of isomaltulose was measured by the gain of solids. Other parameters like water loss, related to fruit dehydration, water activity (aw), soluble solids content (°Brix), color parameters and volumetric shrinkage were also evaluated. After D3S, drying was performed in a tunnel dryer at 50 and 70 °C and air velocity of 1.5 m/s until the moisture content of 0.12 ± 0.01 kg H2O/kg dry matter. The effect of D3S pre-treatment on the final product was evaluated for the moisture content, aw, total phenolic content, antioxidant activity, shrinkage and color parameters. The process that promoted the highest isomaltulose incorporation and water loss consisted of immersion for 25 min in the first stage and ultrasound-assisted impregnation for 40 min in the second stage. A reduction in aw, change in color parameters, and decrease in shrinkage were observed in this condition. However, the drying time increased and there were significant losses in total phenolic compounds, reduction in antioxidant activity and rehydration capacity. In addition, a greater color difference was observed compared to the untreated samples. In summary, the D3S process followed by drying provided an incorporated isomaltulose product, but with altered nutritional and physical properties.

Keywords: Impregnation. Osmotic Dehydration. Phosphomolybdenum Method. Abts Method. D3S process. Sugar substitution. Palatinose.

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CHAPTER ONE

1 GERAL INTRODUCTION

In recent years, the public perception about the impact of the diet on health related to the intake of certain groups of food ingredients has been a driver for changes in food production. Categories commonly used in food preparations, such as fat, sugar, salt and synthetic food additives, are the main targets for substitution, while health promoters are the elected substitutes.

Variation in solutes used in osmotic dehydration (OD) is also becoming frequent and, more than this, the incorporation of alternative carbohydrates can be a strategy for food enrichment. Isomaltulose, commercially known as palatinose, is a calorie-emergent carbohydrate, isomer of sucrose, but it is digested slowly by the body. This characteristic makes this carbohydrate low in glycemic index and insulinemic index, reducing the risk of developing diabetes, obesity, and cardiovascular disease. In addition, it is a non-cariogenic carbohydrate, which contributes to a prolonged feeling of satiety and a sustained release of energy with additional implications for physical and mental performance.

One technology with great prospects for alternative sweetener application in solid food matrices is impregnation, which uses an osmotic gradient pressure to incorporate ingredients of interest into porous matrices. Modifications in the impregnation step are usually performed to optimize the process, especially with respect to solid gain (SG) and water loss (PA).

The process called two-stage sugar substitution (D3S) aims to induce the replacement of native sugar by others of interest. In the first stage, sugars such as sucrose, fructose and glucose are partially removed from the food matrix and, in the second stage, an ingredient is incorporated, which in the case of this research was isomaltulose. The use of ultrasound in these impregnation processes is due to its influence on the cell structure, from the "sponge effect" and cavitation, mainly, favoring mass transfer processes.

The products from the impregnation process fit into the *Intermediate Moisture Foods* (IMF) class, requiring a subsequent process that guarantees microbiological and physicochemical stability during storage and commercialization. Drying is considered one of the most common preservation methods, and when associated with impregnation pre-treatments, it is commonly observed a reduction in drying time and preservation of the food quality.

This work aimed to evaluate the influence of the D3S technique in the incorporation of isomaltulose by apple slices. Moreover, to evaluate the impact of this incorporation on the drying kinetics and quality parameters of apples dried by convective drying.

Mass transfer parameters, solid gain and water loss, were selected as the main variables to define the efficiency of isomaltulose incorporation by D3S. Subsequently, quality parameters such as color, water activity, soluble solid content, and shrinkage were also evaluated aiming at an optimized condition of the technique. A second aim was to reduce the water activity of D3S pretreated apples by convective drying, in order to evaluate the influence of pretreatment on the drying kinetics, drying time, and to verify the influence on a_w, total phenolic content, antioxidant activity, volumetric shrinkage and color parameters of dehydrated apples.

2 THEORETICAL REFERENCE

2.1 Apple - General aspects

The apple is the fruit from the apple tree (*Malus domestica*), it belongs to the family *Rosaceae*, originated in Europe and Asia. Naturally, the apple tree restricts its adaptation in temperate and subtropical regions, especially in places of high altitudes. However, advances in production technology have been achieved in an attempt to expand cultivation in places where environmental conditions are, to some extent, unfavorable. It is important to consider that, when moving away from adequate growing conditions, greater are the difficulties encountered in the production of quality fruit and in ensuring its technical and economic viability. Regarding cultivars, although there is a very wide range, only four of them account for more than half of world production, in which *Delicious, Golden Delicious, Gala* and *Fuji* stand out, being the last two the most favorable for cultivation in southern Brazil (KRETZSCHMAR; RUFATO, 2020).

According to FAO (2020), apple is amongst the 20 most produced crops and third in the class of fruits, with an estimated production of 86 million tons in 2018. China holds most of the world production, about 45%, with 39 million tons/year. Although Brazil has only 1.4% of the total production, it is among the top 15 apple producers, with an estimated production of 1 million tons in 2018. Worldwide, the annual average per capita of the consumption of apple and its products is 9 kg, while in Brazil this amount drops to 5 kg per capita.

It is indisputable that apples play an important role in the world fruit market, and this importance is associated, besides its flavor, with its nutritional value (TABLE 1). In addition, after the WHO recommendation to consume five or more servings of fruits and vegetables per day, there has been an increase in consumption by health-conscious consumers to establish a healthy diet (WHO, 2019).

Table 1 - Centesimal composition and caloric value of *fuji* apples.

Parameter	Value per 100 g
Energy (kcal)	59
Moisture (g)	84.3
Total carbohydrate (g)	15.2
Protein (g)	0.29
Lipids (g)	Tr
Dietary fiber (g)	1.35
Ash (g)	0.22
Saturated fatty acids (g)	Tr
Monounsaturated fatty acids (g)	Tr
Polyunsaturated fatty acids (g)	Tr
Calcium (mg)	1.92
Iron (mg)	0.09
Sodium (mg)	Tr
Magnesium (mg)	2.04
Phosphorus (mg)	9.08
Potassium (mg)	74.7
Zinc (mg)	Tr
Copper (mg)	0.06
Selenium (mcg)	0.00
Vitamin A (RE) (mcg)	7.00
Alpha-tocopherol (Vitamin E) (mg)	0.18
Thiamine (mg)	Tr
Riboflavin (mg)	Tr
Niacin (mg)	Tr
Vitamin B6 (mg)	0.03
Vitamin B12 (mg)	0.00
Vitamin C (mg)	2.41
Folate equivalent (mcg)	3.25

Source: Brazilian Table of Food Composition (TACO, 2011) adapted by the author.

Apple is considered a source of monosaccharides, minerals, dietary fiber, vitamins and various other biologically active compounds, such as antioxidants (WU *et al*., 2007). A diet that includes its consumption can be associated with decreased risk of chronic diseases, such as cardiovascular disease, cancer, and asthma, due to the presence of vitamins and phytochemical compounds that lead to inhibition of cancer cell proliferation, reduced lipid oxidation, and lower cholesterol (BONDONNO *et al*., 2017; BOYER; LIU, 2004).

2.2 Impregnation technology

Major changes have occurred regarding to the population's diet in recent decades, where food consumption patterns have changed to adhere to a healthier lifestyle, including both health promotion and the reduction of disease incidence. Within this scenario, the food industry is encouraged to develop products that meet this new consumer demand.

There are several strategies to deliver healthy processed food to the consumer, such as minimal processing, enrichment of food with ingredients of interest, use of packaging to reduce biochemical and microbiological changes in food, genetically modified products, among others (FITO *et al*., 2001).

In recent years, impregnation has been used as a way to incorporate ingredients of interest into the porous structure of some foods, such as minerals (ERIHEMU *et al*., 2014, 2015; LIMA *et al*., 2016; MORAGA *et al*., 2009), antioxidants (CASTAGNINI *et al*., 2015; NAMBIAR *et al*., 2016), curcuminoids (BELLARY; RASTOGI, 2012; BELLARY; SOWBHAGYA; RASTOGI, 2011), prebiotics (JIMÉNEZ-HERNÁNDEZ *et al*., 2017; NAMBIAR *et al*., 2016) and vitamins (HIRONAKA *et al*., 2011). This process is based on the difference in concentration between the food and a solution, which promotes a parallel flow of ingredient incorporation and water removal. Thus, the composition, physical and chemical properties of the food can be changed to improve some characteristics (TORREGGIANI, 1993). It is important to say that the enrichment of foods with ingredients of interest in liquid and powder matrices is easier when compared to a solid structure, such as fruits and vegetables, therefore, this technology contributes to overcoming this challenge (BELLARY; RASTOGI, 2016).

Among the possibilities of using the impregnation process to manipulate the sensory, technological and/or functional characteristics of a food, there is a trend towards the impregnation of alternative sucrose substitutes (GREMBECKA, 2015; VAN LAAR; GROOTAERT; VAN CAMP, 2020). Low-sugar diets have been increasingly explored not only by people who have some type of comorbidity, such as diabetes, but may also provide metabolic benefits for the general population. Palatinose or isomaltulose is a recent example of an alternative ingredient to sucrose (SAWALE *et al*., 2017).

2.3 Isomaltulose or Palatinose

Isomaltulose, also known by its trade name palatinose, is a disaccharide carbohydrate with the chemical name 6-O-α-d-glucopyranosyl-d-fructofuranose. This carbohydrate is an isomer of sucrose, in which the glucose and fructose portions are linked by an α (1-6) glycosidic bond instead of the α (1-2) glycosidic bond of sucrose (SENTKO; WILLIBALD-ETTLE, 1988). The stability of this α (1-6) bond is what differentiates isomaltulose in its physiological properties, standing out as a non-cariogenic carbohydrate with a low glycemic index and reduced insulinemic response (HOLUB *et al*., 2010).

Isomaltulose exists in extremely small quantities in nature, found in honey and sugar cane, and is very difficult to synthesize chemically (SAWALE *et al*., 2017). Thus, it is produced on an industrial scale from the bioconversion of sucrose from sugar beetroot, by means of the enzyme sucrose isomerase, also known as isomaltulose synthase (SIase; EC 5.4.99.11) produced by different microorganisms (MU *et al*., 2014). Among the microorganisms used for isomaltulose synthesis, *Serratia plymuthica* is the most used for industrial production (GOULTER; HASHIMI; BIRCH, 2012), but other strains also do this bioconversion, such as *Protaminobacter dispersa*, *Erwinia rhapontici*, *Klebsiella planticola*, *Klebsiella spp*. *Klebsiella singaporensis sp.* and *Serratia plymuthica* (LIU *et al*., 2020; SAWALE *et al*., 2017). As by-products of the reaction, there is trehalulose and small quantities of glucose and fructose, which stimulates industries to search for processes that optimize the production of isomaltulose (LIU *et al*., 2020). In addition, research has been conducted to reduce process costs and increase production using low-cost substrates, such as agricultural waste, like soy molasses (WANG et al., 2019), cold-pressed soybean powder (ZHENG *et al*., 2019) and sugarcane by-product (ZHENG *et al*., 2019).

Sucrose is a molecule easily assimilated by the body, providing a rapid rise in blood glucose after ingestion, and its excessive consumption is associated with weight gain and its associated health problems, including type 2 diabetes and heart disease (MU *et al*., 2014). Given this scenario, the use of isomaltulose as a sucrose substitute becomes attractive for having characteristics that surround such disadvantages as:

- It has a caloric value equal to sucrose (4 kcal/g), but with low digestibility and extended energy flow, and its degradation products, glucose and fructose, follow the same classic metabolic pathway as sucrose (HOLUB *et al*., 2010; OKUNO *et al*., 2010). thus, it is indicated for consumers who need glycemic control and for athletes, because it avoids

glycemic and insulinemic peaks and provides energy for a longer time, with the constant release of glucose into the bloodstream (GREMBECKA, 2015; MU *et al*., 2014);

- The complete digestion in the small intestine causes prolonged glucose release without gastrointestinal discomfort, about four to five times slower than sucrose (SENTKO; WILLIBALD-ETTLE, 1988; MARESCH *et al*., 2017). Thus, ingesting a larger amount of isomaltulose does not lead to adverse effects such as flatulence and diarrhea due to its complete hydrolysis, unlike other sweeteners (LINA; JONKER; KOZIANOWSKI, 2002);
- The demineralization process of the teeth is reduced due to the difficulty of the oral flora to use palatinose as substrate for fermentation (MÄKINEN, 2010; PEINADO *et al*., 2013; SENTKO; WILLIBALD-ETTLE, 1988);
- The probiotic action is another benefit that has been explored in relation to isomaltulose, since studies have demonstrated the promotion and selection of the growth of some probiotic strains beneficial to health as *Lactobacillus acidophilus* and *Lactoccocus sp.* (VAN ZANTEN *et al*., 2012) and *Saccharomyces boulardii* (MITTERDORFER; KNEIFEL; VIERNSTEIN, 2001; SHYAM; RAMADAS; CHANG, 2018).

From a range of biochemical and toxicological studies conducted in vitro and in vivo on various species, including humans, it has been concluded that the ingestion of isomaltulose does not result in adverse effects, and it can be used as a food ingredient (LINA; JONKER; KOZIANOWSKI, 2002). Thus, isomaltulose is a "generally recognized as safe" (GRAS) substance by the Food and Drug Administration (GRN No.184). In 2005, its use was approved by the Novel Foods Regulation in the European Union, notified under document number C (2005) 2776, and it is recognized in Japan as a health-promoting ingredient by the program called FOSHU (Food for Specified Health Uses) (LIU *et al*., 2020).

Regarding the properties of isomaltulose, it appears as a white crystalline material in different particle size distributions, has low hygroscopicity, melts at a temperature of 123 - 124 °C. Under acidic conditions, isomaltulose is more stable than sucrose and resists hydrolysis processes. It has a lower solubility than sucrose and its viscosity, water activity, and density of solutions are close to those of sucrose. Its sweetness potential is about 48% with respect to sucrose and presents a sweetness profile similar to that of sucrose, with no aftertaste (SAWALE *et al*., 2017; SENTKO; WILLIBALD-ETTLE, 1988; SHYAM; RAMADAS; CHANG, 2018).

Among the possibilities for the application of isomaltulose as an ingredient in foods, Sawale *et al*. (2017) , there are some of them that are worth to mention, such as:

- The use in the formulation of acidic sports drinks, as the hydrolysis resistance cooperates to avoid isotonic imbalance while maintaining the osmolarity of the product.
- The use in fermented products as a non-fermentable functional sweetener
- The use in the production of powdered products because its glass transition temperature is higher and leads to greater formation of less amorphous parts during drying, generating a less hygroscopic dry product. Besides this, it cooperates in the yield of the drying process.
- The use in nutritional bars, cereals, bakery products, chocolate and chewing gum is associated with its stable crystalline structure and easy application as dry matter in these products. In addition, it behaves like the sugar commonly added to these foods and can completely replace them in formulations.
- The use in dairy products is related to the improvement in consistency and viscosity parameters as well as change in the perception of sweet taste.

2.4 Drying

Drying is considered one of the oldest food conservation techniques and it consists in removing the water present in the food matrix, which reduces the growth of microorganisms and inhibits the enzymatic activity and some chemical reactions. It is an important process to preserve products and increase their stability during storage. However, reducing the changes in physical and chemical composition that can occur during processing is widely studied (HORUZ; MASKAN, 2013).

According to Park *et al*. (2014), one can didactically divide the evolution of heat and mass transfer during drying into three distinct periods:

- In the first period, called the induction period, the food is at a lower temperature than the drying air and, after the exposure to hot air, the food temperature increases, leading to an increase in vapor pressure and drying speed. This occurs until heat transfer outweighs mass transfer. The duration of this period is usually insignificant to the total drying period
- In the second period, called the constant rate period, the food has a large amount of water available for evaporation, in the form of free water present on the surface of the material. This period occurs until the migration of water from the interior to the surface of the product is sufficient to compensate for the loss of water by evaporation at the surface. During this time the drying rate is constant.

- The third period, called the period of decreasing drying rate, is characterized by a reduction in the drying rate. This occurs because heat transfer is no longer compensated by mass transfer, therefore the movement of water inside the solid is insufficient to maintain the evaporation rate at the surface, and this is the limiting factor in this period of drying. Consequently, the temperature on the surface of the material increases. The drying is only finished when the food humidity is equilibrated with the humidity of the drying air.

The drying of foods can lead to physical, chemical, sensory and nutritional changes that must be evaluated to produce a stable food with good properties. These include changes in the porosity of the material, shrinkage, changes in solubility, reduced rehydration, hardening of the food surface, loss of flavor and aroma, enzymatic reactions, lipid oxidation, loss of vitamins, browning reaction, degradation of nutraceutical compounds, and the loss of vitamins and minerals (RATTI, 2009).

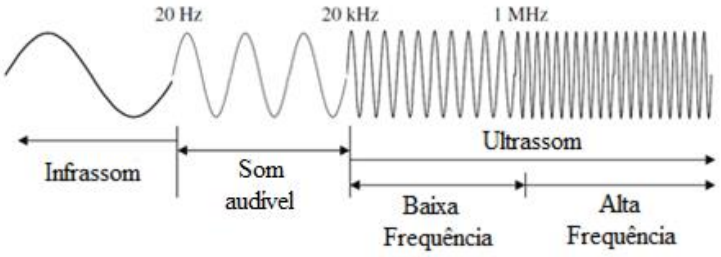
Therefore, the use of pre-treatments such as impregnation in food drying is an interesting alternative to associate the preservation of food quality characteristics with the manipulation of the ingredients of interest.

2.5 Ultrasound

2.5.1 General aspects

The sound spectrum is formed from three main categories, divided according to their frequency, being them the infrasound, which presents very low sound waves (below 16 Hz), imperceptible by human hearing, the sound band, which is the range in which the ears are able to detect (between 20 Hz and 20 kHz), and ultrasound, which is the range of waves that are above the sound band, with higher frequency (above 20 kHz). Within the ultrasonic spectrum, it is possible to further divide it into two main zones: the low-frequency zone also called power ultrasound, which operates between 20 kHz and 1 MHz and the high-frequency zone, which operates above 1 MHz (FIGURE 1) (FENG; BARBOSA-CÁNOVAS; WEISS, 2011).

Figure 1 - The sound spectrum.

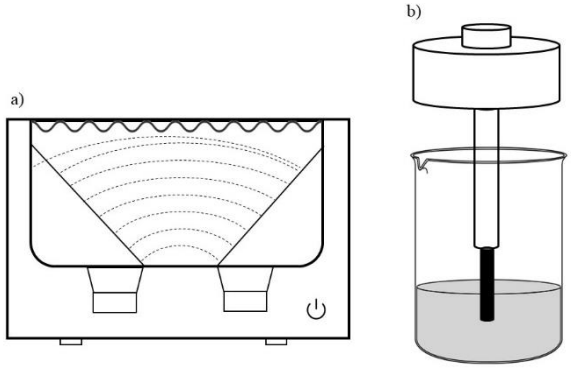


Source: Feng, Barbosa-Cánovas and Weiss (2011) adapted by the author.

Sonication, which is the term referring to the use of ultrasonic waves, is considered an emerging non-thermal technology whose use in industrial processes has shown great promise. Its application presents varied functions, such as improvement in emulsification capacity, solubility and texture, homogenization, viscosity change, extraction and removal of foam, degradation of compounds, aid in mass transfer processes and reduction of microbial load. In this context, low-frequency ultrasound is used to induce these physical and chemical changes of interest. Another prominent function is its use in quality monitoring systems, which include non-destructive testing from the use of high-frequency ultrasound, providing information on physicochemical, structural and compositional properties (BHARGAVA *et al*., 2021; CHEMAT *et al*., 2017; TIWARI; MASON, 2012).

Conventional ultrasound application techniques consist of two main pieces of equipment, being the ultrasonic bath and probe-type ultrasound equipment (FIGURE 2). The ultrasonic bath consists of a stainless steel tank, which can be equipped with temperature control, operating at a frequency of 25 to 40 kHz (TAYLOR; DEMIRDÖVEN; BAYSAL, 2009). They are relatively low-cost and a large volume of products can be treated simultaneously. Probe-type ultrasound, however, consists of a probe inserted into a medium in which the waves will propagate. This system allows direct delivery of the waves into the propagating medium. The use of probe ultrasound in a given process will vary according to the purpose of the application and the volume of the sample to be worked on. For this purpose, there are devices with different probe lengths and different diameters and geometries of the probe tip (CHAROUX *et al*., 2017).

Figure 2 - Ultrasonic equipment: ultrasonic bath (a) and probe type (b).



Source: Miano, Rojas and Augusto (2017) adapted by the author.

The use of ultrasound as a processing technology presents some disadvantages that must be circumvented from studies aiming the optimization of the process, as follows (BHARGAVA *et al*., 2021):

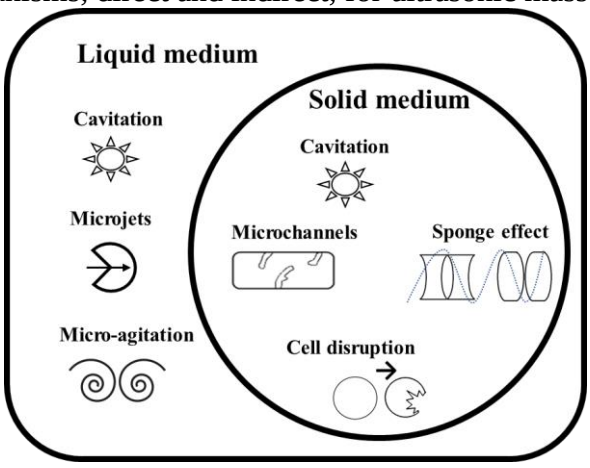
- It can generate heat, raising the temperature of the food, negatively interfering with sensory and nutritional characteristics of the processed food.
- Microbial inactivation is only successful if ultrasound acts with a complementary technology, such as temperature or pressure.
- Depending on the extent and characteristic of the food that has been sonicated, undesired effects such as lipid oxidation, denaturation of proteins and degradation of nutrients may occur.

The chemical and physical effects caused by ultrasound derive from various acoustic phenomena that are related to each medium of propagation. For solid-liquid systems, most authors attribute these phenomena to cavitation and the "sponge effect" (SORIA; VILLAMIEL, 2010). As the ultrasonic waves propagate and move through the solid-liquid medium, regions of fluctuating pressure are created due to successive compression and expansion cycles, also referred to as the "sponge effect". The compression cycles exert positive pressure and push the molecules of the liquid together, while the expansion cycles exert a negative pressure and separate these molecules. When the negative pressure of the expansion cycle exceeds the forces of attraction between the liquid molecules, small vapor-filled voids are formed, called cavitation bubbles. As the application of the ultrasonic waves continues, the bubbles increase in size. This increase in the size of the bubbles produces a micro-agitation at the solid-liquid interface. This bubble increase occurs until a critical size, at that point it becomes unstable and implode, creating a phenomenon known as cavitation. This collapse of the gas bubbles releases large amounts of heat and pressure in a short period and at localized points, referred to as "hot spots.

In addition, with the implosion of the bubbles formed, micro-jets of moisture can be generated that can reach very fast speeds (CHEMAT *et al*., 2017; FENG; BARBOSA-CÁNOVAS; WEISS, 2011; MIANO; ROJAS; AUGUSTO, 2017; RASTOGI, 2011).

Ultrasound has been one of the techniques for intensifying unit operations to improve mass transfer processes in dehydration technology, this is attributed to some effects that are classified as direct and indirect (FIGURE 3).

Figure 3 - Main mechanisms, direct and indirect, for ultrasonic mass transfer enhancement.



Source: Miano, Rojas and Augusto (2017) adapted by the author.

The direct effects are those associated with the sponge effect, removing any boundary layer that may exist in the solid, and keeping the pores of the solid matrix unobstructed. This mechanism is facilitated when applied to foods in a gummy state since the cell structure is more malleable to suffer compression and expansion, and also foods that are highly porous. The indirect effect is related to the formation of microchannels and cavities formed in cavitation, due to the microjets formed that generate surface scaling, erosion, and membrane rupture. This increases the surface area of the solid and favors mass transfer (MIANO *et al*., 2019; MIANO; IBARZ; AUGUSTO, 2016).

2.6 Two-stage sugar substitution (D3S)

Garcia-Noguera *et al*. (2010) proposed a new ultrasonic pretreatment technique for drying, called two-stage sugar substitution (D3S). The aim of these authors was to produce low-calorie dried fruit since this group of foods has a high sugar content due to the concentration that occurs during drying. This process is composed of two steps in which the first one applies an ultrasonic treatment to the fruit from water immersion, which removes naturally present

calorie sugars, while the second step is intended to impregnate a natural sweetener into the fruit. This work consisted in evaluating the process performance in replacing naturally present strawberry sugar with a Stevia-derived natural sweetener using ultrasonic pretreatments in osmotic solutions. They concluded that 45 min ultrasonic pretreatment in distilled water and subsequent osmotic immersion without ultrasonic application in Stevia based solutions was a practical method to do such a substitution.

Similarly, Oliveira *et al*. (2011) used the D3S process to produce low-calorie apples with Stevia-based sweetener. This time, the best process performance was achieved by subjecting the fruit samples to ultrasound in both processing steps. Thus, this condition led to higher sugar removal during the first stage, higher water loss during the process, and higher sweetener incorporation during the second stage of the D3S process.

Medeiros *et al*. (2016) used the D3S technique as a pre-treatment for mango drying. As a result, these authors obtained higher drying rates and less color change, but observed a reduction in phenolic and carotenoid content. Besides this, the sensorial evaluation reported that the pretreated sample obtained good acceptance. Medeiros *et al*. (2019) evaluated the processing of mango slices, this time by changing the ultrasound times used in D3S. They concluded that subjecting the samples to ultrasonic waves in both stages of D3S with immersion in Stevia-based solution in the second stage for 10, 20, and 30 min resulted in higher water losses, while higher values of solids gain were obtained by applying ultrasound only in the first stage. In addition, mango samples with higher concentrations of total phenolics were obtained. When the samples were subjected to the ultrasonic waves in both stages, higher retention of carotenoids was observed.

It is possible to see through these studies that the D3S process can be a good strategy for pre-treatment to drying, however, there are not many studies about it. In addition, it is observed that the results obtained with respect to product quality are variable, according to the matrix studied, serving as a stimulus for further research with other foods. Associated with this variation, a series of other ingredients can be explored in the impregnation stage.

REFERENCES

BELLARY, A. N.; RASTOGI, N. K. Effect of hypotonic and hypertonic solutions on impregnation of curcuminoids in coconut slices. **Innovative Food Science & Emerging Technologies**, [Oxford], v. 16, p. 33–40, Oct. 2012. Disponível em: https://www.sciencedirect.com/science/article/pii/S1466856412000513. Acesso em: 10 jul. 2021.

BELLARY, A. N.; RASTOGI, N. K. Ways and means for the infusion of bioactive constituents in solid foods. **Critical Reviews in Food Science and Nutrition**, [Philadelphia], v. 56, n. 7, p. 1126–1145, May 2016. Disponível em: https://pubmed.ncbi.nlm.nih.gov/25607632/. Acesso em: 07 jul. 2021.

BELLARY, A. N.; SOWBHAGYA, H. B.; RASTOGI, N. K. Osmotic dehydration assisted impregnation of curcuminoids in coconut slices. **Journal of Food Engineering**, [Oxford], v. 105, n. 3, p. 453–459, Aug. 2011. Disponível em: https://www.cabdirect.org/cabdirect/abstract/20113150966. Acesso em: 02 set. 2021.

BHARGAVA, N. *et al*. Advances in application of ultrasound in food processing: A review. **Ultrasonics Sonochemistry**, [Amsterdam], v. 70, p. 105293, Jan. 2021. Disponível em: https://www.sciencedirect.com/science/article/pii/S1350417720310944. Acesso em: 02 set. 2021.

BONDONNO, N. P. *et al*. The cardiovascular health benefits of apples: Whole fruit vs. isolated compounds. **Trends in Food Science & Technology**, [London], v. 69, p. 243–256, Nov. 2017. Disponível em: https://www.sciencedirect.com/science/article/pii/S0924224416305271. Acesso em: 07 jul. 2021.

BOYER, J.; LIU, R. H. Apple phytochemicals and their health benefits. **Nutrition Journal**, [London], v. 3, n. 5, p. 1–45, May 2004. Disponível em: https://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-3-5. Acesso em: 01 set. 2021.

CASTAGNINI, J. M. *et al*. Vacuum impregnation and air drying temperature effect on individual anthocyanins and antiradical capacity of blueberry juice included into an apple matrix. **LWT - Food Science and Technology**, [Amsterdam], v. 64, n. 2, p. 1289–1296, Dec. 2015. Disponível em: https://www.sciencedirect.com/science/article/pii/S0023643815004715. Acesso em: 01 set. 2021.

CHAROUX, C. *et al*. Applications of airborne ultrasonic technology in the food industry. **Journal of Food Engineering**, [Oxford], v. 208, p. 28–36, Sept. 2017. Disponível em: https://www.semanticscholar.org/paper/Applications-of-airborne-ultrasonic-technology-in-Charoux-Ojha/ec62f1a4dce5efc293a152dce88166983f8fdbd7. Acesso em: 01 set. 2021.

CHEMAT, F. *et al*. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. **Ultrasonics Sonochemistry**, [Amsterdam], v. 34, p. 540–560, Jan. 2017. Disponível em: https://www.sciencedirect.com/science/article/pii/S1350417716302358. Acesso em: 01 set. 2021.

ERIHEMU *et al*. Iron enrichment of whole potato tuber by vacuum impregnation. **LWT - Food Science and Technology**, [Amsterdam], v. 59, n. 1, p. 504-509, Nov. 2014. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0023643814002485?casa_token=CT1rmMpw7ckAAAAA:QVAWxNINsHpf30LF2zoK8UZ8eks7_SPGLu59nu08nirBwiybroILAgZySBC2Pje1cVkxVYDk. Acesso em: 07 jul. 2021.

ERIHEMU *et al*. Zinc enrichment of whole potato tuber by vacuum impregnation. **Journal of Food Science and Technology**, [New Delhi], v. 52, n. 4, p. 2352–2358, Apr. 2015. Disponível em: https://pubmed.ncbi.nlm.nih.gov/25829619/. Acesso em: 02 set. 2021.

FAO. **FAOSTAT - Food and agriculture data**. Disponível em: https://www.fao.org/faostat/en/#data. Acesso em: 01 set. 2021.

FENG, H.; BARBOSA-CÁNOVAS, G. V.; WEISS, J. **Ultrasound technologies for food and bioprocessing**. 1st ed. New York: Springer, 2011.

FITO, P. *et al*. Vacuum impregnation and osmotic dehydration in matrix engineering: Application in functional fresh food development. **Journal of Food Engineering**, [Oxford], v. 49, n. 2–3, p. 175–183, Aug. 2001. Disponível em: https://europepmc.org/article/agr/ind23323303. Acesso em: 07 jul. 2021.

GARCIA-NOGUERA, J. *et al*. Dual-stage sugar substitution in strawberries with a Stevia-based sweetener. **Innovative Food Science & Emerging Technologies**, [Oxford], v. 11, n. 1, p. 225–230, Jan. 2010. Disponível em: https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1187&context=biosysengfacpub. Acesso em: 07 jul. 2021.

GOULTER, K. C.; HASHIMI, S. M.; BIRCH, R. G. Microbial sucrose isomerases: Producing organisms, genes and enzymes. **Enzyme and Microbial Technology**, [New York], v. 50, n. 1, p. 57–64, Jan. 2012. Disponível em: https://pubmed.ncbi.nlm.nih.gov/22133441/. Acesso em: 06 ago. 2021.

GREMBECKA, M. Sugar alcohols—their role in the modern world of sweeteners: a review. **European Food Research and Technology**, [New York], v. 241, n. 1, p. 1–14, Feb. 2015. Disponível em: https://link.springer.com/article/10.1007/s00217-015-2437-7. Acesso em: 01 set. 2021.

HIRONAKA, K. *et al*. Ascorbic acid enrichment of whole potato tuber by vacuum-impregnation. **Food Chemistry**, [Oxford], v. 127, n. 3, p. 1114–1118, Aug. 2011. Disponível em: https://www.sciencedirect.com/science/article/pii/S0308814611002184. Acesso em: 06 ago. 2021.

HOLUB, I. *et al*. Novel findings on the metabolic effects of the low glycaemic carbohydrate isomaltulose (Palatinose^{TM}). **British Journal of Nutrition**, [Cambridge], v. 103, n. 12, p. 1730–1737, June 2010. Disponível em: https://pubmed.ncbi.nlm.nih.gov/20211041/. Acesso em: 07 jul. 2021.

HORUZ, E.; MASKAN, M. Hot air and microwave drying of pomegranate (*Punica granatum* L.) arils. **Journal of Food Science and Technology**, [New Delhi], v. 52, n. 1, p. 285–293, May 2013. Disponível em: https://link.springer.com/article/10.1007/s13197-013-1032-9. Acesso em: 06 ago. 2021.

JIMÉNEZ-HERNÁNDEZ, J. *et al*. Osmotic dehydration of mango with impregnation of inulin and piquin-pepper oleoresin. **LWT - Food Science and Technology**, [Amsterdam], v. 79, p. 609–615, June 2017. Disponível em: https://www.sciencedirect.com/science/article/pii/S0023643816306879. Acesso em: 10 jul. 2021.

KRETZSCHMAR, A. A.; RUFATO, L. Maçãs: O que você ainda não sabe sobre a atividade. **Campo e Negócios Online**. 2020. Disponível em: https://revistacampoenegocios.com.br/macas-o-que-voce-ainda-nao-sabe-sobre-a-atividade/. Acesso em: 10 jul. 2021.

LIMA, M. M. de *et al*. Vacuum impregnation and drying of calcium-fortified pineapple snacks. **LWT - Food Science and Technology**, [Amsterdam], v. 72, p. 501–509, Oct. 2016. Disponível em: https://www.sciencedirect.com/science/article/pii/S0023643816302729. Acesso em: 12 jul. 2021.

LINA, B. A. R.; JONKER, D.; KOZIANOWSKI, G. Isomaltulose (Palatinose®): a review of biological and toxicological studies. **Food and Chemical Toxicology**, [Oxford], v. 40, n. 10, p. 1375–1381, Oct. 2002. Disponível em: https://pubmed.ncbi.nlm.nih.gov/12387299/. Acesso em: 12 jul. 2021.

LIU, L. *et al*. Studies on biological production of isomaltulose using sucrose isomerase: current status and future perspectives. **Catalysis Letters**, [New York], v. 151, p. 1868–1881, Nov. 2020. Disponível em: https://link.springer.com/article/10.1007/s10562-020-03439-x. Acesso em: 12 jul. 2021.

MÄKINEN, K. K. Sugar alcohols, caries incidence, and remineralization of caries lesions: A literature review. **International Journal of Dentistry**, [United Kingdom], v. 2010, n. 1, p. 1–23, Jan. 2010. Disponível em: http://www.hindawi.com/journals/ijd/2010/981072/. Acesso em: 12 jul. 2021.

MARESCH, C. C. *et al*. Low Glycemic Index Prototype Isomaltulose - Update of Clinical Trials. **Nutrients**, [Basel], v. 9, n. 381, p. 1–12, Apr. 2017. Disponível em: https://pubmed.ncbi.nlm.nih.gov/28406437/. Acesso em: 06 ago. 2021.

MEDEIROS, R. A. B. de *et al*. Influence of dual-stage sugar substitution pretreatment on drying kinetics and quality parameters of mango. **LWT - Food Science and Technology**, [Amsterdam], v. 67, p. 167–173, Jan. 2016. Disponível em: https://www.infona.pl/resource/bwmeta1.element.elsevier-6bbd8798-6019-35a6-a5a5-7d6c58c36982. Acesso em: 06 ago. 2021.

MEDEIROS, R. A. B. de *et al*. Dual-stage sugar substitution in Tommy Atkins mango. **Revista Brasileira de Engenharia Agricola e Ambiental**, [Campina Grande], v. 23, n. 1, p. 60–64, Jan. 2019. Disponível em: https://www.scielo.br/j/rbeaa/a/SmL3QDJLJ7Nt6dZQPtMKH4P/?lang=en. Acesso em: 06 ago. 2021.

MIANO, A. C. *et al*. Structural changes caused by ultrasound pretreatment: Direct and indirect demonstration in potato cylinders. **Ultrasonics Sonochemistry**, [Amsterdam], v. 52, p. 176–183, Apr. 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/30473479/. Acesso em: 06 ago. 2021.

MIANO, A. C.; IBARZ, A.; AUGUSTO, P. E. D. Mechanisms for improving mass transfer in food with ultrasound technology: Describing the phenomena in two model cases. **Ultrasonics Sonochemistry**, [Amsterdam], v. 29, p. 413–419, Mar. 2016. Disponível em: https://www.sciencedirect.com/science/article/pii/S1350417715300638. Acesso em: 06 ago. 2021.

MIANO, A. C.; ROJAS, M. L.; AUGUSTO, P. E. D. Other Mass Transfer Unit Operations Enhanced by Ultrasound. *In*: BERMUDEZ-AGUIRRE, D. (ed.). **Ultrasound:** advances for food processing and preservation. London: Academic Press, 2017. p. 369–389.

MITTERDORFER, G.; KNEIFEL, W.; VIERNSTEIN, H. Utilization of prebiotic carbohydrates by yeasts of therapeutic relevance. **Letters in Applied Microbiology**, [Malden], v. 33, n. 4, p. 251–255, Oct. 2001. Disponível em: https://pubmed.ncbi.nlm.nih.gov/11559396/. Acesso em: 14 ago. 2021.

MORAGA, M. J. *et al*. Effect of vacuum impregnation with calcium lactate on the osmotic dehydration kinetics and quality of osmodehydrated grapefruit. **Journal of Food Engineering**, [Oxford], v. 90, n. 3, p. 372–379, Feb. 2009. Disponível em: https://www.researchgate.net/publication/222577573_Effect_of_vacuum_impregnation_with_calcium_lactate_on_the_osmotic_dehydration_kinetics_and_quality_of_osmodehydrated_grapefruit. Acesso em: 14 ago. 2021.

MU, W. *et al*. Current studies on sucrose isomerase and biological isomaltulose production using sucrose isomerase. **Applied Microbiology and Biotechnology**, [New York], v. 98, n. 15, p. 6569–6582, May 2014. Disponível em: https://link.springer.com/article/10.1007/s00253-014-5816-2. Acesso em: 14 ago. 2021.

NAMBIAR, S. S. *et al*. Infusion of fructooligosaccharide in Indian gooseberry (*Emblica officinalis*) fruit using osmotic treatment and its effect on the antioxidant activity of the fruit. **Journal of Food Engineering**, [Oxford], v. 190, n. 3, p. 139–146, Dec. 2016. Disponível em: https://www.sciencedirect.com/science/article/pii/S0260877416302102. Acesso em: 14 ago. 2021.

OKUNO, M. *et al*. Palatinose-blended sugar compared with sucrose: different effects on insulin sensitivity after 12 weeks supplementation in sedentary adults. **International Journal of Food Sciences and Nutrition**, [England], v. 61, n. 6, p. 643–651, Sept. 2010. Disponível em: https://pubmed.ncbi.nlm.nih.gov/20367218/. Acesso em: 14 ago. 2021.

OLIVEIRA, F. I. P. *et al*. Dehydration of Malay Apple (*Syzygium malaccense* L.) Using Ultrasound as Pre-treatment. **Food and Bioprocess Technology**, [New York], v. 4, n. 4, p. 610–615, Apr. 2011. Disponível em: https://link.springer.com/article/10.1007/s11947-010-0351-3. Acesso em: 14 ago. 2021.

PARK, B. K. J. *et al*. Secagem: fundamentos e equações. **Revista Brasileira de Produtos Agroindustriais**, v. 16, n. 1, p. 93–126, mar. 2014. Disponível em: https://www.researchgate.net/publication/277926416_SECAGEM_FUNDAMENTOS_E_EQUACOES. Acesso em: 14 ago. 2021.

PEINADO, I. *et al*. Influence of processing on the volatile profile of strawberry spreads made with isomaltulose. **Food Chemistry**, [Oxford], v. 138, n. 1, p. 621–629, May 2013. Disponível em: https://pubmed.ncbi.nlm.nih.gov/23265532/. Acesso em: 13 ago. 2021.

RASTOGI, N. K. Opportunities and challenges in application of ultrasound in food processing. **Critical Reviews in Food Science and Nutrition**, [Philadelphia], v. 51, n. 8, p. 705-722, Sept. 2011. Disponível em: https://pubmed.ncbi.nlm.nih.gov/21838554/. Acesso em: 14 ago. 2021.

RATTI, C. **Advances in food dehydration**. Florida: CRC press - Taylor & Francis Group, 2009.

SAWALE, P. D. *et al*. Isomaltulose (Palatinose) – An emerging carbohydrate. **Food Bioscience**, [Amsterdam], v. 18, p. 46–52, June 2017. Disponível em: https://www.sciencedirect.com/science/article/pii/S2212429216301808. Acesso em: 3 ago. 2021.

SENTKO, A.; WILLIBALD-ETTLE, I. Isomaltulose. *In*: **Sweeteners and Sugar Alternatives in Food Technology**. 1988. p. 397–415

SHYAM, S.; RAMADAS, A.; CHANG, S. K. Isomaltulose: Recent evidence for health benefits. **Journal of Functional Foods**, [Amsterdam], v. 48, p. 173–178, Sept. 2018. Disponível em: https://www.sciencedirect.com/science/article/pii/S1756464618303372. Acesso em: 13 ago. 2021.

SORIA, A. C.; VILLAMIEL, M. Effect of ultrasound on the technological properties and bioactivity of food : a review. **Trends in Food Science & Technology**, [London], v. 21, n. 7, p. 323–331, July 2010. Disponível em: https://www.sciencedirect.com/science/article/pii/S0924224410001056. Acesso em: 3 ago. 2021.

TACO. **Tabela Brasileira de Composição de Alimentos (TACO)**. 4. ed. Campinas: Núcleo de Estudos e Pesquisas em Alimentação (NEPA) – UNICAMP, 2011.

TAYLOR, P.; DEMIRDÖVEN, A.; BAYSAL, T. The use of ultrasound and combined technologies in food preservation. **Food Reviews International**, [Philadelphia], v. 25, n. 1, p. 1–11, Jan. 2009. Disponível em: https://www.tandfonline.com/doi/full/10.1080/87559120802306157. Acesso em: 10 ago. 2021.

TIWARI, B. K.; MASON, T. J. Ultrasound Processing of Fluid Foods. *In*: **Novel Thermal and Non-Thermal Technologies for Fluid Foods**. Amsterdam: Elsevier Inc., 2012. p. 135–165.

TORREGGIANI, D. Osmotic dehydration in fruit and vegetable processing. **Food Research International**, [Amsterdam], v. 26, n. 1, p. 59–68, 1993. Disponível em: https://www.sciencedirect.com/science/article/pii/096399699390106S. Acesso em: 12 jul. 2021.

VAN LAAR, A. D. E.; GROOTAERT, C.; VAN CAMP, J. Rare mono- and disaccharides as healthy alternative for traditional sugars and sweeteners? **Critical Reviews in Food Science and Nutrition**, [Philadelphia], v. 62, n. 5, p. 713-741, Mar. 2020. Disponível em: https://www.tandfonline.com/doi/full/10.1080/10408398.2020.1743966. Acesso em: 13 ago. 2021.

VAN ZANTEN, G. C. *et al*. The effect of selected synbiotics on microbial composition and short-chain fatty acid production in a model system of the human colon. **PLoS ONE**, [San Francisco], v. 7, n. 10, Oct. 2012. Disponível em: https://pubmed.ncbi.nlm.nih.gov/23082149/. Acesso em: 29 jul. 2021.

WANG, Z-P. *et al*. Whole Conversion of Soybean Molasses into Isomaltulose and Ethanol by Combining Enzymatic Hydrolysis and Successive Selective Fermentations. **Biomolecules**, [Basel], v. 9, n. 8, p. 353, Aug. 2019. Disponível em: https://www.mdpi.com/2218-273X/9/8/353. Acesso em: 29 jul. 2021.

WHO - WORLD HEALTH ORGANIZATION. **Increasing fruit and vegetable consumption to reduce the risk of noncommunicable diseases**. 2019. Disponível em: https://www.who.int/elena/titles/fruit_vegetables_ncds/en/. Acesso em: 21 nov. 2021.

WU, J. *et al*. Chemical compositional characterization of some apple cultivars. **Food Chemistry**, [Oxford], v. 103, n. 1, p. 88–93, Dec. 2007. Disponível em: https://www.sciencedirect.com/science/article/pii/S0308814606006157. Acesso em: 08 jul. 2021.

ZHENG, Y. *et al*. Display of a sucrose isomerase on the cell surface of *Yarrowia lipolytica* for synthesis of isomaltulose from sugar cane by - products. **Biotech**, [Heidelberg], v. 9, n. 5, p. 179, May 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31058045/. Acesso em: 29 jul. 2021.

CHAPTER TWO

ARTICLE 1 - REPLACEMENT OF NATIVE SUGARS OF APPLES BY ISOMALTULOSE IN DUAL STAGE

(Paper will be submitted for publication in the journal Innovative Food Science & Emerging Technologies)

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Abstract: The present study aimed to substitute native sugars of apples with isomaltulose by the dual-stage technique. In the first stage, native sugars were partially removed from the fruit tissue. In the second stage, isomaltulose was incorporated into the fruit. The processes were performed at 25 °C and 35 °C and the use of ultrasound (US) was evaluated. The isomaltulose incorporation was measured by the solid gain (SG). Other parameters as water loss (WL), related to the fruit dehydration, water activity (a_w), solid soluble content (°Brix), color parameters and shrinkage were also evaluated. The process that promoted the largest isomaltulose incorporation and higher dehydration consisted in the immersion of the apple slices in distilled water in the first stage (25 min) followed by the impregnation assisted by US for 40 min in the second stage. A reduction in the a_w, a change in color parameters and an increase in shrinkage (Sh) were also observed.

Keywords: D3S process, Ultrasound, Sugar substitution, Palatinose.

1. INTRODUCTION

Removing native sugars from food matrices and replacing them with another ingredient of interest can be a strategy to provide the consumer with healthier food. Dual-stage sugar substitution (D3S) is a technique that can contribute to this process as it consists of two stages, in which, in the first stage, native sugars are partially removed from the food through immersion in water and, in the second one, another substance is incorporated by immersion in hypertonic solution. Ultrasonic waves can be applied in one or both stages, improving the mass transfer rates (Garcia-Noguera et al., 2010; Medeiros et al., 2016, 2019; F. I. P. Oliveira et al., 2012; M. H. da S. B. Oliveira et al., 2021). The ultrasonic waves cause a rapid series of compressions and alternative expansions, also called the sponge effect, along with the cavitation phenomenon, are responsible for the creation of microscopic channels in porous materials, such as food, reducing the boundary layer of the diffusion between the liquid and the solid (J. L. G. G. Corrêa et al., 2015; Fernandes, Oliveira, et al., 2008; L. F. Oliveira et al., 2016).

Isomaltulose (D-glucopyranosyl-1, 6-fructose) is a disaccharide composed of one unit of glucose and one of fructose, being an isomer of sucrose, presenting a caloric value of 4 kcal / g (Holub et al., 2010). Although isomaltulose and sucrose have the same energetic value, isomaltulose provides glucose to the body for a longer time, while sucrose is readily absorbed (Van Laar et al., 2020). This guarantees a low, prolonged and balanced blood glucose supply, with a consequent low insulin demand. Such behavior is a reflection of the presence of the α-1-6 glycosidic bond, which is difficult to be hydrolyzed by gastrointestinal enzymes (Shyam et al., 2018). Isomaltulose is also a non-cariogenic carbohydrate since acids that are harmful to teeth are not produced because the bond is not broken by bacteria in the mouth (Sawale et al., 2017). Besides that, the ingestion of large amounts of isomaltulose does not cause adverse effects such as flatulence and diarrhea due to complete hydrolyzation in the human intestine, differently than other sweeteners (Lina et al., 2002). The prebiotic action is another benefit of this carbohydrate. Some studies presented that the promotion and selection of the growth of some probiotic strains beneficial to health such as *Lactobacillus acidophilus* (Van Zanten et al., 2012), *Lactoccocus sp.* (Van Zanten et al., 2012) e *Saccharomyces boulardii* (Mitterdorfer et al., 2001). Thus, it is a natural sweetener, classified as favorable for consumption by diabetic patients and also suitable as an ingredient in infant formulations. However, it has been little studied in impregnation processes. Replacing natural apple sugars with isomaltulose can result in a food matrix that is low in insulinemic sugar.

This work aimed to use the D3S technique evaluating the influence of immersion in water, the use of ultrasound and process temperature on mass transfer parameters and in the quality of the final pretreated apple. The study evaluated the condition that maximizes, firstly, the solid gain and water loss and then, evaluated the impact of treatments in the color parameters, shrinkage, water activity and solid soluble of the final product.

2. MATERIALS AND METHODS

2.1. Materials

Fuji apples (*Malus domestica L.*) were obtained from a local market (Lavras, MG, Brazil). Only fruits with the same size, weight, color and firmness were used in the experiment. The fruits were peeled and cut into slices (2.0 × 2.0 × 0.4 cm) using cutters designed for this purpose.

2.2 Dual-stage process

In the first stage of the D3S technique, the samples were immersed in distilled water, with or without the application of ultrasound (US) (Unique, model USC-2850, Brazil). The ultrasound frequency was 25 kHz and the power intensity was 8 kWm^{-3}. The ratio between water and the fruit was 4:1(w/w) to maintain a great chemical potential for the mass transfer between water and the food. The time of immersion in water in the first stage was 25 min, determined by preliminary tests, leading to a maximum reduction in native soluble solids content.

The second stage consisted of the immersion of the samples in isomaltulose solution in a concentration of 40 kg isomaltulose/100 kg solution, based on the maximum solubilization achieved under heating to 40 °C. The same weight ratio between the fruit and the liquid medium used in the first stage was applied at this stage to avoid the dilution of the osmotic solution. The total time in this stage was 40 min and, when the US was used, the time of its application was 25 or 40 min. This condition was determined by preliminary tests (data here not shown) aiming for greater incorporation and shorter process time. Both stages were submitted to two different processing temperatures, 25 and 35 °C. The increase in the temperature by the use of US was monitored and controlled to be lower than 2 °C.

The condition of the impregnation without the first stage and US in the second stage was considered as a control treatment. Table 1 presents the experimental codes of the all treatments performed.

Table 1 Experimental conditions.

Test	Description of test
T_1	Impregnation at 25 ° C
T_2	Impregnation at 35 ° C
T_3	Dual-stage process at 25 °C
T_4	Dual-stage process at 35 °C
T_5	Dual-stage process with ultrasound assistance in the first stage and impregnation at 25 °C
T_6	Dual-stage process with ultrasound assistance in the first stage and impregnation at 35 °C
T_7	Impregnation with ultrasound assistance for 25 min at 25 ° C
T_8	Impregnation with ultrasound assistance for 40 min at 25 ° C
T_9	Impregnation with ultrasound assistance for 25 min at 35 ° C
T_{10}	Impregnation with ultrasound assistance for 40 min at 35 ° C
T_{11}	Dual-stage process with ultrasound assistance in the second stage for 25 min at 25 °C
T_{12}	Dual-stage process with ultrasound assistance in the second stage for 40 min at 25 °C
T_{13}	Dual-stage process with ultrasound assistance in the second stage for 25 min at 35 °C
T_{14}	Dual-stage process with ultrasound assistance in the second stage for 40 min at 35 °C
T_{15}	Dual-stage process with ultrasound assistance in the first stage for 25 min and the second stage for 25 min at 25 °C
T_{16}	Dual-stage process with ultrasound assistance in the first stage for 25 min and the second stage for 40 min at 25 °C
T_{17}	Dual-stage process with ultrasound assistance in the first stage for 25 min and the second stage for 25 min at 35 °C
T_{18}	Dual-stage process with ultrasound assistance in the first stage for 25 min and in the second stage for 40 min at 35 °C

After the end of each stage, the samples were drained and placed on absorbent paper to remove the excess water or solution. All experiments were carried out in triplicates.

The moisture content of the samples was determined according to the method 934.06, in a vacuum oven at 70 °C for 24 h (AOAC, 1990). The weight and moisture content data of

each sample were used to calculate the solid gain (SG) and water loss (WL), according to the following equations:

$$SG = \frac{X_f^{ST} M_f^0 - X_0^{ST} M_0^0}{M_0^0} \quad (1)$$

$$WL = \frac{X_0^w M_0^0 - X_f^w M_f^0}{M_0^0} \quad (2)$$

where M is the sample weight, X^w is the moisture content, and X^{ST} is the weight of the solids (dry matter); the sub-indices 0 and f correspond to the initial and impregnated sample, respectively.

2.3. Quality analysis

2.3.1. Volumetric shrinkage (Sh)

The thickness was obtained as an arithmetical average of measurements at five different points on the sample with the use of a digital caliper (Western, 150 mm-DC-60, China). The surface area of the samples was measured directly from the photographs with the aid of image analysis software ImageJ®. The area and thickness measurements were done in quintuplicate. The volume was calculated by multiplying the surface area by the sample thickness. The volumetric shrinkage (Sh) was expressed as the ratio between the volume after and before pretreatment, and the closer to unity, the smaller the volumetric ratio of the sample (Udomkun & Innawong, 2018) (Eq. 7):

$$Sh = \frac{V_o - V_f}{V_o} \quad (3)$$

where V_o is the initial volume of apples (m^3) and V_f is the volume after the immersion process (m^3).

2.3.2. Soluble Solid

Soluble solids were analyzed in samples after impregnation and in raw material employing the refractometric method (RE50 Refractometer, Mettler Toledo) (Instituto Adolfo Lutz, 2008).

2.3.3. Moisture content and water activity

The moisture content of the fruit was determined in a vacuum drying oven at 70 °C until constant weight (AOAC, 2010). The water activity was measured with a dew-point hygrometer (Decagon Devices Inc., Aqualab Series 3, USA). Both analyses were done in triplicate.

2.3.4. Color

The measurement of color parameters was based on the CIE $L^*a^*b^*$ and CIE $L^*C^*h^\circ$ evaluated with D65 illuminant. The measurements were done directly with a Minolta (CR300) in five samples and the average values were reported. The total color difference (ΔE) was also determined (Kroehnke et al., 2018).

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (4)$$

Where L^* indicates the lightness (100 for white to 0 for black), a^* indicates red when positive and green when negative, and b^* indicates yellow when positive and blue when negative. The subscript 0 refers to the fresh fruit color parameters.

2.3.5. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to analyze the internal structure of the vegetables, before and after osmotic processing. Initially, all samples were fixed with a modified Karnovsky solution for 24 h. These samples were immersed in a cryoprotectant solution (30% glycerol in water) for 30 min and transversely sectioned with a scalpel after immersion in liquid nitrogen. These sections were rinsed in distilled water and post-fixed in 1% osmium tetroxide aqueous solution for 1 h and then dehydrated in an increasing series of acetone solution (25%, 50%, 70%, and 90% for 10 min, and thrice for 10 min at 100%). After dehydration, the samples were subjected to drying on a Critical Point Drier (CPD 030®; Balzers, Germany). Then, the samples were placed on aluminum supports and covered with a film of aluminum foil by using double-sided carbon tape. Finally, all samples were covered with a layer of gold using a Sputter coater (SCD 050®; Balzers, Germany) and examined with a SEM scanning electron microscope (LEO Evo 40; Zeiss, Cambridge, UK) operating at 20 kV, with a working distance of 8.5 mm (J. L. G. G. Corrêa et al., 2015).

2.4. Statistical analysis

Initially, the two main responses of the experiments, WL and SG, went through a screening of the factors with the factorial planning. After selecting the most relevant conditions, a response surface analysis was performed. To select the most viable treatment, we considered the responses obtained by the average of the three repetitions concerning the variables SG ($_j$) and WL ($_j$) (j = 1, ..., n), with n being the number of the total treatments.

To have a better interpretation, the transformation (Eq. 5 and 6) was considered, so that the answers were limited to a continuous scale of 0-1, making it possible to interpret them as indices, represented by ySG(j) e yWL(j).

$$y_{SG(j)} = \frac{SG_j - \text{minimum}(SG_j)}{\text{maximum}(SG_j) - \text{minimum}(SG_j)} \quad (5)$$

$$y_{WL(j)} = \frac{WL_j - minimum(PA_j)}{maximum(PA_j) - minimum(PA_j)} \quad (6)$$

To have better confirmation of the similarity between these responses, the graphic technique Q-Qplot was used, in which the probabilities accumulated by the normal distribution in relation to the quantiles (Eq. 7 and 8), defined by yGS (j) and yWL (j) and compared with empirical probabilities (Eq. 2).

$$\left(\frac{j - \frac{1}{2}}{n}\right) = \int_{-\infty}^{y_{gs(j)}} \frac{1}{\sqrt{2\pi}} exp\left(-\frac{z^2}{2}\right) \partial_z) \quad (7)$$

$$\left(\frac{j - \frac{1}{2}}{n}\right) = \int_{-\infty}^{y_{pa(j)}} \frac{1}{\sqrt{2\pi}} exp\left(-\frac{z^2}{2}\right) \partial_z \quad (8)$$

Where *n* is the number of experimental points, the value ½ refers to the continuity correction and z is the standardized value of the normal distribution (0.1).

The linear relationship between these probabilities was represented graphically so that treatments close to the least-squares line were identified as negligible effects to the mean of the response variables.

Subsequently, the biplot technique with predictive axes (Alves, 2012) was used to study the similarity between treatment groups, considering a multivariate approach, using the R (R Team., 2021) with software package biplotGUI.

2.5. RESULTS AND DISCUSSION

The ultrasound condition for the first step of D3S was optimized in a previous study. The initial moisture content for apple was 84.9 ± 1.1% wet basis (w.b) and solid content of 0.151 ± 0.011 kg solids/kg of food. After immersion in distilled water, the moisture content was 7.58±0.25 kg water/kg dry matter and solid contents of 0.12±0.01 kg /kg dry matter. The use of ultrasound in the first stage in the D3S process led to a solid soluble loss of about 22% and an increase in the moisture content by about 3%. A similar loss of native sugars was observed by FERNANDES, LINHARES, RODRIGUES, (2008) in a study of immersion of pineapple in

water assisted by US. It is interesting to note that it was reported that the gained moisture in such a process does not increase the energy demand in further drying (Fernandes & Rodrigues, 2008). Concerning SG, it is common to consider the loss of sugars equal to the loss of soluble solids and, in this case, glucose and fructose were the main sugars lost (F. I. P. Oliveira et al., 2012). The alterations in the moisture and solid content of the food by the immersion in distilled water are consequences of the gradient of chemical potential between the food and the immersion media. In addition, the formation of microscopic channels in the fruit tissue, formed by ultrasonic waves, contributes to the mass transfer (Fernandes & Rodrigues, 2008). Thus, this removal of native sugars could contribute to the production of apple chips as a fast-food option for sugar-restricted consumers.

The analysis for the study of the estimates of the effects of the treatments was carried out with the construction of the QQ_{plot} graphs, in which, each quantile was estimated according to the accumulated probabilities, represented in Figures 1 to 7. The points in Figures 1 to 4 represent the treatments and its possible to observe that some of them are far from the line. This indicates that the effects of these treatments are significantly different from zero. The evaluation of the QQ_{plot} graphs on the SG and WL was made by separating the treatments in which they received or not the ultrasonic waves in the impregnation stage.

Figure 1 presents only the treatments that did not receive ultrasound in the impregnation stage, called ordinary OD. It can be seen that the points T1, T2, T5 and T6 are the treatments that deserve attention in the study as they may have significance. For the other treatments, it can be seen that they are distributed close to the straight line, so there are signs that they are not significant for SG. However, as the focus of the research is the greater incorporation of isomaltulose by apple, and only the treatments T2 and T6 would be those with the greatest chances of presenting some positive and significant difference from the other treatments.

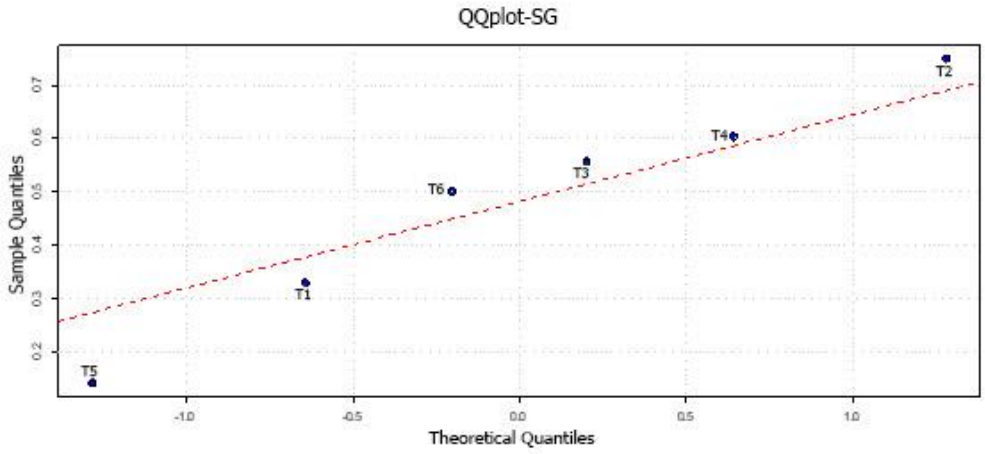

Figure 1 Chart of accumulated normal quantiles for solids gain of treatments submitted to ordinary osmotic dehydration with or without previous water immersion.

T2 represents the treatments where apple slices were subjected to OD for 40 min at 35 °C, while T6 represents treatments in which samples were immersed in water, assisted by ultrasound in the first stage, and submitted to the ordinary OD, for 40 min at 35 °C. T2 led to an SG of 1.927% ±0.360 and T6, 8.084% ±0,919. Thus, the effect of temperature on the behavior obtained is observed, in which at 35 °C it seemed to favor SG when compared to its similar treatment at 25 °C, increasing the SG by at least 11%. Furthermore, it appears that the immersion in water assisted with ultrasound contributed to the increase in GS in the second step, by three times. The increase in the temperature leads to the increase in the permeability of the membranes and the release of the air trapped in the porous structure of the food tissue, improving the removal of water and the absorption of the solids. In addition, the influence of temperature on SG is related to the reduction of the solution viscosity, which intensifies the mass transfer rates (J. L. G. Corrêa et al., 2016; Ramya & Jain, 2016).

The effectiveness of using ultrasound in the water immersion stage may be related to the "sponge effect" and cavitation. These phenomena can modify the structure of cell walls and cell membrane of food tissues, creating microchannels and producing turbulence in the solution, reducing the boundary layer, favoring WL and SG (Nowacka et al., 2021). The trends observed, concerning the SG, were similar to those observed by Prithani and Dash (2020), who also immersed the kiwi slices in water assisted by ultrasound, followed by ordinary OD, concluding that the ultrasound caused an increase in WL and SG.

Figure 2, shows the treatments with ultrasound also during the impregnation stage. It can be observed that the effects of T16 and T17 treatments can be important in SG.

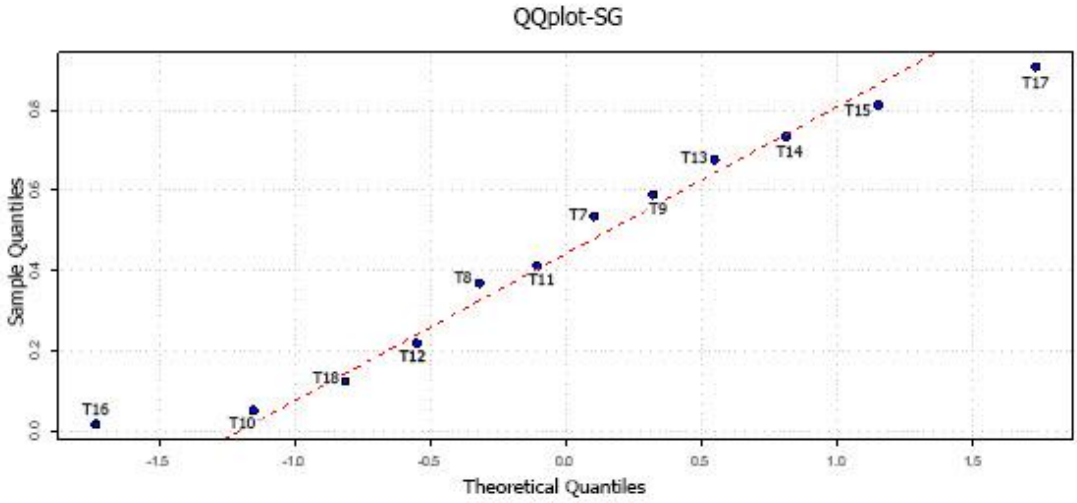

Figure 2 Chart of accumulated normal quantiles for solids gain of treatments submitted to OD assisted by ultrasound, with or without pre-treatment in water.

By focusing on the behavior of interest, that is, maximizing GS, the treatment T17 may lead to significant gains of isomaltulose. This treatment is represented by the samples that were pre-treated in water, assisted by ultrasound and submitted to OD for 25 min also assisted by ultrasound, at 35 °C, resulting in a SG of 8.196 % ±1.862. When comparing T16 and T17, it is possible to observe that the excessive use of ultrasound in processing can drastically affect the SG. A similar result was found by Garcia-Noguera et al. (2010) in the use of the D3S method in strawberry slices incorporated with stevioside and rebaudioside. In this study, the application of ultrasound in both stages led to lower incorporation of the sweetener, indicating that the use of ultrasound in excess is not as advantageous. This behavior was justified by those authors due to the effect of ultrasound on the extraction of solids, which would reduce the incorporation of solute by the sample. This trend about to SG was also observed by Oliveira et al. (2012), in an attempt to replace native Malay apple sugars with stevioside and rebaudioside.

Figure 3 presents the graph of the quantiles of the accumulated normal for WL and, analyzing the arrangement of the points, the results showed that even subjectively it is possible to observe that all points are located close to the straight line. However, some points may supposedly point out doubts concerning this proximity, being represented by T3, T4 and T6, these treatments seem to be important in WL, and a model for confirmatory analysis may be adjusted. As in the SG, the interest of the research is to promote greater WL, so that the moisture content of the food is reduced as much as possible and, consequently, there is a reduction in cost and time in the subsequent stabilization processes. Therefore, the treatments that seem to meet this requirement are T4 and T6. These treatments were the ones in which the apples were immersed in water in the first stage and submitted to OD in the second stage, for 40 min, at 35

°C and to those that were immersed in water assisted by ultrasound in the first stage and submitted to OD in the second stage, for 40 min, also at 35 °C, respectively. T4 led to a WL of 18.614% ± 1.792 and T6 of 18.209% ± 1.760.

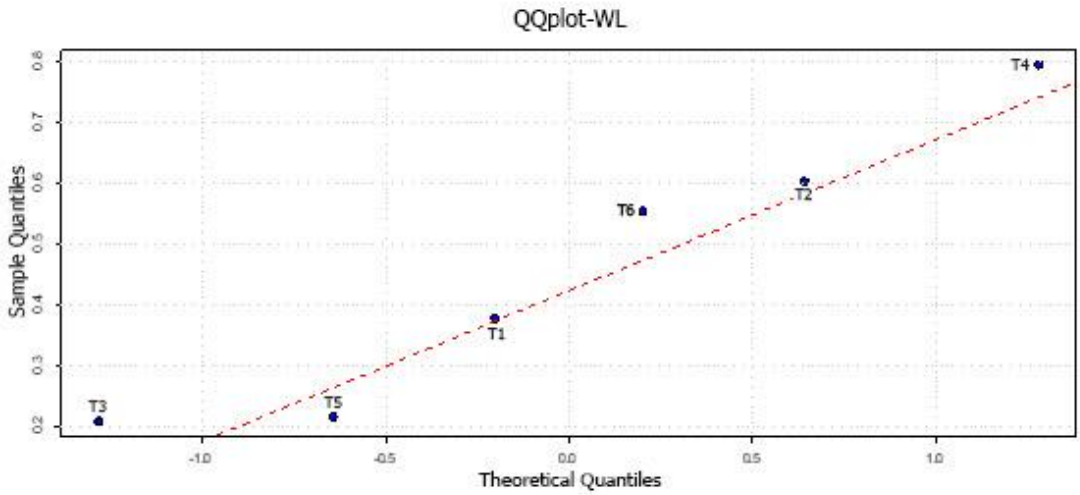

Figure 3 Chart of accumulated normal quantiles for water loss of treatments submitted to ordinary OD, with or without pre-treatment in water

Through this behavior and similarly to solids gain, it can be assumed that temperature at the highest level (35 °C), can be a good strategy for apple dehydration. In addition, the use of pretreatment by water immersion assisted or not by ultrasound can also contribute to WL.

Through the graph of WL obtained by treatments submitted to OD assisted by ultrasound, with or without pre-treatment in water (Figure 4), is possible to notice that there are indications that the effects of treatments T10, T11, T12, T14, T15 and T17 are significant in this parameter. However, only treatments T10, T14 and T17 can be interesting to maximize the WL. Comparing these three treatments, it is possible to verify that all of them were performed at the highest level of temperature studied, confirming that the temperature in an impregnation process favors the mass transfer.

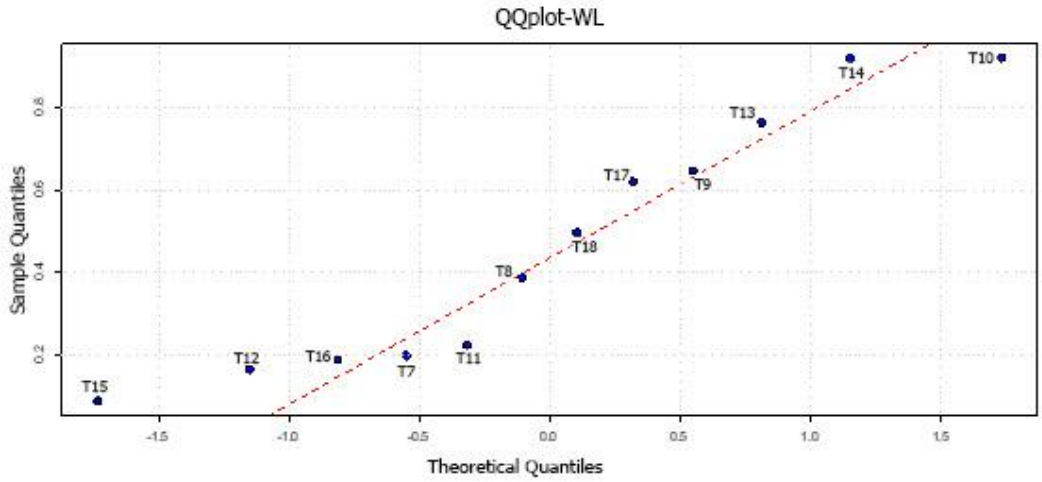

Figure 4 Chart of accumulated normal quantiles for water loss of treatments submitted to OD assisted by ultrasound, with or without pre-treatment in water

In a joint analysis of the graphs of the quantiles of the normal accumulated by WL and SG, treatments T2, T4, T6, T14 and T17 were the ones that showed the most efficiency about these parameters. In these treatments, both parameters were found above 0.5 points, which, on a real scale, represents 15.894% ± 1.556, 18.614 % ± 1.792, 18.209 % ± 1.760, 21.194 % ± 0.497 and 21.699 % ± 2.329 for WL and 1.927% ± 0.360, 7.332% ± 1.096, 8.084% ± 0.919, 8.146 % ± 0.963 and 8.196 % ± 1.862, for SG, respectively. The processing temperature of 35 °C is the common variable between this group of treatments and it is also possible to notice that there is a threshold regarding the use of ultrasound. The number of works that observe a better performance in the parameters of mass transfer with the increase of the OD temperature is extensive and is commonly associated with a decrease in the viscosity of the solution and an increase in cell permeability (Spinei & Oroian, 2021). For the use of ultrasound in the D3S steps, treatments that probably favor mass transfer are those that use ultrasound only in OD for a longer time or those that use ultrasound in both stages of D3S, that is, in the pre-treatment in water and OD, but in a shorter time. Garcia-Noguera et al. (2010) used D3S processing in strawberries to incorporate low-calorie sugar (stevioside and rebaudioside). They also observed that ultrasound in both stages of the D3S did not favor water loss or solids gain. Thus, they concluded that ultrasound-assisted immersion in distilled water and subsequent immersion in an osmotic solution of stevioside and rebaudioside, without the use of ultrasound, was the most viable processing condition.

The second group of responses was analyzed through the multidimensional scaling of the interpretations of the generated biplots. Thus, following the discussion of the results shown in Fig.5, 6 and 7 containing the following variables: final water activity (a_w), soluble solids

(°Brix), shrinkage (Sh) and the color parameters, which are total color difference (ΔE), red/green coordinate (a^*), yellow/blue coordinate (b^*), chroma (C^*) and hue angle (h°). In all figures, we have grouped the treatments and contained them within regions bounded by the variables.

Fig. 5 represents the biplot of treatments that were subjected to ordinary OD at 25 and 35 °C. Samples T7 and T8 show high similarity to each other and are distinct from the other samples. These two samples were subjected to OD assisted by ultrasound for 25 and 40 min, respectively, at 25 °C. These treatments may contribute mainly to the lower a_w of the samples. In addition, these treatments also influenced the ΔE, Sh and h°. Thus, when comparing with T1, without the use of ultrasound, we noticed an interesting influence of T7 and T8 on a_w. This may be related to the modification of the permeability of the apple tissue, which favors the incorporation of solute and, consequently, there is a reduction in a_w. On the other hand, in the literature, it is common to observe a lower a_w with the increase in the temperature of the impregnation process (Spinei & Oroian, 2021). However, this behavior was not observed comparing T7 and T8. These treatments were the ones that had the greatest influence on the ΔE, higher h° and lower Sh. Regarding the color parameters, this behavior can be explained by the fact that T7 and T8 were more yellowish from the incorporation of the solute corroborating the finding of a_w. Complementarily, the Sh of these treatments is contained since the solutes participate in the maintenance of the tissue structure.

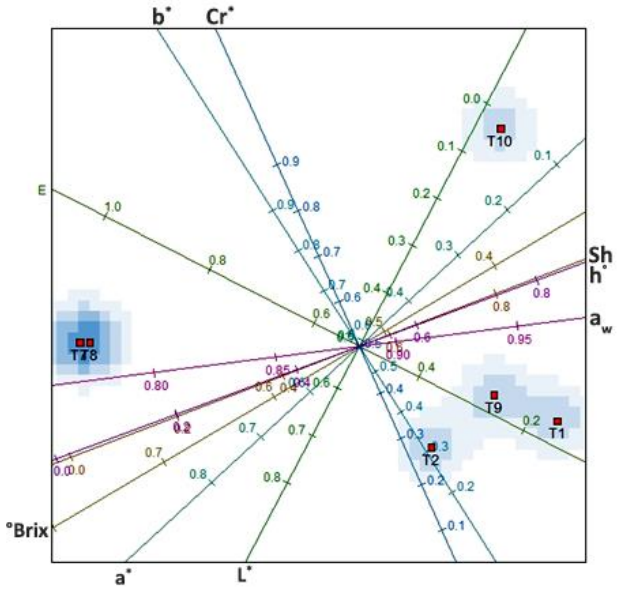

Figure 5 Biplots of treatments that subjected to osmotic dehydration at 25 and 35 °C, considering the following variables: final water activity (aw), soluble solids (°Brix), shrinkage (Sh) and the color parameters, which are total color difference (ΔE), red/green coordinate (a*), yellow/blue coordinate (b*), chroma (Cr*) and hue angle (h°).

It was observed that there was a grouping trend between treatments T1, T2 and T9, suggesting the same behavior about the ΔE, b^*, C* and a_w. The smaller ΔE found may indicate that these treatments are good for maintaining the color of the apple, in which they are slightly yellow and with low saturation, based on the observation of b^* and C* values. However, these treatments were not efficient in reducing the a_w of dehydrated apples. T10 was the most different from the other treatments, producing slightly darkened and greenish apples. Furthermore, this treatment was the one that presented the lowest soluble solids of apples, corroborating with the low value of SG. These apples were obtained in a condition of higher temperature (35 °C) and longer ultrasound time during ordinary impregnation (40 min), suggesting that this condition favored the enzymatic browning due to the extensive alteration in the tissue structure (J. L. G. G. Corrêa et al., 2015).

The second group that was analyzed from the biplot, shown in Fig. 6, was the one in which apples were subjected to immersion in water as a pre-treatment to impregnation. In this case, treatments T3 and T12 were similar to each other and distinct from the other treatments. Both treatments are related to immersed samples in water, submitted to an OD for 40 min, at 25 °C. T3 was conducted in an ordinary OD while T12 was assisted by ultrasound during the 40 min in the OD step. T3 was conducted in an ordinary OD while T12 was assisted by ultrasound for 40 min in the OD step. These samples were the ones that showed a strong correlation with the color parameters L^*, a^* and h°, indicating that the samples were lighter and yellower. In addition, these treatments led to less Sh of the samples. T13 and T14 produced data points grouped based on L^*, h°, and Sh generating darker, yellower, and more shrunken samples. Changes in color parameters could be associated with the increased solids concentration due to the palatinose impregnation, which leads to reduced L* values (J. L. G. G. Corrêa et al., 2015).

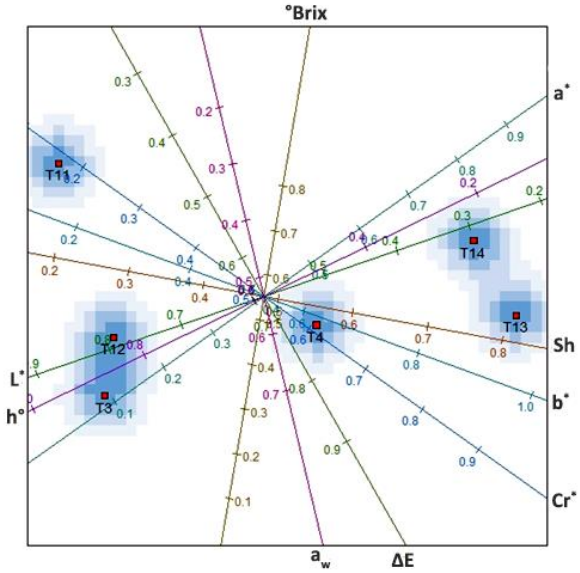

Figure 6 Biplots of treatments that subjected to immersion in water as a pretreatment to osmotic dehydration, at 25 and 35 °C, considering the following variables: final water activity (aw), soluble solids (°Brix), shrinkage (Sh) and the color parameters, which are total color difference (ΔE), red/green coordinate (a^*), yellow/blue coordinate (b^*), chroma (C^*) and hue angle (h°).

T4 and T11 were the treatments most distinct from the others and each other, positioning themselves oppositely from the points of the color parameters, ΔE, b^* and Cr. From the difference between these treatments, which is the use of ultrasound in the impregnation stage, there is an indication that ultrasound favors the maintenance of the apple color parameters, although these have been less vivid and yellowish than fresh fruit.

Figure 7 shows the biplots of treatments that were subjected to immersion in water assisted by ultrasound as a pretreatment to isomaltulose impregnation assisted or not by ultrasound, at 25 and 35 °C. T5 was the one that differs from the others in terms of L^* and h° parameters, indicating that this sample was luminous and yellowish. The T6 treatment also differed from the other treatments and behaved in an opposite way to the T5 treatment, producing apples less luminous but still yellowish only with the change in process temperature. The difference between T5 and T6 is concerning the process temperature, where T5 occurred at 25 °C and T6 occurred at 35 °C. Thus, we can associate the change in color parameters to greater absorption of solutes by the fruit matrix due to the temperature of the process, increasing the concentration of solids. Furthermore, it is also possible to observe that the soluble solids were favored by the increase in temperature, corroborating the QQplot analysis.

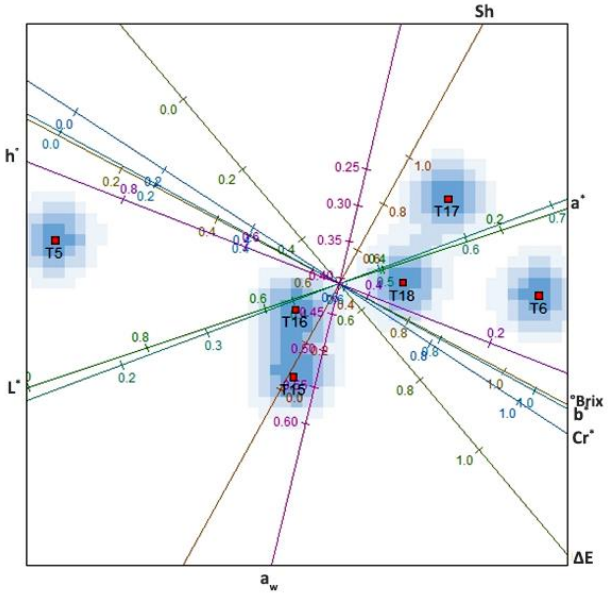

Figure 7 Biplots of treatments that subjected to immersion in water assisted by ultrasound as a pre-treatment to osmotic dehydration, at 25 and 35 °C, considering the following variables: final water activity (a_w), soluble solids (°Brix), shrinkage (Sh), total co color difference (ΔE), red/green coordinate (a^*), yellow/blue coordinate (b^*), chroma (C^*) and h° angle (h).

Treatments T17 and T18 show a certain similarity for Sh, a_w, °Brix and the color parameters L^*, a^*, b^*, C^* and h°. These treatments were the most drastic regarding the temperature and use of ultrasonic waves in D3S stages. Thus, apple slices showed greater Sh, due to the greater extent of damage to the cell wall caused by the treatment. In this case, although they obtained a low a_w and a considerable soluble solids content (°Brix), the incorporation was not efficient to contain the Sh. Regarding the color parameters, it is possible to observe that the apples presented similar behavior to the previous treatments, where the samples remained yellowish, with greater saturation and slightly darkened, also associated with changes caused by the incorporation of solids.

Taking a general approach to the color parameters, it is observed that apples with less ΔE were obtained in those treatments submitted to 25 °C with the presence of the immersion step in the water, assisted or not by ultrasound. The L^* of the samples was also greater at 25 °C, with decreasing order of brightness: impregnation > treatments which used water immersion step > treatments which used water immersion step assisted by US. All treatments resulted in yellowish samples, with h° varying between 93.06 and 98.93 and b^* ranging between 19.91 and 25.73 and also samples with lower color intensity compared to fresh apple (C^* 35.80 ± 0.30), with C^* ranging from 20.05 to 25.67. Regarding Sh, processing at 25 °C in an ordinary OD showed lower values for this variable. The a_w of apples impregnated with isomaltulose was influenced by temperature and also by the presence of ultrasound in the D3S

stages, with behavior similar to WL. The soluble solids content in the samples was mainly favored by the insertion of the immersion step in water-assisted or not by ultrasound.

Assessing the behavior to the secondary variables of treatments T2, T4, T6, T14 and T17, which were those treatments that were shown to influence WL and SG, T14 may be a treatment that contributes to better results, primarily targeting the mass transfer parameters, WL and SG, followed by the quality parameters. This treatment presented, in real values, Sh of 28.7 ± 3.6, °Brix of 20.1 ± 1.1, ΔE of 2.91 ± 0.45, L^* of 73.58 ± 0.82, a^* of -1.36 ± 0.21, b^* of 23.44 ± 0.69, C^* of 23.30 ± 0.77, h° 93.29 ± 0.75, and a_w of 0.971 ± 0.001.

To evaluate the impact of the best impregnation treatment on microstructure, T14, the samples were analyzed using a SEM. The differences between fresh and impregnated tissue are shown in Figs. 8a and 8b.

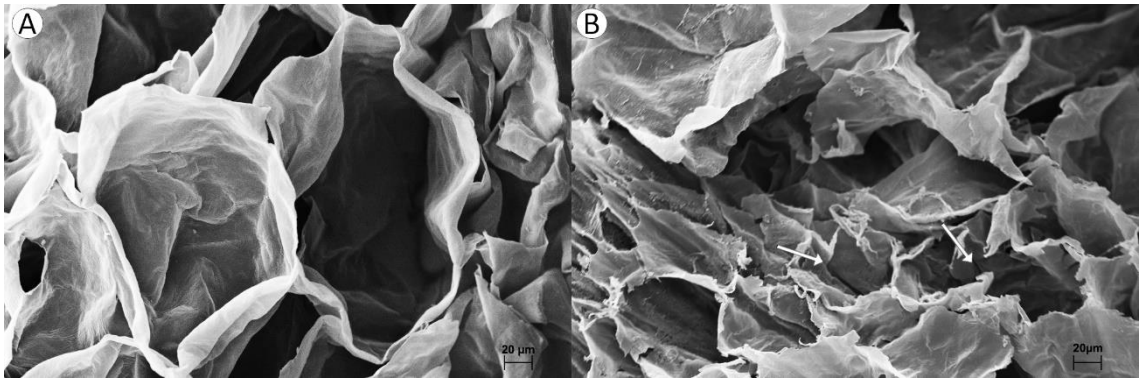

Figure 8 Scanning electron micrograph (600X) of apple slices (A) fresh, (B) treated by the dual-stage process with ultrasound assistance in the second stage for 40 min at 35 °C (T14).

For fresh apple tissues, the cells were round and long in shape, and tightly connected, with defined and structured cell walls. For samples subjected to T14, the cells were irregularly shaped and moderately collapsed with slight tissue disruption. These structural changes are associated with US application and contribute to mass transfer in impregnation processes (J. L. G. G. Corrêa et al., 2015). This behavior justifies the good incorporation of isomaltulose and good dehydration obtained with T14, since the ultrasound only led to moderate tissue damage, not excessively corrupting the tissue.

2.6. CONCLUSION

The dual-stage technique (D3S) carried out with immersion in water in the first stage, followed by osmotic dehydration assisted by ultrasound was the best condition to obtain the substitution of the native sugar of apples for isomaltulose. Regarding the color parameters, the use of a stage with immersion in water leads to lesser color variations, appearing less

luminous and more yellowish. The ultrasound assistance favored shrinkage and increase the soluble solids content.

REFERENCES

Alves, M. R. (2012). Evaluation of the predictive power of biplot axes to automate the construction and layout of biplots based on the accuracy of direct readings from common outputs of multivariate analyses: 1. application to principal component analysis. *Journal of Chemometrics*, *26*(5), 180–190. https://doi.org/10.1002/cem.2433

AOAC. (1990). *Official methods of analysis of AOAC INTERNATIONAL*. method 934.06.

Corrêa, J. L. G., Ernesto, D. B., Mendonça, K. S. de, & de Mendonça, K. S. (2016). Pulsed vacuum osmotic dehydration of tomatoes: Sodium incorporation reduction and kinetics modeling. *Food Science and Technology*, *71*, 17–24.

Corrêa, J. L. G. G., Justus, A., de Oliveira, L. F., & Alves, G. E. (2015). Osmotic Dehydration of Tomato Assisted by Ultrasound: Evaluation of the Liquid Media on Mass Transfer and Product Quality. *International Journal of Food Engineering*, *11*(4), 505–516. https://doi.org/10.1515/ijfe-2015-0083

Fernandes, F. A. N., Linhares, F. E., & Rodrigues, S. (2008). Ultrasound as pre-treatment for drying of pineapple. *Ultrasonics Sonochemistry*, *15*(6), 1049–1054. https://doi.org/10.1016/j.ultsonch.2008.03.009

Fernandes, F. A. N., Oliveira, F. I. P., & Rodrigues, S. (2008). Use of ultrasound for dehydration of papayas. *Food and Bioprocess Technology*, *1*(4), 339–345. https://doi.org/10.1007/s11947-007-0019-9

Fernandes, F. A. N., & Rodrigues, S. (2008). Application of ultrasound and ultrasound-assisted osmotic dehydration in drying of fruits. *Drying Technology*, *26*(12), 1509–1516. https://doi.org/10.1080/07373930802412256

Garcia-Noguera, J., Weller, C. L., Oliveira, F. I. P., Rodrigues, S., & Fernandes, F. A. N. (2010). Dual-stage sugar substitution in strawberries with a Stevia-based sweetener. *Innovative Food Science and Emerging Technologies*, *11*(1), 225–230. https://doi.org/10.1016/j.ifset.2009.07.001

Holub, I., Gostner, A., Theis, S., Nosek, L., Kudlich, T., Melcher, R., & Scheppach, W. (2010). Novel findings on the metabolic effects of the low glycaemic carbohydrate isomaltulose (Palatinose™). *British Journal of Nutrition*, *103*(12), 1730–1737. https://doi.org/https://doi.org/10.1017/S0007114509993874

Instituto Adolfo Lutz. (2008). *Métodos físico-químicos para análise de alimentos* (N. S. P. e P. T. Coordenadores Odair Zenebon (ed.); 4th ed.).

Lina, B. A. R., Jonker, D., & Kozianowski, G. (2002). Isomaltulose (Palatinose®): a review of biological and toxicological studies. *Food and Chemical Toxicology*, *40*, 1375–1381. https://doi.org/https://doi.org/10.1016/S0278-6915(02)00105-9

Medeiros, R. A. B. ., Barros, Zilmar Meireles Pimenta; Carvalho, C. B. O., Fraga Neta, E. G., Maciel, M. I. S., & Azoubel, P. M. (2016). Influence of dual-stage sugar substitution pretreatment on drying kinetics and quality parameters of mango. *LWT - Food Science and Technology*, *67*, 167–

173. https://doi.org/10.1016/j.lwt.2015.11.049

Medeiros, R. A. B. ., Barros, Z. M. P. ., Carvalho, C. B. O. ., Coêlho, A. C. D. ., Maciel, M. I. S. ., & Azoubel, P. M. . (2019). Dual-stage sugar substitution in Tommy Atkins mango. *Revista Brasileira de Engenharia Agricola e Ambiental*, *23*(1), 60–64. https://doi.org/10.1590/1807-1929/agriambi.v23n1p60-64

Mitterdorfer, G., Kneifel, W. ., & Viernstein, H. . (2001). Utilization of prebiotic carbohydrates by yeasts of therapeutic relevance. *Letters in Applied Microbiology*, *33*(4), 251–255. https://doi.org/10.1046/j.1472-765X.2001.00991.x

Nowacka, M., Dadan, M., & Tylewicz, U. (2021). Current applications of ultrasound in fruit and vegetables osmotic dehydration processes. *Applied Sciences (Switzerland)*, *11*(3), 1–22. https://doi.org/10.3390/app11031269

Oliveira, F. I. P., Rodrigues, S., & Fernandes, F. A. N. (2012). Production of low calorie Malay apples by dual stage sugar substitution with Stevia-based sweetener. *Food and Bioproducts Processing*, *90*(4), 713–718. https://doi.org/10.1016/j.fbp.2012.02.002

Oliveira, L. F., Corrêa, J. L. G., de Angelis Pereira, M. C., de Lemos Souza Ramos, A., & Vilela, M. B. (2016). Osmotic dehydration of yacon (Smallanthus sonchifolius): Optimization for fructan retention. *LWT - Food Science and Technology*, *71*, 77–87. https://doi.org/10.1016/j.lwt.2016.03.028

Oliveira, M. H. da S. B., Ferreira Filho, A. J. M., Silva Júnior, E. V., da Silva, E. S., Paim, A. P. S., Honorato, F. A., & Azoubel, P. M. (2021). Impregnation and drying to develop a melon snack enriched in calcium. *Journal of Food Science and Technology*, *58*(2), 672–679. https://doi.org/10.1007/s13197-020-04581-5

Prithani, R., & Dash, K. K. (2020). Mass transfer modelling in ultrasound assisted osmotic dehydration of kiwi fruit. *Innovative Food Science and Emerging Technologies*, *64*(September 2019), 102407. https://doi.org/10.1016/j.ifset.2020.102407

R Team., R. D. C. (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Ramya, V., & Jain, N. K. (2016). A Review on Osmotic Dehydration of Fruits and Vegetables: An Integrated Approach. *Journal of Food Process Engineering*, *40*(3), 1–22. https://doi.org/https://doi.org/10.1111/jfpe.12440

Sawale, P. D., Shendurse, A. M., Mohan, M. S., & Patil, G. R. (2017). Isomaltulose (Palatinose) – An emerging carbohydrate. *Food Bioscience*, *18*(April), 46–52. https://doi.org/10.1016/j.fbio.2017.04.003

Shyam, S., Ramadas, A., & Chang, S. K. (2018). Isomaltulose: Recent evidence for health benefits. *Journal of Functional Foods*, *48*(May), 173–178. https://doi.org/10.1016/j.jff.2018.07.002

Spinei, M., & Oroian, M. (2021). The influence of osmotic treatment assisted by ultrasound on the physico-chemical characteristics of blueberries (Vaccinium myrtillus L.). *Ultrasonics*, *110*,

106298. https://doi.org/https://doi.org/10.1016/j.ultras.2020.106298

Udomkun, P., & Innawong, B. (2018). Effect of pre-treatment processes on physicochemical aspects of vacuum-fried banana chips. *Journal of Food Processing and Preservation*, *42*(8), 1–10. https://doi.org/10.1111/jfpp.13687

Van Laar, A. D. E., Grootaert, C., & Van Camp, J. (2020). Rare mono- and disaccharides as healthy alternative for traditional sugars and sweeteners? *Critical Reviews in Food Science and Nutrition*, *0*(0), 1–29. https://doi.org/10.1080/10408398.2020.1743966

Van Zanten, G. C., Knudsen, A., Röytiö, H., Forssten, S., Lawther, M., Blennow, A., Lahtinen, S. J., Jakobsen, M., Svensson, B., & Jespersen, L. (2012). The Effect of Selected Synbiotics on Microbial Composition and Short-Chain Fatty Acid Production in a Model System of the Human Colon. *PLoS ONE*, *7*(10). https://doi.org/10.1371/journal.pone.0047212

ARTICLE 2 - DRYING OF APPLE ENRICHED WITH ISOMALTULOSE BY THE DUAL-STAGE SUGAR REPLACEMENT TECHNIQUE

(Paper will be submitted for publication in the Journal of Food Science and Technology)

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Abstract: The objective of this work was the evaluation of the dual-stage technique (D3S) as a pre-treatment for the hot air drying (HAD) of apple slices. This pre-treatment consisted of the immersion in distilled water followed by the immersion in an isomaltulose solution assisted by ultrasound. Drying was carried out in a tunnel dryer at 50 and 70 °C, with a velocity of 1.5 m/s. The final moisture content of the product was up to 0.12 ± 0.01 kg H_2O/kg dry matter. The evaluation of the final product was carried out through total phenolic content, antioxidant activity, shrinkage and color of dehydrated apples. The pre-treated dried samples showed higher drying rates. Samples submitted to the D3S process presented lower phenolic content, antioxidants activity and rehydration. Furthermore, greater color difference was observed compared to untreated samples. Shrinkage was reduced by using the D3S process as a pre-treatment.

Keywords: Palatinose, Impregnation, Osmotic Dehydration, Phosphomolybdenum Method, Abts Method.

1 INTRODUCTION

Fruit snacks can contribute to a healthier and more practical diet. Apple is a good alternative for the production of these snacks due to its high nutritional value, containing vitamins such as vitamin C and E, minerals, and other bioactive components, being important in the prevention of chronic non-communicable diseases (e.g., obesity, diabetes, heart disease, and hypertension) [1].

Hot air drying (HAD) is among the simplest techniques for the production of dehydrated food [2, 3]. Conservation takes place due to the removal of water with consequent reduction of the water activity, preventing the growth of microorganisms and slowing down chemical and enzymatic reactions. Besides, emerging technologies have been used as pretreatments to manipulate certain characteristics in foods, improving functional properties and sensory attributes of dried products, from the impregnation of ingredients of interest in the food matrix. In this context, studies that used the impregnation of healthy sweeteners, pigments, minerals, probiotics, prebiotics and bioactive compounds are found in the literature [4–6].

A technique called two-stage sugar replacement (D3S) consists of using ultrasound to remove native sugars from fruits in the first stage and replace them with an ingredient of interest, in the second stage [7]. The removal of native sugars by ultrasound occurs mainly due to the sponge effect, which is a rapid alternation of compression and expansion of the tissue matrix caused by the incidence of ultrasonic waves. The method results in the formation of microchannels and pores unobstructed, favoring the mass transfer [8, 9].

Isomaltulose is a disaccharide, isomer of sucrose, with a low glycemic index, slower digestion, prolonged energy release, and lower cariogenicity [10, 11]. In addition, there are evidences of probiotic action [12]. These properties make isomaltulose an interesting solute in impregnation processes. However, this carbohydrate was not explored as a solute in the impregnation processes.

Thus, this work aimed to investigate the influence of the D3S pretreatment with isomaltulose impregnation in the HAD of apple slices. The effect of the air temperature was also investigated. The quality of the dried fruit in terms of water activity, total phenolic content, antioxidant activity, color parameters and shrinkage were evaluated.

2 MATERIAL E METHODS

2.1 Material and sample preparation

Fresh apples (*Malus Domestica*) used in the experiments were purchased in the local market (Lavras, MG state, Brazil) and stored in a refrigerator at 8 ± 1 °C until use. The fruits were selected visually by uniform appearance, size, color intensity, and firmness. The mean moisture content of the fresh apples was 83.85% ± 1.44 on a wet basis. Subsequently, the apples were cut with the aid of a stainless-steel mold into slices of 2.00 cm length × 2.00 cm width × 0.55 ± 0.04 cm thickness.

2.2 Experimental design

Two types of experiments were performed: HAD of fresh samples and HAD of D3S pretreated samples. The details of each process are described as follows.

2.2.1 D3S pretreatment

The D3S treatment is composed by two stages. In the first one, the slices were immersed in a water bath with distilled water for 25 min using water: fruit ratio of 4:1(w: w). The second stage consisted of the immersion of the samples in an isomaltulose solution in a concentration of 40 kg isomaltulose/100 kg solution. The same weight ratio between the fruit and the liquid medium used in the first stage was applied at this stage to avoid the dilution of the solution. The total time in this stage was 40 min assisted by ultrasound (Unique, model USC-2850, Brazil). The operating frequency of the bath was 25 kHz, and the intensity of ultrasonic energy was 8 kWm^{-3}.

Both stages were performed at 35 °C. The temperature increase by the use of US was monitored to be lower than 2 °C. The D3S conditions were chosen as optimal pretreatment based on a previous study, aiming mainly at greater water loss and isomaltulose gain.

After the impregnation, the samples were removed from the solution, quickly rinsed in a bath of distilled water and had their surface kindly dried with absorbent paper [13].

2.2.2 Hot air drying (HAD)

The HAD was done in a tunnel dryer (Eco Engenharia Educacional, MD018 model, Brazil) with a parallel flow of 1.5 m/s at 50 °C and 70 °C. In each batch, about 73.66 ± 0.39 g of apples were dried. During the drying, the weight of the samples was monitored using a digital balance (Marte Científica, AD33000 model, Brazil) (accuracy ± 0.01 g) coupled to the sample

holder. The drying was maintained until the minimum moisture content of 0.12 ± 0.01 kg H_2O / kg dry matter (DM). The moisture content of the dried apple was determined in a vacuum oven at 70 °C [14]. All the drying experiments were carried out in triplicates.

Moisture ratio (M_R) of the sample was calculated from the experimental moisture data using Eq. (1) as below

$$M_R = \frac{(M - M_e)}{(M_0 - M_e)} \approx \frac{M}{M_0} \quad (1)$$

Where M_R is the moisture ratio. M is the moisture content [kg H_2O/kg DM] at any time. M_0 is the initial moisture content [kg H_2O/kg DM]. The M_e is the equilibrium moisture content [kg H_2O/kg DM] in which the value was much lower with relation to initial moisture content and moisture content at the time [t]. Therefore, its value was assumed to be zero for the drying conditions.

2.3 Quality analysis

2.3.1 Moisture content and water activity

The moisture content of the fruit was determined in a vacuum drying oven at 70 °C until constant weight [15]. The water activity was measured with a dew-point hygrometer (Decagon Devices Inc., Aqualab Series 3, USA). Both analyses were done in triplicate.

2.3.2 Shrinkage coefficient (Sh)

The Sh was measured through image processing. The surface area of the sample was measured using ImageJ® software. The thickness was obtained as an arithmetical average of measurements at five different points with the use of a digital caliper (Western, 150 mm-DC-60, China). Both measurements were made in quintuplicate. The volume was calculated by multiplying the surface area by sample thickness. The Sh was expressed as the ratio between the volume after and before drying, according to CORRÊA et al. (2012) Eq. (2):

$$\text{Sh} = 1 - \frac{V_f}{V_i} \quad (2)$$

Where V_i is the initial volume of apples (m^3) and V_f is the volume after drying (m^3).

2.3.3 Rehydration capacity

Rehydration capacity (RC) was used to measure the water absorption ability of the dried apples. The dried samples were rehydrated in 100 mL of water at 25 °C. The change in the mass of five rehydrated slices was measured after 80 min of immersion. All tests were done in

triplicate and the average values were calculated. The rehydration performance of the dried apple was then calculated as follows Eq. (3) [17]:

$$\text{RC } [\%] = \frac{Weight\ of\ rehydrated\ samples}{Weight\ of\ dried\ samples} \quad (3)$$

2.3.4 Total phenolic content (TPC)

The TPC was measured using filtrates obtained according to the protocol from Larrauri et al. (1997) with modifications. Briefly, samples were homogenized (Turratec TE 102; TECNAL, Piracicaba, SP, Brazil) in a 50% methanol solution (1:10 w/v). After resting for 20 min in the dark, homogenates were held in an ultrasonic bath (USC-1600 A; Unique, Indaiatuba, SP, Brazil) without heating for 15 min then filtered. Filtrate residue was suspended in 10 mL of 70% acetone and the extraction was repeated (20 min in the dark, 15 min in the ultrasonic bath, filtration). An aliquot of the final filtrate was used to determine the total phenolic content according to the Folin-Ciocalteu method adapted for colorimetry as described by Souza et al. (2014), at 720 nm using a microplate reader (EZ Read 2000, Biochrom®). An aqueous solution of gallic acid was used for calibration and results were expressed as mg of gallic acid equivalents (GA)/100 g.

2.3.5 Antioxidant activity

Antioxidant activity was measured using two methods. The first was the 2 2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay, with results expressed as μmol of Trolox/g of the sample [20]. The second method was the formation of the phosphomolybdenum method (% of ascorbic acid), with results expressed as mg ascorbic acid, Ac. Asc /100 g of the sample [21]. The antioxidant activity was expressed relative to that of ascorbic acid. These assays were carried out using filtrates obtained for total phenolic content.

2.3.6 Color

The measurement of color parameters was based on the CIELAB coordinates (L^*, a^*, b^*) evaluated with D65 illuminant. The measurements were done directly with a Minolta (CR300) in five samples and the average values were reported. The color values were expressed as L^* (lightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness). The global color change (ΔE) was also determined Eq. (4) [22].

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (4)$$

Where L^* indicates the lightness (100 for white to 0 for black), a^* indicates red when positive and green when negative, and b^* indicates yellow when positive and blue when negative. The subscript '0' refers to the fresh fruit color parameters.

2.4 Statistical analysis

The results of the quality analysis were evaluated by one-way ANOVA at the 95% probability level. The significant effects ($p < 0.05$), the means were compared using Tukey's test. These analyses were also performed using the software Sisvar 5.6 [23].

3 RESULTS AND DISCUSSION

3.1 Drying kinetics and drying time

The evolution of drying with time is presented in Figure 1 and the drying time in Table 1. Table 1 also presents the final moisture content and water activity to show that these values were statistically similar.

Table. 1: Moisture content final, drying time and effective diffusivity of the pretreated and untreated apples subjected to hot air drying.

Code	Final moisture content [kg H_2O/kg DM]	Water activity final [-]	Drying time [min]
50 °C	0.124±0.005 [a]	0.432±0.119 [a]	217±6[b]
70 °C	0.119±0.005 [a]	0.357±0.025 [a]	143±12[c]
50 °C-D3S	0.117±0.002 [a]	0.438±0.084 [a]	240±0[a]
70 °C-D3S	0.124±0.005 [a]	0.423±0.054 [a]	123±6[d]

Average value ± standard deviation (n = 3). Different letters in the same column show the statistical difference ($p < 0.05$) according to Tukey's test. Code: the number corresponds to the drying temperature and D3S, the dual-stage pretreatment.

All treatments presented similar a_w (Table 1), which is below the critical value of 0.6. Therefore, it can be considered that processing can contribute to the retention of microbial growth, enzymatic activity and non-enzymatic browning [24]. It is important to highlight that the initial moisture content was lowered with the use of the D3S technique, due to the impregnation of isomaltulose in the order of 8.15 % ± 0.96 and water loss in the order of 21.19 % ± 0.50 that occurred in this step.

The variation in moisture content during drying time is presented in Figure 1. It can be observed that the increase in drying temperature reduced the drying time for both conditions

analyzed, in which for untreated apples the time reduction was 52% and for treated apples 95% (Table 1).

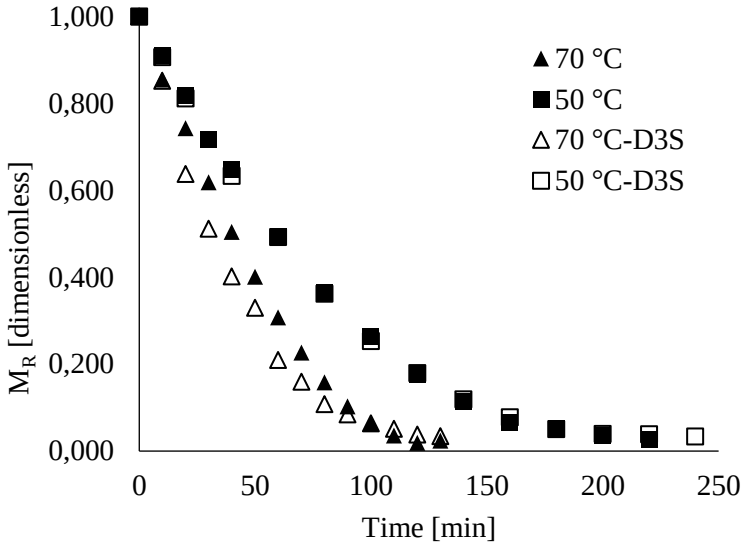

Figure. 1 Evolution of moisture content with time during the drying of apple slices. The number corresponds to the drying temperature and D3S, the dual-stage pretreatment.

The reduction in the drying time due to the increase in the temperature of drying is widely observed in drying works and is attributed to two factors: the reduction in the internal and external resistance. The reduction in internal resistance to moisture transport occurs with the increase in the temperature due to the greater mobility of water molecules within the food. The reduction in external resistance occurs due to the increase in the water pressure gradient between the phases [8, 25].

The use of D3S pretreatment presented different behavior depending on the drying temperature. At 70 °C, there was a 16% reduction in drying time, while at 50 °C there was an inversion of this behavior, that is, drying time increased by 10%. The same behavior was also observed in the drying of sweet potatoes pretreated by ultrasound [26]. The different behavior could be justified by the phenomena in D3S. Alterations in the structure occur by immersion in distilled water and by the ultrasound assistance in the impregnation stage [27]. Such alterations are more preserved in fast moisture removal and consequently the moisture removal and the structural alterations promote one another in a high temperature drying. On the other hand, at 50 ºC, the alterations in the structure are not preserved and the increase in the soluble solid content becomes more relevant.

3.2 Volumetric shrinkage (Sh)

Figure 2 shows the Sh of apples for each experimental condition. All treatments exhibited Sh, but higher were observed for untreated dried samples ($p<0.05$).

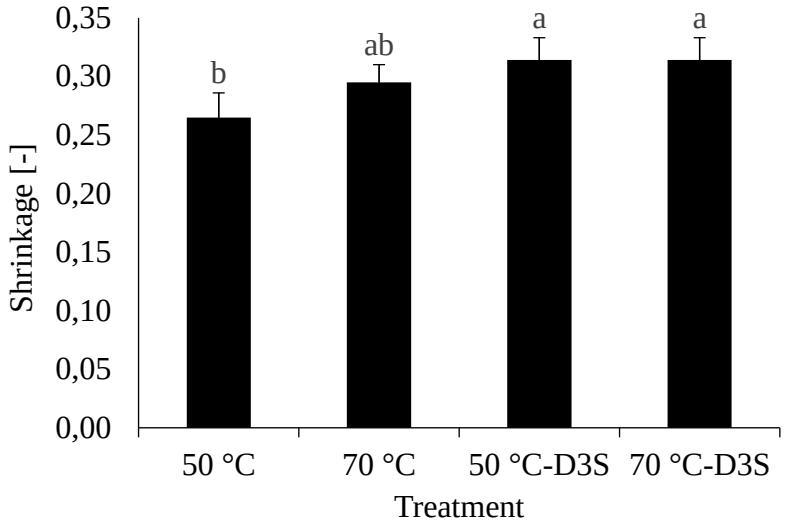

Figure. 2 Shrinkage at different drying conditions for apple slices. The number corresponds to the drying temperature and D3S, the dual-stage pretreatment

This behavior can be associated with the isomaltulose incorporation by the apple, which, in turn, tends to occupy the empty intracellular spaces. This leads to an increase in the structural strength during drying, reducing the final shrinkage [28]. Jointly with the effect of incorporation of solids, the lower shrinkage obtained by the 70 °C-D3S treatment can be justified by the higher drying rate, which leads to a mechanical stabilization of the surface that limits the degree of shrinkage. Similar behavior was obtained in drying of plums impregnated with sucrose, in which the presence of solutes led to lower shrinkage after the drying [29]. Lower shrinkage was also found in the drying of pumpkin slices with higher temperatures [3].

3.3 Rehydration capacity (RC)

The RC of the dried apple slices with time by the different conditions is presented in Figure 3.

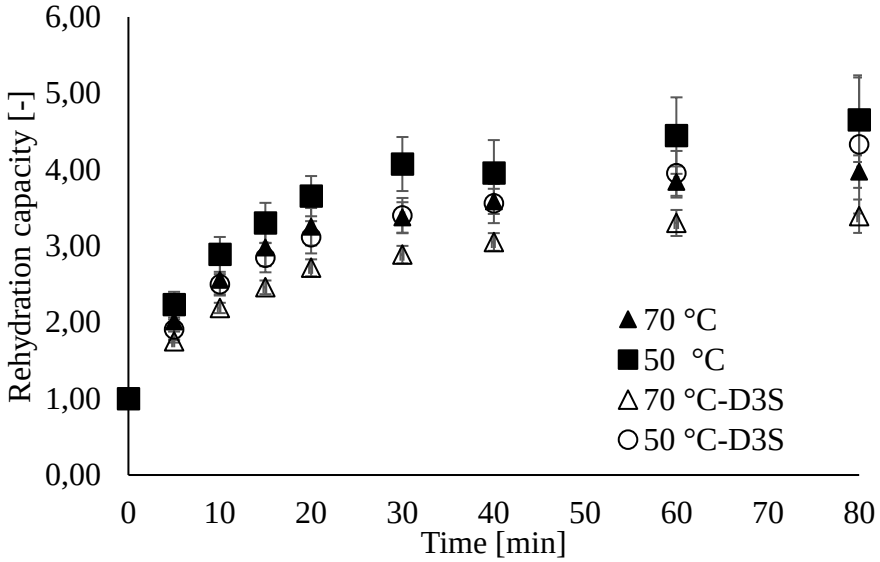

Figure. 3 Rehydration kinetics of dried apples. The number corresponds to the drying temperature and D3S, the dual-stage pretreatment.

The high rate of water gain in the first minutes of the rehydration may be justified by the fast filling up of capillaries and cavities, which are near the surface, with water [25].

A higher rehydration rate was achieved in the 50 °C treatment, in which untreated apples were used to dry at 50 °C ($p<0.05$). It is common to associate the highest degree of rehydration with the least damage to the cellular structure. Therefore, it is noticed that in this condition of lower temperature and absence of D3S treatment, there was greater preservation of the fruit tissue. Softer drying temperatures prevent food surface hardening, contributing to greater rehydration while the use of high temperatures leads to inverse behavior, corroborating with the shrinkage results [30].

The 70 °C-D3S treatment had the lowest CR, about 30% less than the 50 °C treatment. In general, the use of ultrasound as pretreatment leads to the higher the RC. The results obtained in this work were the opposite. In addition to the high temperature favoring the formation of a superficial crust and making rehydration difficult, the presence of ultrasound in the D3S pretreatment can be contributed to this result [25]. A similar result was observed in the rehydration of freeze-dried apples pretreated by ultrasound, and it was justified by the greater degree of cell deformation and the more severe tissue collapse, leading to a lower rehydration rate [31] . Lower rehydration of quinces pretreated by ultrasound in the sucrose solution was justified due to the incorporation of solids may have obstructed the channels produced by ultrasonic waves along with the structural change caused by osmotic stress reduced water absorption [32].

3.4 Total phenolic content (TPC) and antioxidant activity

Polyphenols are an important group of compounds that confer antioxidant activity on apples [33]. The TPC, ABTS and phosphomolybdenum method values of the fresh apple were in the range of 38,212±0.727 mg GA/100 g, 3.574±0.335 µM Trolox/g and 16.964±0.656 mg Ac. Asc/g, respectively. Table 2 shows the total polyphenolic content (TPC) and antioxidant activity (ABTS and phosphomolybdenum method) of dried apples under different conditions. It is important to note that, numerically, dehydrated apples have a higher concentration of TPC and greater antioxidant activity than fresh apples, due to the removal of water after drying and, consequently, the concentration of these compounds.

Table. 2: Effect of drying conditions on the total phenolic content (TPC) and antioxidant activity (ABTS, phosphomolybdenum method).

Code	TPC [mg GA/100 g]	ABTS [mmol Trx/100 g]	Phosphomolybdenum complex [mg ascorbic acid /100 g]
70 °C	106.933±4.859b	9.585±0.875a	75.718±0.420a
50 °C	198.744±8.489a	9.301±0.820a	69.965±2.455a
70 °C-D3S	100.080±2.602bc	6.436±0.973b	69.965±2.455a
50 °C-D3S	85.605±8.207c	5.814±0.762b	72.051±3.789a

Data are expressed as mean ± standard deviation. Different letters in the same column show the statistical difference ($p < 0.05$) according to Tukey's test. Code: the number corresponds to the drying temperature and D3S, the dual-stage pretreatment.

It can be observed that untreated apples had significantly higher TPC ($p < 0.05$) than pretreated dried samples. This may be related to the extraction of phenolic compounds in the D3S process [34]. The use of ultrasound in the D3S step can lead to the release of phenolic compounds due to cavitation, resulting in changes in the cell wall. This exposure of cellular constituents leads to oxidative reactions and intensification of enzymatic activity, decreasing TPC [35]. In addition, the collapse of the bubbles formed during sonication releases high doses of energy in a localized manner, which can contribute to the decomposition of polyphenols [33].

The use of high temperatures is commonly related to lower TPC values due to the thermal degradation of phenolic compounds [36]. However, the opposite behavior was observed. The treatment at 70 °C showed significantly higher TPC ($p<0.05$) when compared to the similar treatment at 50 °C. This behavior may be related to a shorter drying time between

these treatments, 47 min of difference, which would reduce the exposure of the apple to hot air, reducing degradation.

To evaluate the antioxidant activity of the dried apple subjected to different drying conditions (Table 2), ABTS and phosphomolybdenum methods were conducted. Based on the ABTS assay, it is possible to verify the effect of pretreatment on the antioxidant activity of the samples (Table 2). Lower antioxidant activity was observed in samples pretreated with the D3S process, corroborating the findings of TPC. The variation in drying temperature was not significantly different ($p < 0.05$). The decrease in ABTS antioxidant activity due to pretreatment using ultrasound is consistent with the results obtained to husk fruit peel. Concerning the phosphomolybdenum method, no significant difference was observed between treatments ($p<0.05$), presenting an average of 71.925±2.713. The difference in the results obtained between the antioxidant quantification techniques is due to the fact that the methods have different mechanisms of action. In ABTS, the action is based on the capture of the ABTS+ radical by an antioxidant [37]. While the antioxidant activity determined by the phosphomolybdenum complex method is based on the reduction of Mo (VI) to Mo (V) by the analyte [38].

3.5 Color

Table 3 presents the mean values and standard deviations of the parameter chromatics luminosity (L^*), redness (a^*), yellowness (b^*), the total color difference (ΔE), Chroma (C^*) and Hue angle (h°) obtained for apples. In general, it is possible to see that there was a trend of similarity of the color parameters of the treatments to each other and differences about fresh fruit.

Table. 3: Color evaluation of the dried apple fruits in different drying conditions.

Code	Fresh	50 °C	70 °C	50 °C-D3S	70 °C-D3S
L^*	77.95± 2.00^{b}	82.12±2.52^{a}	78.78±2.18^{a}	81.82±0.89^{a}	81.69±0.53^{a}
a^*	-5.27±0.81^{b}	0.49±1.75^{a}	-0.42±0.55^{a}	-1.99±0.99^{a}	-2.43±1.57^{a}
b^*	24.78±2.35^{b}	28.46±1.91^{ab}	28.67±2.54^{ab}	32.10±2.30^{a}	29.73±0.72^{ab}
C^*	102.25±2.32^{b}	28.89±1.57^{ab}	29.18±2.69^{ab}	32.16±2.31^{a}	30.42±0.81^{ab}
h^*	25.4±2.08^{a}	89.12±3.22^{b}	90.44±1.57^{b}	93.58±1.94^{b}	94.55±2.95^{b}
ΔE	-	11.91±1.30^{ab}	9.07±1.18^{b}	12.16±1.43^{a}	10.61±0.05^{ab}

Data are expressed as mean ± standard deviation. Different letters in the same row show the statistical difference ($p < 0.05$) according to Tukey's test. Code: the number corresponds to the drying temperature and D3S, the dual-stage pretreatment.

The dried products differed significantly from the fresh sample to L^* and a^* parameters. The L^* parameter is related to the brightness of the sample, which, in this case, was higher with processing. A similar trend was observed in a^* values. The a^* parameter was found between -6.41 and 0.49 which indicates that the samples were slightly greenish. Thus, there was a reduction in the green color in the dried samples, but not differing from each other concerning to temperature and use of the D3S process ($p<0.05$). Similar behavior was observed in the apple slices dried by hot air-dryer and freeze-dryer [31].

In general, there was an increase in the b^* parameter after drying. However, a significant difference was observed only with the 50 °C-D3S treatment, indicating that there was a yellow highlight of the samples obtained in this condition compared to fresh. Oliveira et al. (2021) also reported the influence of drying on the color of melon samples impregnated with calcium by ultrasound and vacuum, presenting a higher value of b^*, that is, more yellow in relation to fresh samples.

Similar to the b^* parameter, increased color saturation C^* was observed in the 50 °C-D3S treatment. On the other hand, the h° decreased when compared to the fresh sample but showed no significant difference between treatments.

To better understand the effect of drying on apple color, the total color difference was determined. It can be seen that the lower drying temperature resulted in greater color changes. This could be related to the total drying time, which was longer at 50 °C. On the other hand, the apple color was found to be better in 70 °C treatments due to the shorter exposure to hot air, with a reduction of enzymatic and non-enzymatic browning reactions.

4 CONCLUSION

The application of the D3S process results in a different product by the substitution of native sugars by the isomaltulose. The pretreated dried samples presented lower shrinkage. Additionally, pretreatment combined with higher drying temperature led to shorter drying time, however, it was not efficient to protect the samples from phenolic and antioxidant losses. Furthermore, the pretreated samples did not present better rehydration.

REFERENCES

[1] Bondonno, N. P.; Bondonno, C. P.; Ward, N. C.; Hodgson, J. M.; Croft, K. D. The Cardiovascular Health Benefits of Apples: Whole Fruit vs. Isolated Compounds. *Trends Food Sci. Technol.*, **2017**, *69*, 243–256. https://doi.org/10.1016/j.tifs.2017.04.012.

[2] Isquierdo, E. P.; Borém, F. M.; Andrade, E. T.; Corrêa, J. L. G.; Oliveira, P. D.; Alves, G. E. Drying Kinetics and Quality of Natural Coffee. *Trans. ASABE*, **2013**, *v. 56* (3), 995–1001. https://doi.org/http://dx.doi.org/10.13031/trans.56.9794.

[3] Junqueira, J. R. de J.; Corrêa, J. L. G.; Ernesto, D. B. Microwave, Convective, and Intermittent Microwave–Convective Drying of Pulsed Vacuum Osmodehydrated Pumpkin Slices. *J. Food Process. Preserv.*, **2017**, *41* (6), 1–9. https://doi.org/10.1111/jfpp.13250.

[4] Akman, P. K.; Uysal, E.; Ozkaya, G. U.; Tornuk, F.; Durak, M. Z. Development of Probiotic Carrier Dried Apples for Consumption as Snack Food with the Impregnation of Lactobacillus Paracasei. *LWT - Food Sci. Technol.*, **2019**, *103* (December 2018), 60–68. https://doi.org/10.1016/j.lwt.2018.12.070.

[5] Mendonça, K.; Correa, J.; Junqueira, J.; Angelis-Pereira, M.; Cirillo, M. Mass Transfer Kinetics of the Osmotic Dehydration of Yacon Slices with Polyols. *J. Food Process. Preserv.*, **2017**, *41* (1), 1–8. https://doi.org/10.1111/jfpp.12983.

[6] Rojas, M. L.; Augusto, P. E. D.; Cárcel, J. A. Combining Ethanol Pre-Treatment and Ultrasound-Assisted Drying to Enhance Apple Chips by Fortification with Black Carrot Anthocyanin. *J. Sci. Food Agric.*, **2021**, *101* (5), 2078–2089. https://doi.org/10.1002/jsfa.10830.

[7] Garcia-noguera, J.; Oliveira, F. I. P. P.; Gallão, M. I.; Weller, C. L.; Rodrigues, S.; Fernandes, F. A. N. Ultrasound-Assisted Osmotic Dehydration of Strawberries: Effect of Pretreatment Time and Ultrasonic Frequency. *Dry. Technol.*, **2010**, *28* (2), 294–303. https://doi.org/10.1080/07373930903530402.

[8] Corrêa, J. L. G.; Rasia, M. C.; Mulet, A.; Cárcel, J. A. Influence of Ultrasound Application on Both the Osmotic Pretreatment and Subsequent Convective Drying of Pineapple (Ananas Comosus). *Innov. Food Sci. Emerg. Technol.*, **2017**, *41* (April), 284–291. https://doi.org/http://dx.doi.org/10.1016/j.ifset.2017.04.002.

[9] Jansrimanee, S.; Lertworasirikul, S. Ultrasonics - Sonochemistry Synergetic e Ff Ects of Ultrasound and Sodium Alginate Coating on Mass Transfer and Qualities of Osmotic Dehydrated Pumpkin. *Ultrason. - Sonochemistry*, **2020**, *69* (July), 105256. https://doi.org/10.1016/j.ultsonch.2020.105256.

[10] Shyam, S.; Ramadas, A.; Chang, S. K. Isomaltulose: Recent Evidence for Health Benefits. *J. Funct. Foods*, **2018**, *48* (May), 173–178. https://doi.org/10.1016/j.jff.2018.07.002.

[11] Sawale, P. D.; Shendurse, A. M.; Mohan, M. S.; Patil, G. R. Isomaltulose (Palatinose) – An Emerging Carbohydrate. *Food Biosci.*, **2017**, *18* (April), 46–52. https://doi.org/10.1016/j.fbio.2017.04.003.

[12] Van Zanten, G. C.; Knudsen, A.; Röytiö, H.; Forssten, S.; Lawther, M.; Blennow, A.; Lahtinen, S. J.; Jakobsen, M.; Svensson, B.; Jespersen, L. The Effect of Selected Synbiotics on Microbial Composition and Short-Chain Fatty Acid Production in a Model System of the Human Colon. *PLoS One*, **2012**, *7* (10). https://doi.org/10.1371/journal.pone.0047212.

[13] Viana, A. D.; Corrêa, J. L. G.; Justus, A. Optimisation of the Pulsed Vacuum Osmotic Dehydration of Cladodes of Fodder Palm. *Food Sci. Technol.*, **2014**, No. 49, 726–732. https://doi.org/10.1111/ijfs.12357.

[14] AOAC. Official Methods of Analysis of AOAC INTERNATIONAL. **1990**, method 934.06.

[15] AOAC. *Official Methods of Analysis*, 18th ed.; Washington, DC, 2010.

[16] Corrêa, J. L. G.; Braga, A. M. P.; Hochheim, M.; Silva, M. A. The Influence of Ethanol on the Convective Drying of Unripe, Ripe, and Overripe Bananas. *Dry. Technol.*, **2012**, *30* (8), 817–826. https://doi.org/10.1080/07373937.2012.667469.

[17] Junqueira, J. R. de J.; Corrêa, J. L. G.; Oliveira, H. M.; Avelar, R. I. S.; Pio, L. A. S. Convective Drying of Cape Gooseberry Fruits: Effect of Pretreatments on Kinetics and Quality Parameters. *LWT - Food Sci. Technol.*, **2017**, *82*, 404–410. https://doi.org/10.1016/j.lwt.2017.04.072.

[18] Larrauri, J. A.; Rupérez, P.; Saura-Calixto, F. Effect of Drying Temperature on the Stability of Polyphenols and Antioxidant Activity of Red Grape Pomace Peels. *J. Agric. Food Chem.*, **1997**, *45* (4), 1390–1393. https://doi.org/10.1021/jf960282f.

[19] Souza, V. R.; Pereira, P. A. P.; Da Silva, T. L. T.; De Oliveira Lima, L. C.; Pio, R.; Queiroz, F. Determination of the Bioactive Compounds, Antioxidant Activity and Chemical Composition of Brazilian Blackberry, Red Raspberry, Strawberry, Blueberry and Sweet Cherry Fruits. *Food Chem.*, **2014**, *156*, 362–368. https://doi.org/10.1016/j.foodchem.2014.01.125.

[20] Rufino, M. do S. M.; Alves, R. E.; Brito, E. S. de; Morais, S. M. de; Sampaio, C. de G.; Pérez-Jiménez, J.; Saura-Calixto, F. D. Metodologia científica: Determinação da

Atividade Antioxidante Total em Frutas pelo Método de Redução do Ferro (FRAP).

[21] Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a …. *Anal. Biochem.*, **1999**, *269*, 337–341. https://doi.org/10.1037/a0037168.

[22] Kroehnke, J.; Szadzińska, J.; Stasiak, M.; Radziejewska-Kubzdela, E.; Biegańska-Marecik, R.; Musielak, G. Ultrasound- and Microwave-Assisted Convective Drying of Carrots – Process Kinetics and Product's Quality Analysis. *Ultrason. Sonochem.*, **2018**, *48* (May), 249–258. https://doi.org/10.1016/j.ultsonch.2018.05.040.

[23] Ferreira, D. F. Sisvar: A Computer Statistical Analyses System. *Cienc. e Agrotecnologia*, **2011**, *35*, 1039–1042.

[24] Cuccurullo, G.; Metallo, A.; Corona, O.; Cinquanta, L. Comparing Different Processing Methods in Apple Slice Drying. Part 1. Performance of Microwave, Hot Air and Hybrid Methods at Constant Temperatures. *Biosyst. Eng.*, **2019**, *188*, 331–344. https://doi.org/10.1016/j.biosystemseng.2019.10.021.

[25] Corrêa, J. L. G.; Lopes, F. J.; Mello Junior, R. E.; Junqueira, J. R. de J.; Mendonça, K. S. de; Macedo, L. L.; Salvio, L. G. A. Drying of Persimmon Fruit (Diospyros Kaki L.) Pretreated by Different Osmotic Processes. *J. Food Process Eng.*, **2021**, No. July, 1–12. https://doi.org/10.1111/jfpe.13809.

[26] Infante, R. A.; Azoubel, P. M.; de Lima, M. A. B.; Stamford, T. C. M.; Araújo, A. S.; de Mendonça, W. S.; da Silva Vasconcelos, M. A. Ultrasound Pretreatment Application in Dehydration: Its Influence on the Microstructure, Antioxidant Activity and Carotenoid Retention of Biofortified Beauregard Sweet Potato (Ipomoea Batatas Lam). *J. Food Sci. Technol.*, **2021**. https://doi.org/10.1007/s13197-020-04938-w.

[27] Fernandes, F. A. N.; Linhares, F. E.; Rodrigues, S. Ultrasound as Pre-Treatment for Drying of Pineapple. *Ultrason. Sonochem.*, **2008**, *15* (6), 1049–1054. https://doi.org/10.1016/j.ultsonch.2008.03.009.

[28] Dehghannya, J.; Farshad, P.; Heshmati, M. K. Three-Stage Hybrid Osmotic–Intermittent Microwave–Convective Drying of Apple at Low Temperature and Short Time. *Dry. Technol.*, **2018**, *0* (0), 1–24. https://doi.org/10.1080/07373937.2018.1432642.

[29] Dehghannya, J.; Gorbani, R.; Ghanbarzadeh, B. Shrinkage of Mirabelle Plum during Hot Air Drying as Influenced by Ultrasound-Assisted Osmotic Dehydration. *Int. J. Food Prop.*, **2016**, *19* (5), 1093–1103. https://doi.org/10.1080/10942912.2015.1055362.

[30] Ricce, C.; Rojas, M. L.; Miano, A. C.; Siche, R.; Augusto, P. E. D. Ultrasound Pre-Treatment Enhances the Carrot Drying and Rehydration. *Food Res. Int.*, **2016**, *89*, 701–

708. https://doi.org/10.1016/j.foodres.2016.09.030.

[31] Deng, Y.; Zhao, Y. Effect of Pulsed Vacuum and Ultrasound Osmopretreatments on Glass Transition Temperature, Texture, Microstructure and Calcium Penetration of Dried Apples (Fuji). *LWT - Food Sci. Technol.*, **2008**, *41* (9), 1575–1585. https://doi.org/10.1016/j.lwt.2007.10.018.

[32] Noshad, M.; Mohebbi, M.; Shahidi, F.; Mortazavi, S. A. Kinetic Modeling of Rehydration in Air-Dried Quinces Pretreated with Osmotic Dehydration and Ultrasonic. *J. Food Process. Preserv.*, **2012**, *36* (5), 383–392. https://doi.org/10.1111/j.1745-4549.2011.00593.x.

[33] Wiktor, A.; Sledz, M.; Nowacka, M.; Rybak, K.; Witrowa-Rajchert, D. The Influence of Immersion and Contact Ultrasound Treatment on Selected Properties of the Apple Tissue. *Appl. Acoust.*, **2016**, *103*, 136–142. https://doi.org/10.1016/j.apacoust.2015.05.001.

[34] Sakooei-Vayghan, R.; Peighambardoust, S. H.; Hesari, J.; Peressini, D. Effects of Osmotic Dehydration (with and without Sonication) and Pectin-Based Coating Pretreatments on Functional Properties and Color of Hot-Air Dried Apricot Cubes. *Food Chem.*, **2020**, *311* (November 2019), 125978. https://doi.org/10.1016/j.foodchem.2019.125978.

[35] Pirce, F.; Vieira, T. M. F. S.; Augusto-Obara, T. R.; Alencar, S. M.; Romero, F.; Scheuermann, E. Effects of Convective Drying Assisted by Ultrasound and Osmotic Solution on Polyphenol, Antioxidant and Microstructure of Murtilla (Ugni Molinae Turcz) Fruit. *J. Food Sci. Technol.*, **2021**, *58* (1), 138–146. https://doi.org/10.1007/s13197-020-04523-1.

[36] Yap, J. Y.; Hii, C. L.; Ong, S. P.; Lim, K. H.; Abas, F.; Pin, K. Y. Effects of Drying on Total Polyphenols Content and Antioxidant Properties of Carica Papaya Leaves. *J. Sci. Food Agric.*, **2020**, *100* (7), 2932–2937. https://doi.org/10.1002/jsfa.10320.

[37] Rufino, M. D. S. M.; Alves, R. E.; Brito, E. S.; Morais, S. M.; Sampaio, C. G.; Jimenez, J. P.; CALIXTO, F. D. S. Determinação Da Atividade Antioxidante Total Em Frutas Pela Captura Do Radical Livre DPPH. Comunicado Técnico Embrapa. 2007, pp 1–4.

[38] Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.*, **1999**, *269* (2), 337–341. https://doi.org/10.1006/ABIO.1999.4019.

[39] Oliveira, M. H. da S. B.; Ferreira Filho, A. J. M.; Silva Júnior, E. V.; da Silva, E. S.;

Paim, A. P. S.; Honorato, F. A.; Azoubel, P. M.; da Silva Barros de Oliveira, M. H.; Ferreira Filho, A. J. M.; da Silva Júnior, E. V.; et al. Impregnation and Drying to Develop a Melon Snack Enriched in Calcium. *J. Food Sci. Technol.*, **2021**, *58* (2), 672–679. https://doi.org/10.1007/s13197-020-04581-5.

GENERAL CONCLUSION

The most suitable condition for the dual-stage technique (D3S) was performed from immersion in water in the first stage, followed by ultrasound-assisted osmotic dehydration, resulting in better replacement of native apple sugar by isomaltulose. This incorporation led to an increase in the soluble solids content. On the other hand, the use of the treatment contributed to the shrinkage. About the color parameters, lesser color variations were observed, being less luminous and more yellowish.

When drying apples, the use of pre-treatment combined with a higher drying temperature led to a shorter drying time. This contributed to the lesser observed shrinkage and lesser rehydration. However, it was not efficient to protect the samples from the loss of phenolic compounds and antioxidants, making it important to study other drying methods to minimize such losses.