UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS

CURSO DE ENGENHARIA DE ALIMENTOS

EVALUATION OF DRYING TEMPERATURE ON BIOACTIVE COMPOUNDS RETENTION IN PASTA ENRICHED WITH MICROALGAE BIOMASS

Isadora Cafruni

Porto Alegre

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Trabalho de Conclusão de Curso apresentado ao curso de Engenharia de Alimentos da UFRGS, para a obtenção do título de Engenheira de Alimentos.

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ABSTRACT

Fruits and vegetables intake are associated with prevention of chronical diseases. Although they are aware about its health benefits, the greatest part of the worldwide population do not eat it in enough amounts. Incorporating ingredients with high nutritional value into frequently consumed food, such as pasta, is a viable alternative to increase these nutrients intake. However, it is important to consider nutrient losses during food processing. The aim of this study was to verify the influence of drying temperature on bioactive compounds retention in pasta enriched with 2% of *Chlorella* biomass powder. Two drying condition, HT (high temperature, 75-80ºC and 75% HR) an LT (low temperature, 50ºC and 81% HR) were tested. Pasta samples analyzed were: uncooked fresh pasta (UF), cooked fresh pasta (CF), uncooked low temperature dry pasta (ULT), cooked low temperature dry pasta (CLT), uncooked high temperature dry pasta (UHT) and cooked high temperature dry pasta (CHT). Cooking properties (Optimum cooking time (OCT), solid loss, cooked weight and volume gain), color, total carotenoids and carotenoids identification and quantification were evaluated. HT drying resulted in pasta with low cooking losses and better nutritional value after cooked. The thermal treatment increased the nutrients bioaccessibility and contributed to a stronger and denser protein network.

Keywords: Pasta, High temperature drying; microalgae; *Chlorella vulgaris*, carotenoid

1 INTRODUCTION

Great changes in world's diet have been happening both in industrial regions and developing countries. In the past decades, people have been eating more carbohydrates, fat and sugar and less fruits and vegetables. This fact is leading to an increase in chronical diseases, malnutrition, diabetes and obesity. The greatest part of the population is aware of the nutritional importance of eating fruits and vegetables and still do not consume it in the recommended daily amounts. This results in a diet with low intake of bioactive compounds. On the other hand, pasta is highly consumed by people from all ages and social classes, being present in almost 100% of Brazilian's homes. Therefore, incorporating ingredients with high nutritional value into frequently consumed food, such as pasta, is a viable alternative to increase these nutrients intake. Enriching pasta with vitamins and minerals is an easy way to improve its nutritional value and, consequently, increase bioactive compounds consumption in people who regularly eat pasta products. Usually, only pasta with small amount of vegetables with the purpose of coloring are found in market. It was only more recently that the possibility to enrich pasta with amounts that provide a portion of the daily recommended vegetable intake has appear. However, it is important to consider nutrient losses during food processing. In addition to that, substitution of part of the flour tend to damage the gluten network and decrease pasta quality. It can result in a product with lower texture properties and higher cooking loss. Applying high temperatures drying and using ingredients that bring high nutritional value even if small amounts are used, might be a solution for these limitations. Microalgae biomass, for example, are rich in bioactive compounds and can be used for this purpose. Microalgae are high in carotenoids, compounds that are associated with health benefits such as antioxidant activity, anti-inflammatory activity and provitamin A action.

1.1 General Objectives

The aim of this study was to verify the influence of drying temperature on bioactive compounds retention in pasta enriched with 2% of *Chlorella* biomass powder.

1.2 Specific Objectives

- Production of pasta with addition of microalgae biomass;
- Pasta drying in two different conditions: 75-80ºC and 75% HR; 50ºC and 81% HR;
- Evaluation of cooking properties of fresh pasta, low temperature dry pasta and high temperature dry pasta;
- Evaluation of bioactive compounds in fresh pasta, low temperature dry pasta and high temperature dry pasta.

2 BIBLIOGRAPHIC DEVELOPMENT

2.1 PASTA

Pasta, or alimentary paste, is one of the simplest cereal products. It exists in the human diet for millennia. Evidences show that noodles exist in China since, at least, 3000 BC. Spaghetti, macaroni, vermicelli and noodles are among the types of alimentary pastes (BROCKWAY, 2001). It is a good source of complex carbohydrates and a moderate source of protein and some vitamins. A portion (2 oz. or 56.7 g) of dry pasta has about 210 cal (878.64 J) and its composition is of 75% percent carbohydrate, 13% protein, and 1.5% fat. Pasta is a good seller even in bad economic times (MARCHYLO and DEXTER, 2001).

Pasta can be found in markets in many varieties, shapes and sizes. They can be classified as short or long goods and can be dried, fresh, frozen, microwavable, instant or restorable (MARCHYLO and DEXTER, 2001).

Dried pasta shelf-life is usually of two years. Nutritional levels claimed in labeling is what basically determines the storage time of these products, once vitamins degrade over time (HUI, 2006).

2.1.1 Definitions

Pasta is defined as the non-fermented product of several shapes, stuffed or not, obtained from doughs from wheat flour, whole wheat flour, wheat semolina, *durum* wheat flour, *durum* wheat semolina or *durum* whole wheat flour or derivatives from cereals, legumes, roots or tubers, added or not of other ingredients, and with or without seasonings or complements separately or added directly to the pasta (BRASIL, 2000).

Wheat flour for industry application has no more than 1.35% of ash content on dry basis and 98% of the product needs to pass through a sieve with mesh of 250 µm (BRASIL,1996).

Whole wheat flour for industry application has no more than 2.5% of ash content on dry basis (BRASIL,1996).

Durum wheat semolina, durum wheat flour and durum whole wheat flour are the products obtained from the milling of grains from *Triticum durum* Desf. species (BRASIL,1999).

Durum wheat semolina has no more than 0.92% of ash content on dry basis and all the product needs to pass through a sieve with mesh of 841 µm and, at most, 10% through a sieve with mesh of 150 µm (BRASIL, 1999).

Durum wheat flour has no more than 1.5% of ash content on dry basis and at least 98% of the product needs to pass through a sieve with mesh of 250 µm (BRASIL 1999).

Durum whole wheat flour has no more than 2.1% of ash content on dry basis (BRASIL, 1999).

Durum wheat semolina is the most adequate type for pasta production. It has larger particles, what positively affects water absorption. Besides, *T. durum* wheat semolina provides the characteristic yellow color. However, because of its soil and climate conditions, durum wheat production is limited in Brazil. That is why wheat flour from *T. aestivum* is usually used for pasta production in Brazil, even though it is more appropriate for bread making (GUERREIRO, 2006).

2.1.2 Classification

Pasta can be classified by the moisture content or by its composition.

According to the moisture content, pasta can be classified in dried pasta, fresh pasta or instant pasta. Dried pasta is the pasta product that undergoes a drying process resulting in a product with no more than 13.0% (g/100g) moisture (BRASIL, 2000), while fresh pasta is the pasta product that undergoes or not a partial drying process resulting in a product with no more than 35.0% (g/100g) moisture (BRASIL, 2000). Instant pasta – dehydrated by frying is the pasta product that undergoes or not a cooking process and that is dried by a frying process resulting in a product with no more than 10.0% (g/100g) moisture (BRASIL, 2000). Instant pasta – dehydrated by hot air or other methods is the pasta product that undergoes a cooking process and a hot air drying or other drying method (that not frying) resulting in a product with no more than 14,5% (g/100g) moisture (BRASIL, 2000).

On the other hand, in relation to composition, pasta can be obtained from wheat flour, wheat semolina, durum wheat flour or durum wheat semolina (BRASIL, 2000).

Whole wheat pasta is the product obtained from whole wheat flour or durum whole wheat flour or a mixture of whole wheat flour or durum whole wheat flour or wheat bran or durum wheat bran with wheat flour or wheat semolina or durum wheat flour or durum wheat semolina (BRASIL, 2000).

There are also types of pasta with replacement of some of the wheat flour by other ingredients and types of pasta produced exclusively with derivatives from legumes, roots, tubes or other cereals that not wheat (BRASIL, 2000).

2.1.3 Ingredients

The only required ingredient to pasta production is wheat flour or whole wheat flour or wheat semolina or durum wheat flour or durum wheat semolina or durum whole wheat flour or derivatives from cereals, legumes, roots or tubers, according to the classification of the product as described in item 2.1.2. However, other ingredients may be used: water, eggs, vegetables, wheat/durum bran, milk and dairies, salt, seasonings, complements, spices, animal and vegetable protein, oils and fats, stuffing, sauces and other ingredients that do not deprive the product characteristics (BRASIL, 2000).

2.2 PASTA MARKET

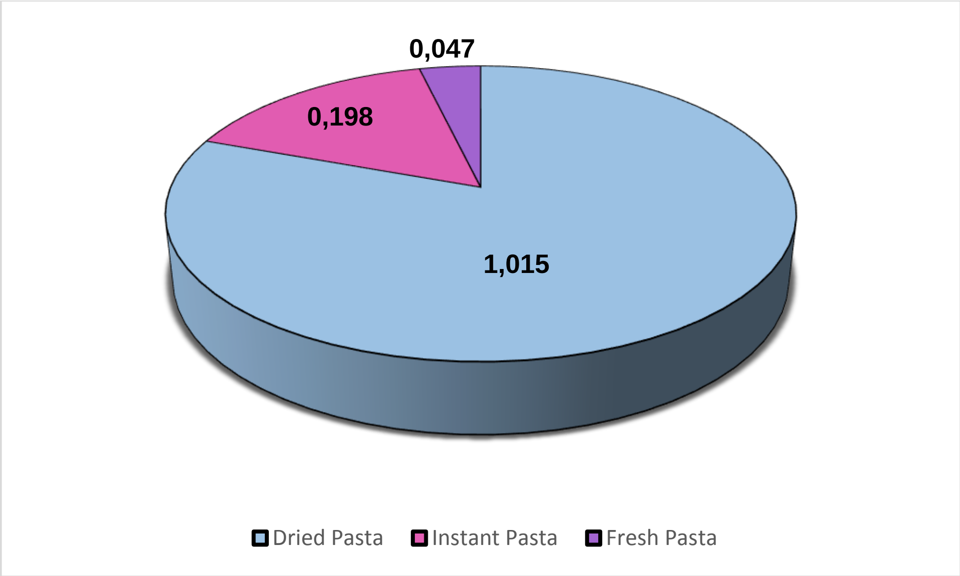
Pasta is one of the oldest food in the world, being produced by humans for more than 6000 years. In Brazil, pasta came with Italian immigrants in XIX century and became one of Brazilian's favorite foods (ABIMAPI, 2015). It is very versatile: can be served as the main dish or as a side and can be produced in different formats, sizes and colors. It has high acceptability, low cost and it is easy to prepare. The technology involved in its production is also simple (REIS, 2013).

Pasta is present in 99.6% of Brazilian's homes and it's incorporated in eating habits of people of all ages and social classes. Brazil is the third country in the ranking of pasta consumption in the world. Italy comes first, followed by the United States (ABIMAPI, 2015).

Brazil experienced a growth of 10.35% in pasta market profits from 2013 to 2014. Instant pasta was the segment with greatest growth (12.3%), followed by dried pasta (9.6%). This was possible even with the increase in commodities price due to the very low price of these products. In 2015, 1.26 millions of tons of pasta were marketed in Brazil. This is equivalent to R$ 8.28 billion. The consumption per capita is estimated in 6.17 kg per year (ABIMAPI, 2015).

Dried pasta correspond to 80.56% of the pasta market in Brazil (ABIMAPI, 2015). It's the most consumed type of pasta among all age groups and more than half of each group is considered “heavy consumers” (REPORT BUYER, 2014). Figure 1 illustrates the sales of each segment: fresh, dried and instant pasta, according to ABIMAPI (2015).

Figure 1: Pasta sales in millions of tons by segment in Brazil.



Source: Adapted from ABIMAPI (2015).

2.3 PASTA PRODUCTION

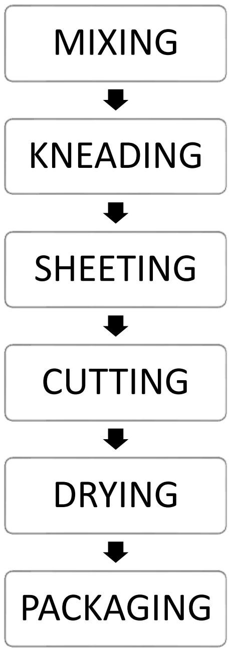
In the beginning of pasta production, it was a very simple procedure where flour and water were mixed and kneaded into doughs. Years later it was discovered that the dough could be sheeted, cut into strips and sun dried, resulting in a product that could be safely stored and easily transported. With the industrial revolution, mechanization started to be included into pasta processing. Extrusion presses, mixers, kneaders, drying cabinets were built in order to increase efficiency. However, the processing was still in batches, productivity was still restricted and the final product did not have the same consistency as today. Continuously developments were made, until the first continuous automatic production line was built in Swiss in 1946. Other developments appeared along the years. Vacuum application in mixing and extrusion helped to minimize oxidation of pigments. High temperature drying and computer control helped to improve efficiency, productivity, capacity, product diversity and quality (MARCHYLO and DEXTER, 2001).

Pasta can be process in two different ways: by sheeting or by extrusion. The first one is a discontinuous method, while the other one is a continuous and more commonly used in industries. Both of them will be described below.

2.3.1 Sheeting process

Figure 2 illustrates the process flow diagram of pasta production by sheeting process.

Figure 2: Process flow diagram for pasta sheeting process.



The author (2016).

2.3.1.1 Mixing

This operation consist in mixing dry and liquid ingredients. The dry ingredients, such as wheat flour and semolina and powder eggs, are mixed for a few minutes. Then, the liquid ingredients, such as water and eggs, are added into the mixture (GUERREIRO, 2006). Gluten should not be formed during mixing. The dough formed should look dry and crumbled (BROCKWAY, 2001).

Water amount depends on wheat variety, protein content, particles size, initial water content and other ingredients (GUERREIRO, 2006). The measure is usually about 25 to 30 kg of water to 100 kg of semolina (MARCHYLO and DEXTER, 2001). The dough formed should have 28% to 30% (w/w) of moisture (BROCKWAY, 2001).

If liquid eggs are added, they need to be homogenized and filtered prior to its addition. The amount of water required in this case is smaller, on the other hand powder eggs demand higher amount of water. Salt and additives have to be dissolved in the mixing water for better uniformity (GUERREIRO, 2006).

Manufacturers usually apply vacuum during mixing because the presence of air can compromise the mechanical strength and appearance of the final product. Also, the lack of air limit the activity of enzymes that catalyze the oxidation of natural durum semolina pigments. Thus, vacuum is important to keep the characteristic pale yellow color of good quality pastas (BROCKWAY, 2001).

In addition, water temperature is important to process efficiency and quality. If it is slightly hotter than room temperature, it gets easier to mold and mixing time decreases. Oxidation and loss of coloration are lower when this step is faster (GUERREIRO, 2006).

2.3.1.2 Kneading

It is during kneading that dough structure is developed and becomes homogeneous. The proteins absorb water and create the gluten network, providing elasticity and resistance, important characteristics to dough molding (GUERREIRO, 2006).

2.3.1.3 Sheeting

In this unit operation, the dough passes through a series of sheeting rolls in order to reduce its thickness. The distance between the rolls get smaller each time the dough passes through it, until it reaches the desired thickness and a smooth, uniform and non-brittle appearance (GUERREIRO, 2006). Research shows that the more the dough passes through the rolls or the smaller the gap between rolls is, more damage is caused to the protein network. Thus, the sheeting process is a highly relevant for the quality of the pasta (BROCKWAY, 2001). Extra hygienic care is important in this operation, once there is a lot of handling (GUERREIRO, 2006).

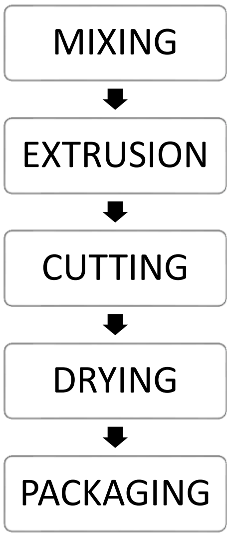
2.3.1.4 Cutting

After sheeting, the pasta is then cut in strips of desired length and width, which can be done manually or mechanically. Cutting cylinders can be used with different gaps to reach different product sizes. Common size is of about 2 mm for spaghetti and can reach up to 7.5 mm for tagliatelli. For lasagna, the sheets of dough are stacked one above other with a plastic film in between them and, then, manually cut. The common size found in market is 17.5 cm × 5.5 cm (GUERREIRO, 2006).

2.3.2 Extrusion

The other pasta production method is extrusion, which is illustrated in Figure 3.

Figure 3: Process flow diagram for pasta extrusion process.



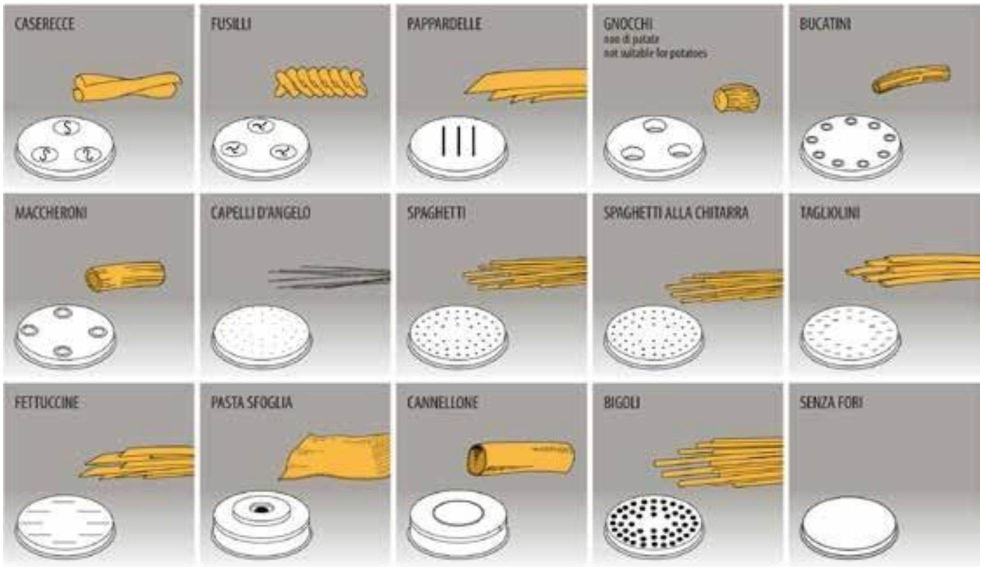
Source: The author (2016).

Extrusion is a continuous process, more frequently used in dry pasta manufactures because it is faster and allows less handling then sheeting process.

In this process, all mixing, kneading and molding steps are made in the same equipment (GUERREIRO, 2006). The extruder can be equipped with a variety of dies depending on the shape of pasta desired (EPA, 1995). Figure 4 illustrates different shapes of dies for some pasta types. Pasta passes through cylinder fitted with a screw, is kneaded, compressed in the orifices of a die made of Teflon or Bronze. At this point, the gluten is formed in pasta production. As the product exits the extruder, rotating knives coupled to the outer surface of the die cut it. The speed of rotation, which is constant and controlled by independent motor, determines the length of the product. The faster the knives rotate, the smaller the final product is (BROCKWAY, 2001; GUERREIRO, 2006). The high pressure applied in the extrusion provide the necessary compactness for a good quality cooked pasta (KRUGER, MATSUO, and DICK, 1996).

Dough temperature during extrusion should not be higher than 50 °C to avoid damage to the gluten network and poor cooking quality pasta (MARCHYLO and DEXTER, 2001), because high temperatures cause irreversible protein denaturation (BROCKWAY, 2001). However, extrusion generates frictional energy that is converted into heat, what can quickly heat the dough and the equipment (BROCKWAY, 2001). Therefore, extrusion barrels contain water cooling jacket to maintain a constant temperature, dissipating the heat generated in this step (EPA, 1995). Also, mixing water should be at room temperature or cooler when flour with smaller particles is used, preventing the dough to get excessively hot (GUERREIRO, 2006).

Figure 4: Extrusion dies and its products.



Source: Shop Equip (2015). http://www.shop-equip.com/metcalfe-pm1-5-pasta-maker.html

Figure 5: Extruder Machine with 15 kg capacity

Source: Italvisa (http://www.italvisa.com.br/).

2.3.3 Drying

Pasta is usually dried from 30% w/w to about 10-12% w/w moist. Drying directly affects the quality of the final product. Pasta dough contracts during this step and uneven drying causes pasta cracking (BROCKWAY, 2001). Either quality of the product and process costs depend on proper drying conditions. Moisture is released slowly due to adsorptive and osmotic phenomena (TSCHEUSCHNER, 2001).

Drying is important to obtain a strong pasta with long shelf life. It is essential to have a constant drying rate, avoiding the edges to completely dry and harden before the inside. This creates a barrier making the moisture diffusion difficult. As a result, the product can have fracture lines, the chances of breakage in package are higher and cooking quality is inferior. The alternative to avoid this is to apply different drying zones with specific temperatures and relative humidity. Even higher attention is required when long pasta is dried. The first stage consist in submitting the pasta to pre-drying, decreasing the moisture from 30% to about 17-19%. The final drying, then, reduces the moisture to about 12.5%. After drying, there is the stabilization stage, in which the remaining water is evenly distributed along the pasta. This eliminates moisture gradients and prevent further fractures and cracking. At the end, the product has to be cooled to 28-32 ºC (MARCHYLO and DEXTER, 2001). Conventional drying is held at temperatures between 40 to 55 ºC, with pre-drying varying from 40 ºC and 55% relative humidity to 75 ºC and 99% relative humidity and taking from 45 mins to 1,5 h. Water activity decreases to about 0.5 to 0.8, what improves product stability (GUERREIRO, 2006).

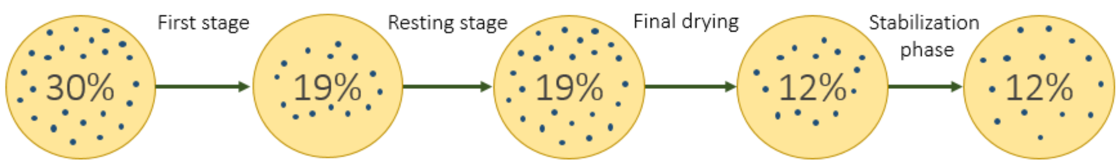
If drying occurs too quickly, pasta can crack, and the product will have bad appearance and low mechanical strength. On the other hand, if drying process is too slow, pasta can become moldy. Therefore, drying is the most critical step to control in order to obtain a firm but also flexible pasta (MARCHYLO and DEXTER, 2001).

One of the pasta quality problems that can be caused in drying process is checking, which is stress cracks. It occurs when stress is higher than the product strength. The three pasta drying stages are predrying, final drying and cooling/stabilization. Initial predrying occurs when the product exits the extruder dies. It only removes up to 1% of the moisture, but it is important to avoid pieces of sticking together, for example. Then, the product follows to the predrying section of the dryer, where one third of the total water is removed, reaching about 18-21% moisture content. High drying rates are possible without stressing the product because it is still in the plastic state. Following, the product will enter the final dryer, where the moisture content needs do decrease to about 12%. Once it is in elastic state in this stage, the rate of drying is critical and it has to be adjusted according to the product thickness. After final drying, comes the cooling/stabilizing stage, in which the product is cooled to close to room temperature to minimize stresses. Some of the water in the center of the pieces migrate to the surface, better distributing the water content (HUI, 2006).

It is also important to consider a resting stage during drying. In this stage, temperature and air humidity are such that the product does not lose moisture to the air. The goal of this stage is to evenly distribute the water content as the core loses water to the surface, releasing stress (HUI, 2006).

Drying stages are illustrated in figure 6.

Figure 6: Drying stages



Source: The Author (2016)

2.3.3.1 Drying Temperatures

In the beginning of dry pasta manufacture, only low temperatures (LT), of no more than 60 ºC, were used. It used to take at least 18 h to dry long goods. In the 1970s, however, higher temperatures of 60-85 ºC started to take place to produce pasta containing eggs with better microbiological control. It also decreased drying time to about 8 h. This allowed industrial lines to reduce its sizes for the same capacity and reduced plant costs. As these higher temperatures were applied, other benefits in the final product started to show up, such as better cooking quality and better color. Thenceforth, high temperature (HT) drying became worldwide applied for dry pasta processing. More recently, ultra-high temperature (UHT) drying of 85-110 ºC have been applied. It allows short drying times, of 4 to 5 h for long goods and 2 to 3 h for short goods. Superior cooking quality and color can be reached with this process, even if lower-grade ingredients are used. This enable good quality pasta to be process and commercialized at affordable prices (MARCHYLO and DEXTER, 2001).

Comparing to the low-temperature drying process, high-temperatures result in better color and firmness, lower cooking loss and less stickiness, bulkiness and adhesiveness. Higher temperatures also lead to lower starch damage, what can be associated with better cooking quality as well. This can be attributed to the lower amylolytic enzymes activity. Therefore, when high temperature is applied, raw material quality is not as important and low gluten content semolina or flour can be used. Industry productivity, time reduction and microbiological control are other advantages of drying using higher temperatures (RODA, 2013).

Starch and protein changes and surface properties are directly associated with pasta cooking properties. High temperature drying tends to improve pasta cooking quality. It favors protein crosslinking through disulfide bond, promoting their aggregation. Drying temperature above 80 ºC, thus, decrease the extractability of storage proteins (gliadin and glutenin). As a tight and dense protein matrix is formed, there is less free amylose on pasta surface and starch swelling during cooking is minimized (ZWEIFEL, 2001).

When pasta is dried at high temperatures, the protein network is formed in the raw pasta, before starch is hydrated in boiling water. This results in a good quality product, because starch is surrounded by the coagulated protein network. Quality of the raw materials are not as important in this case. However, at lower temperatures drying, both starch gelatinization and protein coagulation happen during boiling. In this case, the quality of proteins in the raw material is very important in order to maintain the final product expected quality (RESMINI and PAGANI, 1983).

Because pasta stays in a more plastic states when drying is held at HT, stress is relief and the risk of cracking is lower. Higher drying temperatures lead to increased breaking strength due to the formation of a more compact and dense protein network. HT also causes higher adhesion between starch and protein (ZWEIFEL, 2001). The breaking strength is the indicator of the gluten strength, semolina quality and how vulnerable the pasta is of breaking during packaging and distribution.

In a study with pasta processed with the same raw materials and process conditions, when higher temperatures were applied, the final product was less sticky and firmness was increased. According to the authors, this was probably due to the formation of strong insoluble aggregates of protein that resulted in less water absorbed by the starch (LAMACCHIA et Al., 2007).

D'Amico et al. (2015) studied the effects of high temperature drying in gluten-free pasta properties, since its benefits in weak gluten pasta quality had already been verified by other researchers. The results confirm that gluten free pasta could have its quality improved when higher drying temperatures are used, including better structural integrity, leading to lower levels of cooking loss and protein solubility. Furthermore, high drying temperatures positively affect texture properties, improving firmness and tensile strength. However, elasticity similar to regular wheat pasta was still not reached.

Petitot et al. (2010) noticed advantages in using HT drying in pasta fortified with legumes, once its structure showed to be stronger. It resulted in lower cooking loss and higher pasta hardness, cohesiveness and breaking energy. According to the authors, the only problem with very high temperature drying was the promotion of Maillard reaction that can be visually prejudicial due to its brownish color and can negatively affect nutritional value.

Regarding pasta enriched with vitamin C, HT drying might lead to higher vitamin degradation during the drying process, but might also lead to lower losses of the vitamin in the cooking water. On the other hand, LT drying has the opposite effect with lower losses during drying, but higher losses during cooking (OLIVIERO and FOGLIANO, 2016).

2.3.4 Packaging

After drying, pasta will absorb environment moisture, especially in places with high relative humidity. It may cause the surface to expand, leading to stress. If humidity is higher than 74%, checking will occur. To avoid this, pasta has to be packed in no longer than 3 h after leaving the dryer (HUI, 2006).

Packaging protects the product from breakage and contamination. It is also the mean of communication with the consumer (MARCHYLO and DEXTER, 2001).

The most usual packaging material for pasta is the cellophane bag. It functions as a barrier for moisture and can be easily used on packaging machines. However, it is not easy to stack on grocery shelves. Therefore, many manufacturers packs pasta with boxes instead of bags. Besides being easy to stack, it protects fragile products very well and is more convenient for advertising (EPA, 1995).

2.4 FRUITS AND VEGETABLES INTAKE

High amounts of fruits and vegetables consumption leads to lower risks of chronic diseases, such as cancer and cardiovascular disease (SERDULA et Al., 2004). About 60% of the deaths in 2001 were associated with chronic diseases. Most of them were cardiovascular diseases. Diabetes and obesity are also worrisome once they affect a large portion of the population and have been appearing in young people too. Unhealthy diet is one of causes of chronic diseases. Other factors also contribute, such as physical inactivity, tobacco use and alcohol consumption.

Since the second half of twentieth century, great changes in world's diet have been happening. What used to be plant-based diets have been replaced by high fat and energy diets, both in industrial regions and developing countries. Approximately 30% of the population suffers from malnutrition. It is the cause of about 60% of the 10.9 million deaths of children under five years old in the developing countries. Contrary of what was thought in the past, it is clear now the undernutrition and chronic disease problems are strongly associated.

The diet changes in the world include higher energy density diet with higher amounts of fat and sugars, greater saturated fat intake, lower intakes of complex carbohydrates and dietary fiber, and reduced fruit and vegetable intakes. The great majority of the world does not consume the recommended average intake of fruits and vegetables. The increase in urbanization may reduce the consumption of primary food, but, on the other hand, it can facilitate the access to a diverse and varied diet (FAO, 2002).

Some studies observed that the most part of the population have knowledge of the benefits of fruits and vegetables intake and, even so, don't eat it in enough amounts. This indicates the importance in studying and intervening in promoters and barriers of its consumption (SILVA, 2011).

SERDULA et al. (2004) studied the trends in fruit and vegetable consumption in the United States from 1994 to 2000. 75% of the respondents didn't eat fruits and vegetables five or more times a day, as established as healthy by the National Cancer Institute and the Produce for Better Health Foundation. The group of people from 18 to 24 years was the only group to show increase in fruits and vegetables consumption during the years of study.

RASMUSSEN et al. (2006) made a review of 98 papers about the determining factors in fruits and vegetables intake among children and adolescents between six and eighteen years old. According to this study, it was possible to conclude that gender, age, socioeconomic position, preferences, parental intake, and home availability/accessibility are strong determinants. All papers that analyzed preference observed a positive relation between preferences and fruit and vegetables intake.

Another review was made to analyze qualitative studies about these determinants in fruits and vegetables consumption among children and adolescents. It was concluded that taste/flavor is the main reason why some kids dislike fruits and vegetables, once they claim that some unhealthy foods taste better. It was also noticed that they tend to like fruits better than vegetables. Vegetables were associated with negative characteristics: bitter, sour, taste of nothing, bland, dull, tart, too strong. Some studies also showed that the method of preparation can influence in acceptability of the vegetable and the addition of toppings or another food can facilitate its consumption by children. Besides taste, texture, appearance, color and smell also appeared as determinants in children’s liking or disliking of fruits and vegetables (KROLNER et Al., 2011).

In Brazil, malnutrition in early stages of life and overweight and obesity in all ages are huge problems. A survey applied in Brazil indicated that child malnutrition is present especially in families with low income and in the north region of the country. Overweight and obesity, on the other hand, are frequently present in the population aged five years or older in all income groups and all Brazilian regions. These problems have been increasing along the years. The survey also shows an increasing trend in replacement of traditional foods in Brazilian diet, such as rice, beans and vegetables, for beverages and industrial foods, such as soda, cookies, processed meats and ready-to-eat food (IBGE, 2010). In addition, another study indicated that only 18.2% of the Brazilians consume at least five daily portions of fruits and vegetables, what is equivalent to 400 g, as recommended by the World Health Organization (BRASIL, 2010).

SILVA (2011) studied fruits and vegetables intake of adults living in Brasilia, Brazil. The results confirm that the greatest majority of the interviewers consumes less than the three daily portions of fruits and vegetables that are considerable as healthy by the Eating Guide for the Brazilian Population. According to the author, the main reasons why the interviewers feel motivated to eat fruits and vegetables are health benefits, good taste and contribution in maintenance or weight loss, while bad taste, lack of habit, available time and high perishability are among the list of reasons why they do not consume enough fruits and vegetables. The knowledge of these motivations and barriers is important when elaborating nutritional programs.

Considering the low consumption of fruits and vegetables by people of all ages all over the world and its much known importance to health, it is necessary to think in strategies to increase its intake. Also, by the studies cited in this section, it was possible to conclude that taste preference is an important factor that contributes to this low consumption. Therefore, incorporating ingredients with high nutritional value into food with high rates of consumption, such as pasta, bread and cookies, is a viable strategy to increase nutrients intake.

2.5 ENRICHED PASTA

Enriched food is any food to which a substance was added in order to increase its nutritional value, either to reset nutrients amount that were lost in the processing and storage or to add nutrients in higher amounts than its usual content (BRASIL, 1988). According to the FDA (2015), a food can be claimed as "enriched" with a nutrient when it is at least ten percent higher in the Daily Value of that nutrient than a regular food of the same type.

Brazilians have been eating less fruits and more baked products, however, a balanced diet with proteins, carbohydrates, fats, vitamins and minerals is essential to a healthy life. Thus, it is important to think in solutions to improve population's diet without huge changes in its habits, but adding nutritional quality to them. One convenient way to do that is to incorporate ingredients with high nutritional value into foods with great consumption, such as pasta (NICOLETTI, 2007).

Pasta enriched with vitamins and minerals is a cheap way to improve people's diet and to minimize hunger (REIS, 2013). However, pasta products with fruits or vegetables with high content are not common. Usually, this kind of product is found in the market either with low content of dried vegetables or with incorporation of vegetable pulp, which is normally made of up to 90% of water (Silva et al., 2012). The main purpose of adding vegetables in pasta is usually for obtaining a product with different color. For this reason, low amounts of spinach, beet, tomato and carrot are added. More recently, however, the possibility to enrich pasta with amounts that provide the recommended vegetable intake or the recommended dietary allowance (RDA) has appeared (OLIVIERO and FOGLIANO, 2016).

Worried about their health, people have been increasingly interested for food products with additional nutritional value. Once it can help to prevent some chronic diseases, it would be convenient if children ate more fruits and vegetables. However, they tend to avoid it. Considering this and that children usually like pasta products, adding vegetables into pasta and noodles may be a good strategy to increase their vegetable consumption. SILVA et al. (2013) studied the incorporation of broccoli powder in sweet potato starch noodles and durum wheat semolina pasta in order to add glucosinolates, component associated with health benefits. They concluded that incorporation of 20% of broccoli powder can improve the nutritional value of pasta-like products without losing its acceptability (SILVA et al., 2013).

In order to achieve the nutritional recommendation of certain nutrient, components losses must be taken into account. For example, hydrophilic vitamins and phytochemicals are very likely to be leached in the cooking water, and drying can cause degradation of heat sensitive bioactive compounds (OLIVIERO and FOGLIANO, 2016). Moreover, if it is necessary to add a very high concentration of a vegetable in order to obtain the recommended amount of a nutrient in the final product after cooked, it might not be viable, once it affects its rheological properties by weakening the gluten network and interfering with starch gelatinization (OLIVIERO and FOGLIANO, 2016).

Although it can be a lot beneficial to the nutritional point of view, incorporating other ingredients, such as vegetables, can negatively affect pasta quality. PETITOT et al. (2010) noticed this effect on pasta cooking quality when studied the incorporation of split pea and faba bean flour. Adding a non-gluten protein and fibers weakens the gluten and, thus, affect pasta cooking quality and texture properties. When dried at low temperatures, cooking loss was even higher and breaking energy was lower.

SILVA et al. (2012) also found problems when adding dried broccoli powder to starch noodles. Because of its swelling capacity, adding dried broccoli causes excessive increase in volume, what damages the starch matrix. The higher the concentration of broccoli added, the lower the starch fraction is and, therefore, there is less starch available to form a matrix. When the same replacement was made in durum wheat semolina pasta, no significant impact was noticed. The gluten formed in this kind of product is a strong network that prevents broccoli powder from swelling (SILVA et al., 2013).

In general, the advantages of adding vegetables in pasta are: increase of vegetable intake of pasta consumers, stabilization of phytochemicals during storage in dried pasta, possible new line of pasta products and increase of product portfolio. While the possible disadvantages are: low acceptance by pasta consumers, technological complications and lower cooking quality, big changes in sensory properties (OLIVIERO and FOGLIANO, 2016).

The addition of hydrocolloids with high water binding capacity, such as xanthan gum (SILVA et al., 2013) and the use of High Temperature drying (PETITOT et Al., 2010) in pasta production can be a solution for these problems in fortified pasta properties. Moreover, another possibility to increase nutritional value without compromising technological and sensory properties is the addition of ingredients that bring health benefits even when small quantities are used. This is the case of microalgae incorporation. These organism are rich in nutrients such as carotenoids, vitamins and fatty acids and small quantities applied in food are enough to bring good benefits (LIRA, 2011).

2.6 MICROALGAE

Algae is a large group of organisms, usually photosyntesizers, found in aquatic or humid systems. It includes unicellular and multicellular forms that can be either planktonic or benthic. The microalgae group, which consists in algae with microscopic characteristics, is mainly formed by phytoplankton, although some species are benthic or terrestrial. These microalgae make more than 90% of all photosynthesis realized in the oceans. They are the main marine primary producer. Origin, morphology and chemical composition of algae groups are very variable (LOURENÇO, 2006).

The number of species of microalgae is uncertain and can vary from 200.000 to millions. As they have very different biochemical composition, the number of products originated from these species is also unlimited (PULZ and GROSS, 2004).

2.6.1 Aplications

Microalgae can be used in human and animal feeding, for extraction of pharmaceutics compounds, for coloring, cosmetic production, as environmental indicator, methane production, water treatment, among others (LOURENÇO, 2006).

The oldest reports of human using of microalgae for feeding come from China 2000 years ago, when food was scarce and they ate cyanobacteria. Indians from Mexico and people from Africa also used to consume some species centuries ago. However, it was only in XIX century that microalgae started to be studied indeed. For almost a century, production of algae biomass have been studied for animal feeding (LOURENÇO, 2006). Since the last years, some microalgae have been commercialized as powder, tablets, capsules or extracts or incorporated in foods such as pastas, snacks, candies and beverages for nutritional purpose or as natural coloring (TRIPATHI et al., 1999).

Microalgae have high contents of protein, vitamins and fatty acids, including polyunsaturated fatty acids. Some of these important polyunsaturated fatty acids can also be obtained from oils of fishes that consume algae. However, because of its bad flavor, they are not sensory acceptable for humans (LOURENÇO, 2006). Some microalgae are rich in vitamin B-12, vitamin E and β-carotene, for example. Some studies want to prove that microalgae can be a valuable source of all important vitamins (NIELSEN, 2006).

Microalgae can also be cultivated for pigment production. Carotenoids, for example, are accessory pigments in photosynthesis, can be commercially applied as they have food coloring capacity, antioxidant and anti-inflammatory activity and are provitamin A. Other possible health benefits associated with these substances are better muscle injury recovery, reduction of menstrual cramps and cancer prevention. β□carotene, astaxanthin, lutein, zeaxanthin, lycopene and bixin are the small portion of carotenoids found in nature that are commercially used (LOURENÇO, 2006). This large variety of pigments produced is one of the most economical advantage of microalgae (NIELSEN, 2006). The main pigments present in these organisms are chlorophylls, carotenoids and phycobiliproteins (ABALDE et al., 1955).

From the environmental point of view, microalgae are also of great importance. Its application in effluent treatment have been worldwide studied and applied. They are capable of generating oxygen, can produce biomass for methane production and help to remove CO_2 from the atmosphere. Besides, this integrated system of algae and bacteria for waste decomposition, removes ammonium, nitrate and phosphate and precipitates calcium, magnesium and metals (LOURENÇO, 2006).

The two figures bellow illustrate the composition of microalgae species (figure 7) and microalgae products and applications (figure 8).

Figure 7: Comparative board of the composition of different foods and microalgae in percentage of dry matters.

Source	**Proteins (%)**	**Carbohydrates (%)**	**Lipids (%)**
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soy	37	30	20
Anabena Cilíndrica	43-56	25-30	4-7
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaria	51-58	12-17	14-22
Dunadiella salina	57	32	6
Porphyridium cruentum	28-39	40-57	9-14
Scenedesmus obliquus	50-56	10-17	12-14
Spirulina maxima	60-71	13-16	6-7
Synechoccus sp.	63	15	11

Source: Adapted from Becker (2004).

Figure 8: Micoalgae products and its applications

	Products	Applications
Biomass	Biomass	Health food Functional food Feed additive Aquaculture Soil conditioner
Coloring substances and antioxidant	Xantophylls (astaxanthin and canthaxanthin) Lutein B-carotene Vitamins C and E	Food and feed additives Cosmetics
Fatty acids-FA	Arachidonic acid – AA Eicosapentaenoic acid-EPA Docosahexaenoic acid-DHA ɣ-linolenic acid-CGA Linoleic acid-LA	Food additive
Enzymes	Superoxide dismutase-SOD Phosphoglycerate kinase-PGK Luciferase and Luciferin Restriction enzymes	Health food Research Medicine
Polymers	Polysaccharids Starch Poly-β-hydroxybutyric acid-PHB	Food additives Cosmetics Medicine
Special products	Peptides Toxins Isotopes Aminoacids (prolines, arginine, aspartic acid) Sterols	Research Medicene

Source: BARBOSA, 2003.

2.6.2 Microalgae Cultivation

With the growing application of microalgae species by man, it is of great importance its cultivation in order to reach enough quantity of its biomass. Different systems and conditions can be used to generate different substances, such as proteins, pigments, fatty acid and carbohydrates, even if the same species are cultivated (LOURENÇO, 2006). Desirable compounds production can be increased by manipulating the cultivation conditions (BOROWITZKA, 1993).

Only a small portion of the species cultivated in labs are applied in bigger scale. High rates of growth, cells shape and size, and chemical composition are the main factors to consider when scaling up a microalgae cultivation in order to make it productive (LOURENÇO, 2006).

Different production systems can be used when cultivating microalgae. Usually, open systems are used, with the disadvantage of having lack of control in temperature and lighting parameters. Common tanks are shallow, made of concrete, fiberglass, polycarbonate, ground-based or plastic-coated. In the last years, however, closed systems have been applied. Photobioreactors are used with the advantages of having a more controllable system, where pH, temperature, lighting and nutrients can be controlled, increasing process productivity (TREDICI, 2004).

Industrial effluent as a medium for algae cultivation is a solution for cost reductions. For food industry application, though, it is important to have a clean condition with no contamination. Therefore, effluents cannot be used for algae growth in this case (CHIA et Al., 2013).

2.6.3 Chlorella

The genus *Chorella* belong to the phylum Chlorophyta. They are green algae because of its photosynthetic pigments chlorophyll (LOURENÇO, 2006).

They are single-celled and have spherical shape and micro-size diameter (PANAHI et Al., 2016).

More than 70 industries produce *Chlorella* for human alimentation. They are commercialized as pills, powder for food incorporation, juices, concentrated liquid extracts and gelatinous bars. Β-1,3-glucan is the most important substance in *Chlorella,* being associated with several health benefits (LOURENÇO, 2006). Other nutritional benefit associated with *Chlorella* is the presence of antioxidant compounds: α-carotene, chlorophyll, β-carotene, α-tocopherol, ascorbic acid, lycopene, lutein, zeaxanthin, and trace elements such as zinc, copper, and magnesium (PANAHI et Al., 2016). This algae can also be used as food additive to enhance flavor and add color. Powder *Chlorella* digestibility is about 80% in humans (LOURENÇO, 2006).

Fradique et al. (2010) studied the incorporation of *Chlorella vulgaris* biomass in pasta products. The result was a product richer in chemical composition with higher protein, total fat and ash content. Cooking loss and the cooking properties were not affected, indicating that high pasta quality was achieved. Moreover, once it was higher in protein, firmness was increased. It also had good sensory acceptance.

Gouveia et al. (2007) added *Chlorella vulgaris* biomass in cookies for coloring. It was verified that low concentrations of 1% (w/w) were enough for this purpose. Color of cookies remained stable during the three months of study. The cookies had high acceptability in the sensory evaluation and better firmness.

3 ARTICLE

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Evaluation of drying temperature on bioactive compounds retention in pasta enriched with microalgae biomass

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Abstract

The aim of this study was to verify the influence of drying temperature on bioactive compounds retention in pasta enriched with 2% of *Chlorella* biomass powder. Two drying conditions, HT (high temperature, 75-80 ºC and 75% HR) an LT (low temperature, 50 ºC and 81% HR) were tested. Pasta samples analyzed were: uncooked fresh pasta (UF), cooked fresh pasta (CF), uncooked low temperature dry pasta (ULT), cooked low temperature dry pasta (CLT), uncooked high temperature dry pasta (UHT) and cooked high temperature dry pasta (CHT). Cooking properties (Optimum cooking time (OCT), solid loss, cooked weight and volume gain), color, total carotenoids content and carotenoids identification and quantification were evaluated. HT drying resulted in pasta with low cooking losses and better nutritional value after cooked. The thermal treatment increases the nutrients bioaccessibility and contributes to a stronger and denser protein network.

Keywords: Pasta, High temperature drying; microalgae; *Chlorella*, carotenoid

1 INTRODUCTION

Pasta, or alimentary paste, is one of the simplest cereal products. It exists in the human diet for millennia. Evidences show that noodles exist in China since at least 3000 BC. (BROCKWAY, 2001). It is very versatile: can be served as the main dish or as a side and can be produced in different formats, sizes and colors. It has high acceptability, low cost and it is easy to prepare. The technology involved in its production is also simple (REIS, 2013). It is a good source of complex carbohydrates and a moderate source of protein and some vitamins. Pasta is a good seller even in bad economic times (MARCHYLO and DEXTER, 2001).

Since the second half of 20th century, great changes in world's diet have been happening both in industrial regions and developing countries. The diet changes in the world include higher energy density diet with higher amounts of fat and sugars, greater saturated fat intake, lower intakes of complex carbohydrates and dietary fiber, and reduced fruit and vegetable intakes. The great majority of the world does not consume the recommended average intake of fruits and vegetables. The increase in urbanization may reduce the consumption of primary food, but, on the other hand, it can facilitate the access to a diverse and varied diet (FAO, 2002). Some studies observed that the most part of the population have knowledge of the benefits of fruits and vegetables intake and, even so, do not eat it in enough amounts. (SILVA, 2011). Considering the low consumption of fruits and vegetables by people of all ages all over the world and its much known importance to health, it is necessary to think in strategies to increase its intake. Therefore, incorporating ingredients with high nutritional value into food with high rates of consumption, such as pasta, is a viable strategy to increase nutrients intake.

Pasta enriched with vitamins and minerals is a cheap way to improve people's diet and to minimize hunger (REIS, 2013). Usually, only pasta with small amount of vegetables with the purpose of coloring are found in market. The possibility to enrich pasta with amounts that provide the recommended vegetable intake or the recommended dietary allowance (RDA) has only more recently appeared (OLIVIERO and FOGLIANO, 2016). The incorporation of high amounts of vegetables, however, can weaken the protein network, leading to a product with poor quality, with lower texture properties and higher cooking loss. The addition of hydrocolloids with high water binding capacity, such as xanthan gum (SILVA et al., 2013) and the use of High Temperature drying (PETITOT et Al., 2010) in pasta production can be a solution for these problems in fortified pasta properties. Comparing to the low-temperature drying process, high-temperatures result in better color and firmness, lower cooking loss and less stickiness, bulkiness and adhesiveness (RODA, 2013). When pasta is dried at high temperatures, the protein network is formed in the raw pasta, before starch is hydrated in boiling water. This results in a good quality product, because starch is surrounded by the coagulated protein network. Quality of the raw materials are not as important in this case. However, at lower temperatures drying, both starch gelatinization and protein coagulation happen during boiling. In this case, the quality of proteins in the raw material is very important in order to maintain the final product expected quality (RESMINI & PAGANI, 1983).

Microalgae incorporation is another viable possibility to increase foods nutritional value without compromising technological and sensory properties. These organism are rich in nutrients such as carotenoids, vitamins and fatty acids and small quantities applied in food are enough to bring good benefits (LIRA, 2011). *Chlorellas*, for example, is associated with several health benefits because of its high content of bioactive compounds, such as antioxidant compounds: α-carotene, chlorophyll, β-carotene, α-tocopherol, ascorbic acid, lycopene, lutein, zeaxanthin, trace elements such as zinc, copper, and magnesium (PANAHI et Al., 2016). This algae can also be used as food additive to enhance flavor and add color (LOURENÇO, 2006).

Nevertheless, in order to achieve the nutritional recommendation of certain nutrient, components losses must be considered. For example, hydrophilic vitamins and phytochemicals are very likely to be leached to the cooking water, and drying can cause degradation of heat sensitive bioactive compounds. A strong gluten network, however, can minimize these losses (OLIVIERO and FOGLIANO, 2016).

The aim of this study was to investigate the effect of drying temperature in the retention of carotenoids in pasta incorporated with microalgae *Chlorella luteoviridis*. Low temperature (LT) drying (50 ºC) and high temperature (HT) drying (75-80 ºC) were tested. Cooking quality, color and carotenoids content were evaluated.

2 MATERIALS AND METHODS

All experiments were held at the Universidade Federal do Rio Grande do Sul (UFRGS) at the Instituto de Ciência e Tecnologia de Alimentos (ICTA), in Porto Alegre, Brazil.

2.1 Chemicals

The carotenoids were extracted with 95 % ethanol for total carotenoids analysis. Acetone, potassium hydroxide, ethyl ether and petroleum ether were used for samples preparation for carotenoids identification and quantification. HPLC grade solvents were methyl tert-butyl-ether and methanol. Millipore water was used in the samples undergoing HPLC analysis.

2.2 Pasta preparation

Common wheat flour (protein 11.5 g/100 g, ash 0.61 g/100 g, moisture 14.1 g/100 g), 500 g, water, 170 mL, and ChlP (*Chlorella luteoviridis* biomass powder), 10 g, were used as components of pasta. Wheat flour, ChlP and water were mixed using an industrial mixer (G.Paniz, Mod 90334, Brazil) for 10 min, to obtain homogeneous dough. The premixed dough was extruded (40 ± 2 °C), through a die, in the same equipment, to obtain the fettuccini shaped pasta.

As the pasta exited the extruder, it was manually cut in approximately 100 mm length. At the end, the product was hanged in a pasta rack and air dried for 20 min and, then, packed in plastic bags and stored under refrigeration. To define the drying conditions, two preview assays were performed. In the first one, a predrying at 40 ºC was held for 1 h, followed by a 30 min resting stage at 27 ºC and 85 % RH and finished by the final drying at 75-80 ºC for 35 min. In the other trial, the whole drying was held at the temperature of 75-80 ºC with 30 min resting stage at 27 ºC and 85 % RH in the middle of the drying process. Then, cooking loss was measured for both trials and, as there was no difference in this aspect, it was defined that no predrying would take place for the purpose of this study. Thus, the real effect of each temperature tested could be observed. Thereby, pasta samples were dried at two drying condition, HT (high temperature, 75-80 ºC and 75 % HR) or LT (low temperature, 50 ºC and 81 % HR).

Pasta samples (fettuccini about 2.0 mm thickness, 100 mm length) were dried in a tray batch dryer until the moisture of pasta reached about 12 g/100 g (wb). Both of them had a resting stage of 30 min at 27 ºC and 85 % HR at the middle of the drying. In addition, the drier was cooled to 30 ºC before the pasta was removed to avoid a "thermal chock" and consequent pasta cracking.

At the end of the pasta preparation, six pasta samples were analyzed: uncooked fresh pasta (UF), cooked fresh pasta (CF), uncooked low temperature dry pasta (ULT), cooked low temperature dry pasta (CLT), uncooked high temperature dry pasta (UHT), cooked high temperature dry pasta (CHT).

2.3 Pasta Moisture

Fresh and dry pasta moisture was determined based on AOAC (1990) methods. Five grams of fresh pasta were weighted, placed into dry crucible and put into oven at 110 ºC until constant weight (dry sample) was reached. Moisture content (%) was measured using equation 1. Three measurements were performed for each analysis, and the mean values were obtained.

$$\% \, Moisture = \frac{(wet\ sample\ weight - dry\ sample\ weight)}{wet\ sample\ weight} \quad (1)$$

2.4 Pasta Cooking Properties

Optimum cooking time (OCT), solid loss, cooked weight and volume gain methodology were determined by the American Association of Cereal Chemists Official Methods 16-50 and 16-51 (AACC, 2000).

The pasta sample (10 g) was broken into 5-cm pieces and cooked in boiling distilled water (170 mL). Boiling was maintained during the cooking period. The time when the inner white core of the pasta disappeared was identified as the OCT (*al dente point*). After cooking, the pasta was rinsed with water for 30 s and drained for 1 min to expel the residual water. An aliquot of 25 mL of cooking water was put into a metal capsule and dried at 105 ºC (DeLeo, model 48 TLK, Brazil) until constant weight to evaluate the presence of solids from the pasta. The solid loss was expressed as a percentage of the raw pasta, according to Equation 2.

$$\%\ cooking\ loss = solids\ weight \times \frac{140\ mL}{25\ mL} \quad (2)$$

At this stage, pasta samples were weighed to determine the cooked weight and the percentage of weight increase (Equation 3).

$$weight\ gain\ = \frac{weight\ after\ cooked}{weight\ before\ cooked} \quad (3)$$

For determination of the volume gain, ten grams of pasta were immersed into a measuring cylinder filled with 140 mL of distilled water and the volume was read. After that, the pasta was removed from the measuring cylinder and cooked (at OCT) in 140 mL of boiling distilled water. Then, the cooked pasta was immersed into the measuring cylinder filled with 140 mL of distilled water and the volume was read again to determine the volume gain by equation 4. All tests were performed in duplicate.

$$volume\ gain\ = \frac{volume\ after\ cooked}{volume\ before\ cooked} \quad (4)$$

2.5 Total carotenoids

The pasta samples (CF, UF, CHT, UHT, CLT and ULT) were lyophilized and grinded by hand using gral and pistil. For each sample, 0.5 g were weighted and put into a Falcon tube. An aliquot of 10 mL of 95% ethanol was added to each tube, mixed in a vortex agitator and stored in refrigerator overnight. The next day, all tubes were centrifuged (Sigma Laboratory Centrifuge 4K15) for 20 min and the supernatant was read in a spectrophotometer (Amersham Biosciences, Ultrospec 3100 Pro) at 664 nm (DO_{664}), 649 nm (DO_{649}) and 470 nm (DO_{470}). LINCHTENTHALER and BUSCHANN (2001) equations were used to determine chlorophyll a, chlorophyll b and total carotenoids.

$$C_a = 13.36 \times DODO_{664} - 5.19 \times DO_{649} \quad (5)$$

$$C_b = 27.43 \times DO_{470} - 8.12 \times DO_{664} \quad (6)$$

$$C_{Ct} = \frac{1000 \times DO_{470} - 2.13 \times C_a - 97.64 \times C_b}{209} \quad (7)$$

Where:

C_a = Chlorophyll a content in mg/L

C_b = Chlorophyll b content in mg/L

C_{Ct} = Total Carotenoids content in mg/L

Once the experiment was realized with 0.5 g of sample and 10 mL of solvent, the final result was divided by 0.5 g and multiplied by 0.01 L and 1000 ug/mg, in order to obtain μ

g/g as the result unit.

2.6 Carotenoids identification and quantification

Approximately 2 g of each sample, previously lyophilized, were separately weighted and grinded manually using gral and pistil with acetone as solvent. The sample and solvent were transferred to a Falcon tube and centrifuged for 12 min. Afterwards, the supernatant phase was put into a Beaker glass and the solid was macerated with acetone again, put back into the tube and centrifuged for another 12 min. The same procedure was done about four times, until almost all coloration was extracted.

The liquid phases that were accumulated in the Beakers were transferred to a round-bottom flask and put in a rotary evaporator until all the solvent evaporated. Then, ethyl ether was used to rinse the flask so that all the sample could be transferred to a Beaker. KOH in the same quantity of the liquid present in the Beaker was added for saponification. Aluminum paper was used to cover the glasses, which were stored overnight.

The next day, the samples were slowly added to a separating funnel previously filled with half water and half ethyl ether and petroleum ether. Distilled water was used to repeatedly wash the samples until pH 7 was reached. At this point, all water was removed from the funnel, and the extract was transferred to a clean Beaker. Anhydrous sodium sulfate was added to the glass in order to absorb the left water. Afterwards, all the liquid phase was put into a round-bottom flask and evaporated in the rotary evaporator. After the samples were dried, they were removed with ether and put into an amber flask using a funnel. Then, the samples were dried with nitrogen gas and stored in freeze.

The samples were analyzed by high-performance liquid chromatography (HPLC) (Waters Alliance® E2695) using a reversed phase column C30 250 mm × 4.6 mm ID, 3 µm (YMC, modelo CT99SO3-2546WT). Previous to injection, the samples were diluted in methyl tert-butyl-ether (MTBE) and methanol, put in the ultrasound (Unique, USC 1400) and filtered (Millex LCR 0,45, 13 mm). The injection volume was 20 µL. The mobile phase was water/methanol/MTBE with a flow rate of 1 mL/min in a total running time of 67 min. Carotenoids were detected at a wavelength of 450 nm and identified based on retention time compared with reference materials.

2.7 Color

Color analyses was measured with a Hunter Lab Colorimeter (MiniScan XE Plus, Reston, VA). Color was expressed in *L**, *a**, *b** Hunter scale parameters. Results were expressed as color differential (ΔE) between fresh and cooked pasta, calculated as Equation 8. Results are the means of independent duplicate determinations.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \tag{8}$$

Where, ΔL was calculated as : $L^*_{\text{fresh sample}} - L^*_{\text{cooked sample}}$;

Δa was calculated as : $a^*_{\text{fresh sample}} - a^*_{\text{cooked sample}}$;

Δb was calculated as : $b^*_{\text{fresh sample}} - b^*_{\text{cooked sample}}$.

3 RESULTS AND DISCUSSIONS

3.1 Moisture and Cooking Properties

Uncooked pasta moisture and cooking properties (optimum cooking time, cooking loss, weight gain and volume gain) are shown in Table 1.

Table 1: Moisture and cooking properties for uncooked fresh pasta (UF), uncooked low temperature dry pasta (ULT) and uncooked high temperature dry pasta (UHT).

	Moisture (%)	Optimum cooking time (min)	Cooking loss (%)	Weight gain (%)	Volume gain (%)
UF	29.35 ± 0.07	5.0	11.68 ± 1.4 [a]	198 ± 12 [a]	107.0 ± 0.5 [a]
ULT	13.23 ± 0.13	8.0	9.86 ± 0.05 [a,b]	225 ± 02 [a]	106.5 ± 1.0 [a]
UHT	11.16 ± 0.03	7.5	6.73 ± 0.34 [b]	212 ± 08 [a]	107.1 ± 0.5 [a]

Results indicate mean values ± SD.
Means with the same letter in the same column are not significantly different (Tukey test, $p \leq 0.05$).

Moisture content for UF and UHT pasta were within the range stablished by ANVISA (BRASIL, 2000) for fresh (≤ 35.0 %) and dry (≤ 13 %) pasta, respectively. ULT pasta moisture content was slightly higher than the maximum of 13 % for dry pasta. It might be attributed to water absorbance after drying, either before packaging or during samples preparation for analysis.

Cooking loss was lower than 12 %, what indicates good quality pasta according to Hummel (1966). It indicates that the amount of flour substituted was not enough to damage the gluten network. These results were similar to what was found by Del Bem et al. (2012) when producing pasta with substitution of 35 % of wheat semolina by peas and chickpeas flours. High temperature drying lead to a decrease in solid loss to the cooking water. This was also concluded in other studies: Padalino et al. (2016) observed an average cooking loss of 5.09 % for pasta dried at low temperature (50 ºC), decreasing to 4.25 % when dried at very high temperature (90 ºC); Dexter, Matsuo and Morgan (1981) experienced decrease in solid losses in all three semolina samples studied when higher temperatures were used specially in the final drying stage; D`Amico et al. (2015) achieved lower cooking loss in gluten-free pasta when higher drying temperatures were used. This effect is a consequence of the stronger protein network that is formed in the uncooked pasta when HT drying is applied (Zweifel, 2001).

No significant difference was noticed between the three samples regarding weight gain and volume gain. Weight gain of about 200 % and volume gain of about 300 % are common results (Casagrandi, 1999). Thus, results for weight gain indicate good quality pasta. Increase in volume, however, was low considering this reference and some pasta studies (Del Bem, 2012; Menegassi & Leonel, 2006; Casagrandi, 1999). One possible explanation for this is the quality of the ingredients. If a better quality flour was used, durum wheat semolina for example, a stronger gluten network would be formed.

Consequently, more water would be retained, leading to higher increases in weight and volume.

3.2 Total Carotenoids

Total carotenoids of all pasta samples are summarized in Table 2.

Table 2: Total carotenoids in uncooked fresh pasta (UF), cooked fresh pasta (CF), uncooked low temperature dry pasta (ULT), cooked low temperature dry pasta (CLT), uncooked high temperature dry pasta (UHT), cooked high temperature dry pasta (CHT) incorporated with 2% of *Chlorella luteoviridis* biomass powder.

	Total Carotenoids (μg/g)
UF	13.6 ± 0.7 [b]
CF	2.0 ± 0.3 [d]
ULT	17.4 ± 0.2 [a]
CLT	5.4 ± 0.5 [c]
UHT	13.2 ± 0.6 [b]
CHT	7.0 ± 0.8 [c]

Results indicate mean values ± SD.
Means with the same letter in the same column are not significantly different (Tukey test, $p \leq 0.05$).

For uncooked samples, fresh and HT pasta had no significant difference, while ULT was higher in total carotenoids. Some nutrients can be degraded by heat, while thermal treatment can increase its bioaccessibility (Van Boekel et al., 2010). Thus, it can be assumed that in LT, the moderate thermal treatment was enough to increase carotenoids bioaccessibility without high losses. However, it is important to consider the results for the cooked samples, once this is how people consume it. Lower losses were observed in HT pasta, leading to a final product with higher carotenoids content than the fresh pasta. This was caused due to the increase in bioaccessibility caused by drying and cooking (Van Boekel et al., 2010) in addition to the formation of a more compact, dense and strong protein network because of the drying temperature applied (ZWEIFEL, 2001). Statistically, CHT and CLT did not present difference in total carotenoids content. The tests should be repeated once the standard deviation was high and it might have compromised the statistical analysis. Also, higher temperatures could be tested in order to verify a possible greater difference between drying temperatures.

Figure 1 better illustrates these results.

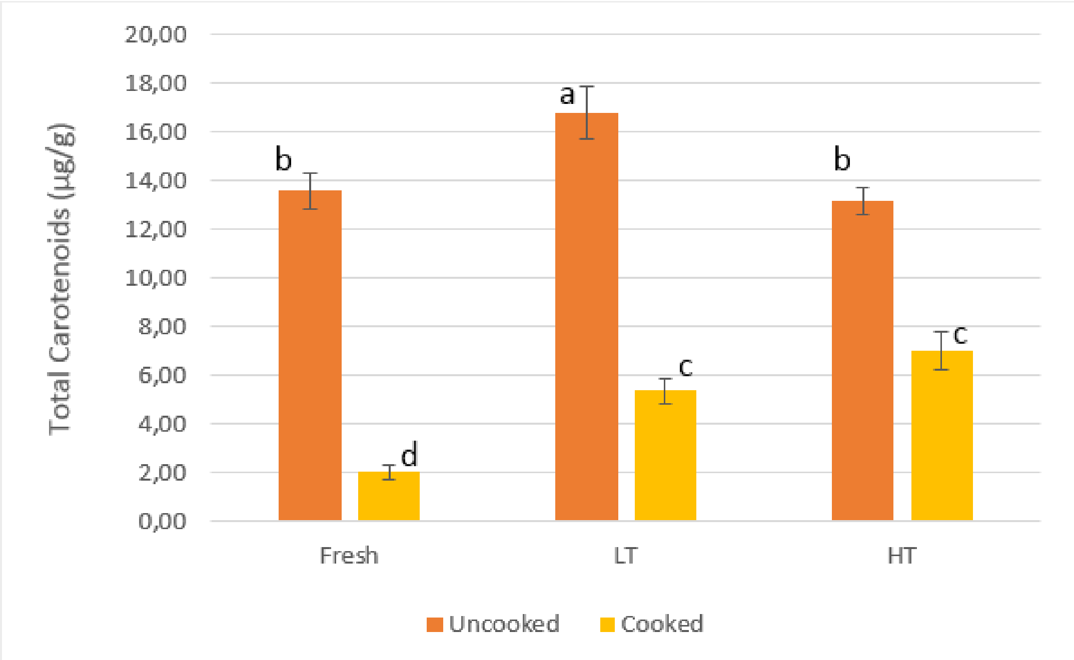

Figure 1: Total carotenoids in uncooked fresh pasta (UF), cooked fresh pasta (CF), uncooked low temperature dry pasta (ULT), cooked low temperature dry pasta (CLT), uncooked high temperature dry pasta (UHT), cooked high temperature dry pasta (CHT) incorporated with 2% of *Chlorella luteoviridis* biomass powder.

3.3 Carotenoids Identification and Quantification

HPLC results indicated that the most relevant carotenoids present in pasta with incorporation of 2 % of *Chlorella luteoviridis* biomass powder were lutein, trans-zeaxanthin, all trans-α-carotene and all-trans-β-carotene. Therefore, these were the carotenoids analyzed. Table 3 and Figure 2 show the results of this analysis.

Table 3: Main carotenoids content in uncooked fresh pasta (UF), cooked fresh pasta (CF), uncooked low temperature dry pasta (ULT), cooked low temperature dry pasta (CLT), uncooked high temperature dry pasta (UHT), cooked high temperature dry pasta (CHT) incorporated with 2% of *Chlorella luteoviridis* biomass powder in µg/g.

	Lutein	*trans*-zeaxanthin	*all-trans*-α-carotene	*all-trans*-β-carotene
UF	$1.15 \pm 0.50^{b,c}$	0.24 ± 0.08^{c}	0.45 ± 0.11^{c}	$0.47 \pm 0.13^{b,c}$
CF	0.60 ± 0.16^{c}	0.16 ± 0.03^{c}	0.24 ± 0.11^{c}	0.25 ± 0.11^{c}
ULT	$2.06 \pm 0.06^{a,b}$	$0.47 \pm 0.04^{a,b}$	$0.70 \pm 0.04^{a,b}$	$0.77 \pm 0.03^{a,b}$
CLT	$1.30 \pm 0.17^{a,b,c}$	0.24 ± 0.03^{c}	0.38 ± 0.01^{c}	$0.40 \pm 0.02^{b,c}$
UHT	$1.63 \pm 0.38^{a,b,c}$	$0.32 \pm 0.06^{b,c}$	$0.60 \pm 0.19^{b,c}$	$0.64 \pm 0.19^{a,b,c}$
CHT	2.60 ± 0.26^{a}	0.46 ± 0.07^{a}	0.95 ± 0.06^{a}	1.02 ± 0.06^{a}

Results indicate mean values ± SD.
Means with the same letter in the same column are not significantly different (Tukey test, $p \leq 0.05$).

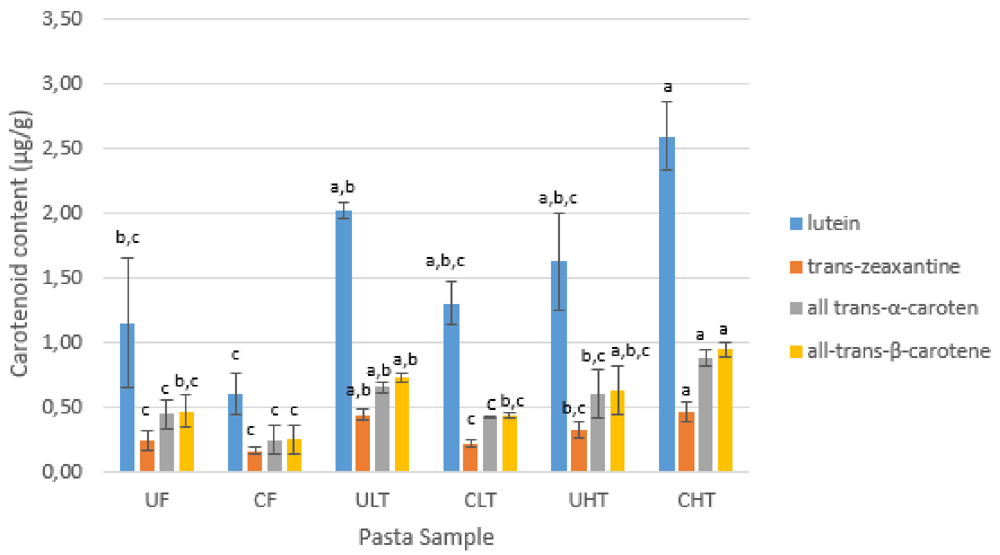

Figure 2: Main carotenoids content in uncooked fresh pasta (UF), cooked fresh pasta (CF), uncooked low temperature dry pasta (ULT), cooked low temperature dry pasta (CLT), uncooked high temperature dry pasta (UHT), cooked high temperature dry pasta (CHT) incorporated with 2% of *Chlorella luteoviridis* biomass powder.

Cooked and uncooked fresh pasta had no difference in the content of each carotenoid. This indicates that the nutrient loss during cooking was equivalent to its increase in accessibility. No nutrient losses was noticed for HT samples during cooking, indicating good carotenoids retention when pasta is dried at high temperatures. While there was no difference between CF and CLT samples, CHT showed to have higher amounts of trans-zeaxanthin, all trans-α-carotene and all-trans-β-carotene when the product was ready-to-eat. Similar results were found in a study (Oliviero and Fogliano, 2015) with pasta incorporated with carrots. β-carotene content reduced with drying by heat degradation, but did not decreased after cooking. β-carotene has lipophilic behavior and, therefore, leaching to the cooking water is low (Oliviero and Fogliano, 2015). This relation was different in a study with pasta enriched with broccoli powder, in which the glucosinolates (GLs) content was evaluated. In this study, drying did not have the expected effect in retaining these compound. GLs are water soluble and higher amounts (10 %, 20 % and 30 %) of broccoli were added, having negative effect in the protein matrix (Silva et al., 2013). Therefore, it is necessary to study the specific nutrient in question, as well as the ingredient added.

In general, high temperature drying showed to have a better effect in carotenoids retention. The results for specifics carotenoids quantification and total carotenoids are different because of the different methodology and solvents that are used for extraction in each method. It is hard to extract carotenoids from the compact pasta matrix. This can cause a high variability among the results. Further work suggestion is improvement in carotenoids extraction technique for this kind of product.

3.4 Color

ΔE values between uncooked and cooked pasta samples are shown in Table 4. No statistic difference was observed between samples. Fradique et al. (2010) found lower color losses in pasta with addition of 2 % of *Chlorella luteoviridis* incorporation. They attributed this decrease in color to pigment diffusion into cooking water and oxidation caused by thermal treatment.

Table 4: Color differential (ΔE) between uncooked and cooked pasta for Fresh, HT and LT samples.

	ΔE
Fresh	11.4 ± 1.5^{a}
HT	17.4 ± 4.7^{a}
LT	13.0 ± 1.0^{a}

Results indicate mean values ± SD.
Means with the same letter are not significantly different (Tukey test, $p \leq 0.05$).

4 CONCLUSION

Incorporation of 2 % of ChlP did not affect cooking quality, indicating that the protein network was not damaged by adding this small amount of biomass. Cooking loss was lower for HT sample, because of the fortification of the gluten network formed in the raw pasta caused by the high temperature drying.

LT showed higher amounts of total carotenoids in the uncooked sample because the heat degradation by the moderate thermal treatment applied was low, but its losses to the cooking water were higher. It is convenient to consider the cooked samples, once that is how they are usually consumed. Both dried samples (CHT and CLT) had higher amounts of total carotenoids than the fresh sample (CF) when cooked. HT pasta showed lower total carotenoids losses during cooking. The high temperature drying increased the biocompounds accessibility at the same time that favored its retention in the pasta matrix.

Lutein, *trans*-zeaxanthin, *all*-*trans*-α-carotene and *all*-*trans*-β-carotene were the carotenoids identified in higher amount in pasta incorporated with ChlP. CHT was the sample with greatest content of almost all carotenoids. It shows the importance of using high temperature drying in enriched pasta for the retention of carotenoids. Further studies are needed for different nutrients.

In conclusion, pasta incorporated with 2 % of *Chlorella luteoviridis* powder dried at high temperatures appear to have higher nutritional value concerning carotenoids content. However, tests should be repeated in order to reduce standard deviation. Also, two further studies are recommended: improvement in extraction techniques and evaluation of higher drying temperatures.

This study indicates the importance of studying the processing parameters for the nutritional value of the products. In this case, drying temperature lead to higher bioactive compounds content, even if it could be expected otherwise due to heat degradation. It is important to have similar studies for different nutrients or other types of enriched food.

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