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Avaliação *in vitro* da cinética de Álcool desidrogenase em frutos de *Ziziphus joazeiro* para aliviar os efeitos deletérios do álcool

In vitro evaluation of Álcool desidrogenase kinetics in *Ziziphus joazeiro* fruits to alleviate the harmful effects of alcohol

Evaluación *in vitro* de la cinética de la Alcohol deshidrogenasa en frutos de Ziziphus joazeiro para aliviar los efectos nocivos del alcohol

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Resumo

O Ziziphus joazeiro é endêmico da Caatinga brasileira e seus frutos podem ser utilizados como complemento alimentar, acelerando o metabolismo do etanol no organismo e reduzindo os efeitos nocivos do álcool devido à alta atividade da desidrogenase do álcool (ADH). O objetivo foi determinar a cinética da atividade do ADH, em diferentes tempos de incubação, de frutos maduros de Z. joazeiro como suplemento alimentar. Os frutos de Ziziphus joazeiro, no quarto estágio de maturação, foram incubados por 0 (sem incubação), 3, 6, 12, 24 e 48 horas em temperatura ambiente. A atividade de ADH foi determinada. A atividade do ADH foi maior nos frutos incubados por 0 e 3 h. A atividade do ADH foi maior nos primeiros tempos de incubação, provavelmente devido à maior disponibilidade de NAD +, que após ser reduzido a NADH atrasou a regeneração. Sem o cofator na forma oxidada, a atividade enzimática diminui. O fruto de Ziziphus joazeiro tem potencial para ser utilizado como complemento alimentar, acelerando o metabolismo do álcool e reduzindo os efeitos nocivos.

Palavras-chave: Acetaldeído; Álcool desidrogenase; Aldeído desidrogenase.

Abstract

Ziziphus joazeiro is endemic to the brazilian Caatinga and its fruits can be used as a food supplement by accelerating the ethanol metabolism in the body and reducing the alcohol harmful effects due to high alcohol dehydrogenase (ADH) activity. The objective was to

determine the kinetics of ADH activity, in different incubation times, of *Z. joazeiro* mature fruits as a food supplement. *Ziziphus joazeiro* fruits, at the fourth maturation stage, were incubated for 0 (no incubation), 3, 6, 12, 24 and 48 hours at room temperature. The ADH activity was determined. ADH activity was higher in fruits incubated for 0 and 3 h. The ADH activity was higher in the early incubation times, probably due to the greater availability of NAD^+, which after being reduced to NADH delayed regeneration. Without the cofactor in the oxidized form, the enzymatic activity decreases. *Ziziphus joazeiro* fruit has the potential to be used as a food supplement accelerating the alcohol metabolism and reducing the harmful effects.

Keywords: Acetaldehyde, Alcohol dehydrogenase, Aldehyde dehydrogenase.

Resumen

Ziziphus joazeiro es endémico de la Caatinga brasileña y sus frutos se pueden usar como complemento alimenticio al acelerar el metabolismo del etanol en el cuerpo y reducir los efectos nocivos del alcohol debido a la alta actividad de la deshidrogenasa de alcohol (ADH). El objetivo fue determinar la cinética de la actividad de ADH, en diferentes tiempos de incubación, de las frutas maduras de Z. joazeiro como suplemento alimenticio. Los frutos de Ziziphus joazeiro, en la cuarta etapa de maduración, se incubaron durante 0 (sin incubación), 3, 6, 12, 24 y 48 horas a temperatura ambiente. Se determinó la actividad de ADH. La actividad de ADH fue mayor en frutos incubados durante 0 y 3 h. La actividad de ADH fue mayor en los primeros tiempos de incubación, probablemente debido a la mayor disponibilidad de NAD +, que después de reducirse a NADH retrasó la regeneración. Sin el cofactor en la forma oxidada, la actividad enzimática disminuye. La fruta de Ziziphus joazeiro tiene el potencial de ser utilizada como un complemento alimenticio que acelera el metabolismo del alcohol y reduce los efectos nocivos.

Palabras clave: Acetaldehído; Alcohol deshidrogenasa; Aldehído deshidrogenasa.

1. Introduction

Alcohol consumption is increasing rapidly in the world (Wang *et al*., 2016) and is a global health problem (UK Medical Officer, 2019). Health benefits from alcohol consumption at low levels are outweighed by the increased risk of further harm, including cancer (Burton and Sheron, 2018). Alcoholic liver disease, for example, is due to the daily consumption of alcohol for a time greater than or equal to 5 years (Singal and Bailey, 2018). There is no safe level of alcohol consumption because it compromises the mental, behavioral and

physiological capabilities of drinkers even in small doses. Therefore, global public health policies induce a reduction in alcohol consumption (UK Medical Officer, 2019).

The absorption rate of alcohol, a phenomenon different from tolerance, varies with the amount of alcohol ingested, fractionation and dose spacing, alcohol concentration of the beverage, presence of food in the stomach and absorption capacity of the individual (Cederbaum, 2012). After ingestion, alcohol begins to be absorbed through the digestive tract, passing directly into the portal vein and into the liver, into the blood and lymphatic circulation, distributing itself through the tissues (Kiela and Ghishan, 2017). When the amount consumed exceeds caloric production, the excess alcohol is impregnated in fat-soluble tissues, predominantly in the brain, producing a narcotic effect, the first symptom of which is excitation and then depression. From there the human organism begins detoxification through continued stages of oxidation (Shabtai *et al*., 2018).

Alcohol is eliminated from the body by metabolic degradation by enzymatic and non-enzymatic pathways (Wang *et al*., 2016). More than 90% of the alcohol is metabolized in the liver (Ramchandani *et al*., 2001). The metabolism of alcohol by enzymatic oxidation consists of two successive reactions that require nicotinamide adenine dinucleotide (NAD^+) as a cofactor (Shabtai *et al*., 2018). The first reaction is catalyzed by alcohol dehydrogenase (ADH) which converts ethanol to acetaldehyde by reducing NAD^+. Acetaldehyde, reactive and hepatotoxic (Singal and Bailey, 2018), is metabolized to acetic acid by the aldehyde mitochondrial dehydrogenase (ALDH), which also reduces NAD^{+1}. The acetate produced is rapidly converted to carbon dioxide and water in the tricarboxylic acid cycle. Changes in these metabolic pathways may modulate the toxicity of the alcohol as its efficiency determines its bioavailability (Wang *et al*., 2016).

The consumption of food and non-alcoholic beverages with alcohol can increase the activity of ADH and ALDH and thus accelerate the metabolism of ethanol. Alcohol is metabolized more rapidly in fed individuals because ADH levels are higher and the ability of the mechanisms of transport of reducing substrates in the mitochondria is high (Lee *et al*., 2013). Food can increase blood flow in the liver and provide sugars that increase the metabolism of alcohol for providing substrates that convert NADH to NAD^+ by increasing mitochondrial uptake of oxygen. The increase in alcohol for food elimination rate was similar in different compositions for meals (Cederbaum, 2012).

Fruits are rich in ADH that contribute to the development of aroma by the interconversion of aldehydes and volatile alcohols from lipids and amino acids, but not all have a beneficial effect (Sieso *et al*., 1976). The concentration of acetaldehyde and ethanol,

pyruvate decarboxylase activity (PDC) and ADH in fruits stored in anerobic conditions is high (Pesis, 2005; Espino-Diaz *et al*., 2016) and can be a potential food supplement to accelerate the metabolism of alcohol (Ramchandani *et al*., 2001b).

Fruits of *Ziziphus joazeiro* Mart. (Rhamnaceae), are globular drupes 1 to 1.5 cm in diameter, with yellow-brown bark and white flesh. The pulp is sweet and mucilaginous, with a high content of vitamin C (Silva *et al*, 2018a) and pleasant taste and flavor (Leon-Sanchez *et al*., 2009) and can be used as dietary supplements and source of ADH assisting in a diet to eliminate alcohol and preventing liver damage (Wang *et al*., 2016).

The objective was to determine the kinetics of ADH activity, at different incubation times, of ripe fruits of Z. joazeiro, as a potential food supplement to combat the harmful effects of alcohol.

2. Material and Methods

Seventy-two fruits, at the fifth maturation stage (Figure 1). At this stage, the fruits were ripe and with organoleptic characteristics suitable for consumption. The fruits were harvested from adult trees at the no Centro de Ciência e Tecnologia Agroalimentar (6º 48' 16" S and 37º 49' 15" W) from Universidade Federal de Campina Grande, Pombal, Paraíba, Brazil. Three fruits, in four replicates, were packed in plastic-coated pots and incubated for 0 (no incubation), 3, 6, 12, 24 or 48 hours after harvesting at room temperature, simulating storage. The experiment was repeated three times.

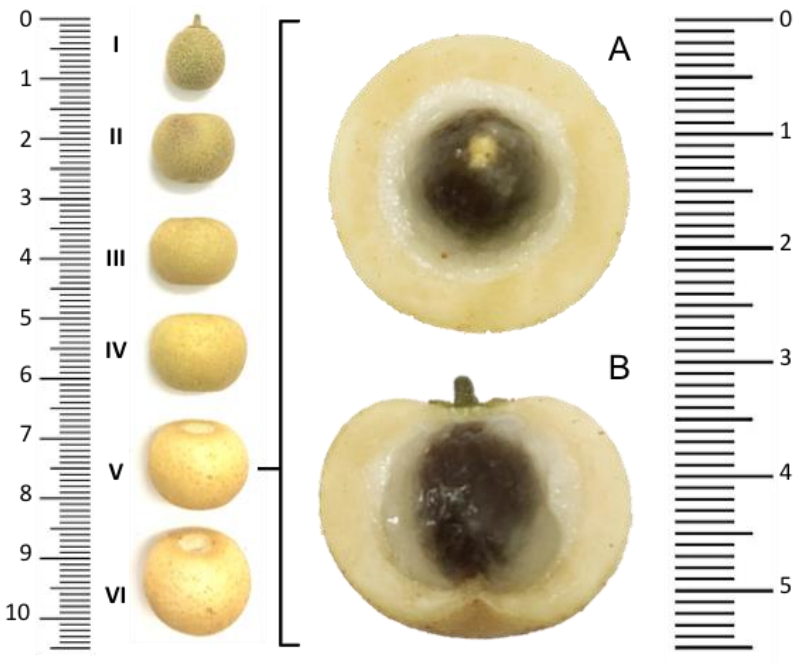

Figure 1. General appearance of the *Ziziphus joazeiro* fruits highlighting the fifth stage. Scale in cm.

Determination of ADH activity

The fruits were peeled, weighed and macerated with 100 mM MES solution + 10 mM β-mercaptoethanol for enzymatic extraction. The macerate was filtered and centrifuged at 3500 rpm for 60 minutes at 4 °C. Forty microliters of the supernatant were collected, homogenized, and 800 µL of 100 mM MES buffer solution, 50 µL of 1.6 mM NADH and 50 µL of 80 mM acetaldehyde were added. The ADH activity was determined by spectrophotometry (CT 5000R - Cientec®) at 340 nm every 90 seconds for 15 minutes to obtain activity curve in each incubation time.

Experimental design and data analysis

The experiment was arranged in a completely randomized design. The results were evaluated with Software AgroEstat® and means compared by Tukey test at 5% probability.

3. Results and Discussion

ADH activity was higher in fruits incubated for 3 and 0 hours and lower in those incubated for 12 and 24 h (Figure 2).

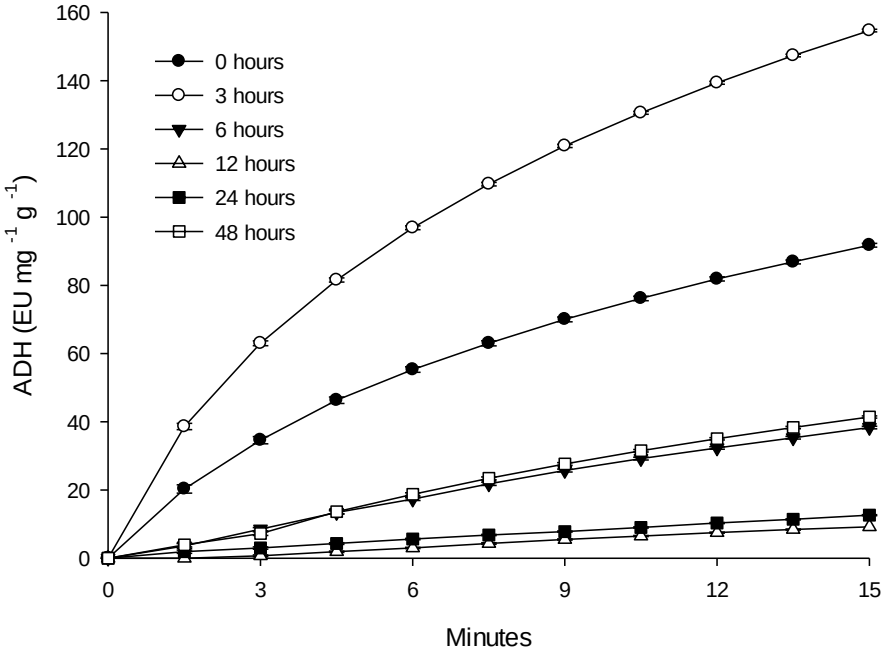

Figure 2. ADH activity in fruits of *Ziziphus joazeiro* harvested at the fifth maturation stage at different incubation times.

The highest activity of ADH in the fruits of *Z. joazeiro* incubated for 3 and 0 hours is due to the metabolization of the alcohol accumulated by the fermentation. Pyruvates formed during glycolysis are decarboxylated by pyruvate decarboxylase, forming acetaldehyde during anaerobic respiration. Acetaldehyde is converted to ethanol by ADH reducing NAD^+ to NADH (Wang *et al*., 2016) (Figure 3). The highest detection of enzymatic activity occurs precisely because of the availability of NAD^+ in its oxidized form. However, the reduction of NAD^+ to NADH reduces the amount of this molecule, limiting the activity of ADH and explaining the decrease in activity between 6 and 12 hours of incubation. For ADH activity to continue, NAD^+ needs to be regenerated (Tacin, 2015). Regeneration of NAD^+ is one of the major problems that reduce the activity of dehydrogenase enzymes, since the reaction requires its oxidized form. Properly balanced catalytic amounts of the oxidized and reduced form of NAD^+ coenzyme is sufficient to allow the dynamic process (Knaus and Mutti, 2017).

One possible solution to maintain or prolong the activity of ADH and hence its positive effect on the metabolism of alcohol would increase the pH of the reaction medium, driving the consumption of H^+ ions, forcing the oxidation of NADH to NAD^+ (Smith, 2011). The regeneration of NAD^+ can also occur through the use of catalysts which accelerate the electron transfer kinetics as the use of organic dyes or by heterogeneous catalysis using

modified electrodes (Smith, 2011). The possibility of efficiently replacing NAD^+ coenzymes with synthetic biomimetics may contribute to the overall economic profitability of the hydrogen lending cascades applied to the food industry (Knaus *et al*., 2016; Höfler *et al*., 2018).

Bi-catalytic redox reactions, such as those catalyzed by ADH, are receiving increasing attention in preparative organic synthesis (Bornscheuer *et al*., 2012). Specifically, stereospecific ketoreductions and reductive aminations are well established, especially in the pharmaceutical industry (Huisman *et al*., 2010) and could be better applied to the food industry. Being reductive in nature, these reactions need to be constantly supplied with reducing equivalents in the form of the reduced nicotinamide cofactors NAD(P)H. Effective reaction schemes (cost) inevitably involve sub-stoichiometric amounts of the NAD(P)H cofactors and their *in situ* regeneration (Höfler *et al*., 2018).

The speed of ethanol oxidation is critical because the metabolism pathway itself is toxic (Garcia, 2009). The acetaldehyde formed can induce DNA deletions chains, chromosomal aberrations, generation of proteins and DNA adduct, besides the potential carcinogenicity often supported by animal and in vitro evidence (Scoccianti *et al*., 2014). The maximum activity of the dehydrogenases in the liver is similar, occurring in the same way (Garcia, 2014), however, the consumption of foods, mainly fruits, is a viable alternative to increase the activity of the enzymes dehydrogenases, ADH and ALDH, accelerating metabolism of ethanol and acetaldehyde (Sieso *et al*., 1976; Li *et al*., 2014). The consumption of mixed fruit and vegetable juice demonstrated a significant reduction in the blood alcohol content of adult humans (Figure 3), as well as reducing thirst and headache scores (Lee et al., 2013).

Intake of citrus juices of lemon, *Averrhoa carambola*, *Pyrus* spp. and *Syzygium samarangense* decreased blood alcohol concentrations in rats (Zhang *et al*., 2016).

However, ingestion of some juices may potentiate the effect of alcohol, as reported for *Chaenomeles sinensis* which increased blood alcohol concentrations of rats (Zhang *et al*., 2016). Therefore, further research should be performed to evaluate the effect of *Z. joazeiro in vivo*.

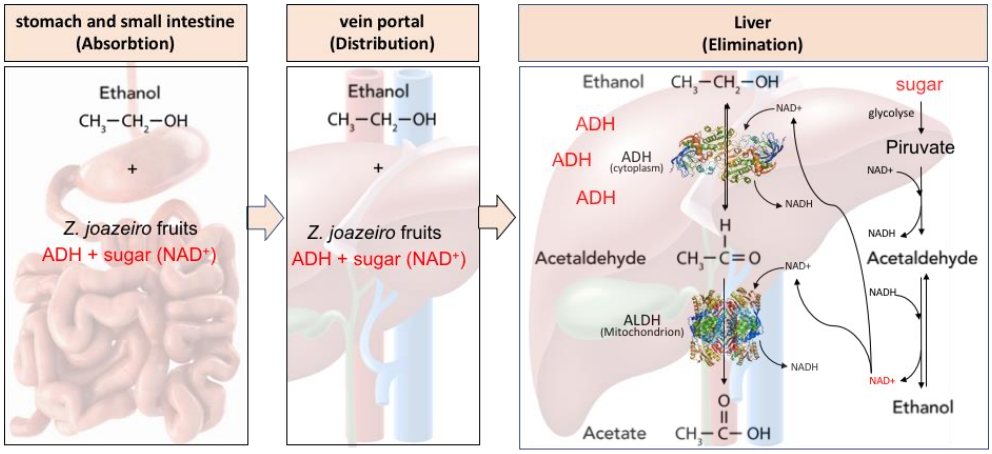

Figure 3. General scheme demonstrating the increase in the ADH and NAD^+ pool in the human liver with the ingestion of fruits *Ziziphus joazeiro*.

4. Conclusion

Fruits of *Z. joazeiro* has the potential to be used as a food supplement accelerating the metabolism of alcohol and reducing the harmful effects on the liver, especially when incubated for 3 h. The limitations of the study refer to the failure to perform the *in vivo* evaluation, to ensure greater consistency in the results. The *in vivo* evaluation of the effects of Z. joazeiro in combating the harmful effects of alcohol is a suggestion for future work.

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