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AMARANTH EXTRACTS AS A SUBSTRATE FOR THE GROWTH OF *LACTOBACILLUS PLANTARUM* A LACTIC ACID BACTERIA WITH PROBIOTIC CHARACTERISTICS

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— Abstract—

Amaranth, due to its nutritional composition (carbohydrates 50-66%, lipids 1.9-9.7% and proteins 12-22%) has been selected by FAO as the best food of vegetable origin, it has also been reported that amaranth exerts beneficial effects on health such as lowering the blood cholesterol level, stimulating the immune system, having antioxidant activity as well as protecting against cancer. In some researches, it has been mentioned that thermal pre-treatments such as roasting, extrusion and explosion, applied to amaranth grain before flour processing, have an impact on nutrient content and bioavailability of nutrients. The high content of proteins makes amaranth an attractive substrate for the growth of probiotic lactic acid bacteria, which would complement the benefits, provided by the nutrients of amaranth and would be an alternative of probiotic consumption for those people who cannot consume them regularly (lactose intolerant, allergic to milk protein, gluten or soy). Therefore, the objective of this research was to evaluate the effect of pretreatments on the functionality of amaranth seed extracts as a substrate for the growth of probiotic lactic acid bacteria. To achieve this goal, extracts of amaranth flour were prepared: untreated, washed, cooked and burst seeds. The protein content, reducing sugars and total carbohydrates were determined to the extracts. Growth kinetics of *Lactobacillus plantarum* were developed by the pouring plate method. The results show that amaranth extracts are functional for the culture of *L. plantarum* and that only the explosion of the seeds seems to affect the availability of nutrients since it was in the only extract where a significantly lower maximum cell growth was observed (1.44 ± 0.04 Log UFC/UFC_0), nevertheless the rate of growth was not affected.

Keywords

Amaranth; lactic acid bacteria; probiotics.

Amaranth is considered a pseudocereal because it has a chemical composition similar to that of cereals. It has a relatively high protein content (14-17%), and in some varieties it becomes higher than in cereals (22%). Amaranth presents an amino acid profile with more lysine, tryptophan and sulfur amino acids than cereal proteins (Escudero *et al*., 2004), which is why it is considered a source of high nutritional value proteins. Amaranth oil also has fatty acid content similar to that of corn and other oilseeds, about 6% of the fatty acids of amaranth are unsaturated, of which 40% is linoleic acid which is an essential fatty acid in human nutrition (Saunders and Becker, 1984, Sánchez, 2007).

It has been reported that amaranth can have beneficial effects on health such as lowering the blood cholesterol level, strengthen the immune system, have antioxidant activity, as well as protect against cancer; these effects are promoted by the presence of active peptides, terpenoids and polyphenols (Venskutonis and Kraujalis, 2013). The insoluble and soluble fiber of amaranth acts favorably by reducing the serum and liver cholesterol levels in animals (Danz and Lupton, 1992), this fiber can be considered prebiotic, since it can be used by probiotic microorganisms to improve its growth (Charalampopoulos *et al*. ., 2002).

The interest in using amaranth as a substrate for the production of fermented products lies, among other characteristics, in its high protein content with a good pattern of essential amino acids and its high lysine content (Pedersen, 1987). For the mentioned characteristics the amaranth has been selected by the FAO (1986) as the best food of vegetable origin. Its nutritional value makes amaranth an attractive substrate for the growth of lactic bacteria with probiotic characteristics, which are currently distributed mainly in dairy products. The addition of potentially probiotic bacteria such as *Lactobacillus plantarum* (Molin, 2001, Cebeci and Gürakan, 2003), would complement the benefits provided by the nutrients of amaranth and would be an alternative of probiotic consumption for all those people who cannot consume the usual products (lactose intolerant, allergic to milk protein, gluten or soy) (Prado *et al*., 2008). Amaranth, being a gluten-free pseudocereal, also represents a consumption option for people with celiac disease (Pantanelli 2001, García 2008, Soteras 2011).

It has been reported that the different thermal processes applied to amaranth grain, such as roasting, extrusion and explosion of the grain, before the flour is elaborated, have an impact on the nutritional content and bioavailability of the nutrients, but that, despite the reduction in the content of phenolic compounds, the antioxidant activity remains without significant differences

with respect to the untreated grain (Bresani, 1992). This highlights the importance of ensuring that the processing of amaranth grains affects the content and availability of its nutrients as little as possible, so the objective of this research was to evaluate the effect of processing conditions on the content and bioavailability of nutrients in amaranth extracts and its functionality as a substrate for the growth of *Lactobacillus plantarum*, a potentially probiotic lactic bacteria.

METHODOLOGY

Preparation and characterization of the extracts

Unprocessed seeds of amaranth *Amaranthus hypochondriacus* grown organically in the states of Puebla, Oaxaca and Hidalgo were used; acquired in the virtual shop of the Quali group in packaging of 250 g. Flours were obtained from burst seeds (240-270 °C, 35 s), from seeds washed with a solution of sodium bicarbonate (NaCOH3) at 5% for 7 minutes with agitation, of seeds subjected to cooking (water at 100 °C, 25 min) and from untreated seed as a control. The washed and cooked seeds were placed for 15 minutes in racks to remove excess water and dried in a vacuum oven at 60 ° C for 24 h prior to grinding. The treated and untreated amaranth seeds were ground in a 200 pulvex mill with a 0.5 mm sieve, the milled product was sieved (No. 80 mesh) to homogenize the particle size. Four different extracts were elaborated according to the amaranth seeds flours (burst amaranth flour, washed amaranth flour, cooked amaranth flour and untreated amaranth flour), mixing 10g of flour with 100ml of distilled water, mixtures were left at rest for 20 min and subjected to a process of homogenization and filtration, later the extracts were partially characterized by their protein content (Kjeldahl method), reducing sugars (Miller 1959) and total carbohydrates (Dubois, 1956). Once characterized, the extracts were subjected to thermal treatment (121 ºC, 10 min.) for the elimination of the microbial load.

Development of growth kinetics

The bacterial strain of *Lactobacillus plantarum* NRRL B-4496 (isolated from cabbage in vinegar), donated by the United States Department of Agriculture (USDA, Peoria, Illinois, USA), was previously adapted to the extracts by cultivating it in a 50:50 mixture of amaranth-broth extract MRS. The inoculum was taken when the strains reached their maximum growth at the end of the exponential phase (7.55 x 10^8 CFU/mL) for the development of the growth kinetics in the amaranth extracts. The amaranth extracts were inoculated with 0.5% inoculum previously standardized. During the fermentation, samples were taken every hour during the first 6 hours and then every 2

hours until 24 hours, to evaluate the cell concentration (colony forming units per milliliter, CFU/mL) by the plate casting method, the evolution of pH, titratable acidity (AOAC, 1990), the concentration of reducing sugars (Miller 1959) and total carbohydrates (Dubois, 1956).

Mathematical modeling and statistical analysis

The growth parameters were calculated by adjusting the logistic model to the data of UFC obtained during the development of the kinetics and the significance between them was evaluated by analysis of variance with a level of significance ($p \leq 0.05$).

RESULTS

Partial characterization of obtained extracts

The thermal treatments applied to a plant matrix are an important step to obtain extracts since they influence their nutritional content. The evaluation of the effect of the thermal treatments applied to the amaranth seed on the nutritional content has been reported only for the amaranth flour (Bressani, 1983); however, this effect has not been reported in extracts. For this reason and to define the extract to be fermented, in this work it was considered to evaluate the effect of washing, bursting and cooking of the seed on the nutritional content of the extracts, as well as its capacity to be used as a culture medium for lactic acid bacteria.

Table 1 shows the effect of the treatments applied to the amaranth seed on the protein content, of reducing sugars and of total sugars in the extracts. As can be observed, the amaranth extract showed the highest protein content, but one of the lowest reducing sugar contents, only above the content of cooked amaranth extract and both lower compared to the content of reducing sugars in the extract of seeds without heat treatment.

Table 1. Protein content, reducing sugars and total carbohydrates in amaranth extracts

Samples	Proteins (%)	Reducing sugars (mg/mL)	Total carbohydrates (mg/mL)
Untreated amaranth extract (UN)	0.690 ± 0.003^{c}	10.953 ± 0.300^{a}	35.96 ± 2.60^{a}
Washed amaranth extract (WA)	0.670 ± 0.028^{c}	9.330 ± 0.212^{b}	18.05 ± 1.69^{b}
Cooked amaranth extract (CO)	0.815 ± 0.007^{b}	3.135 ± 0.503^{c}	23.93 ± 1.67^{c}

Burst amaranth extract (BU)	1.130±0.014[a]	3.325±0.106[c]	43.57±8.42[a]

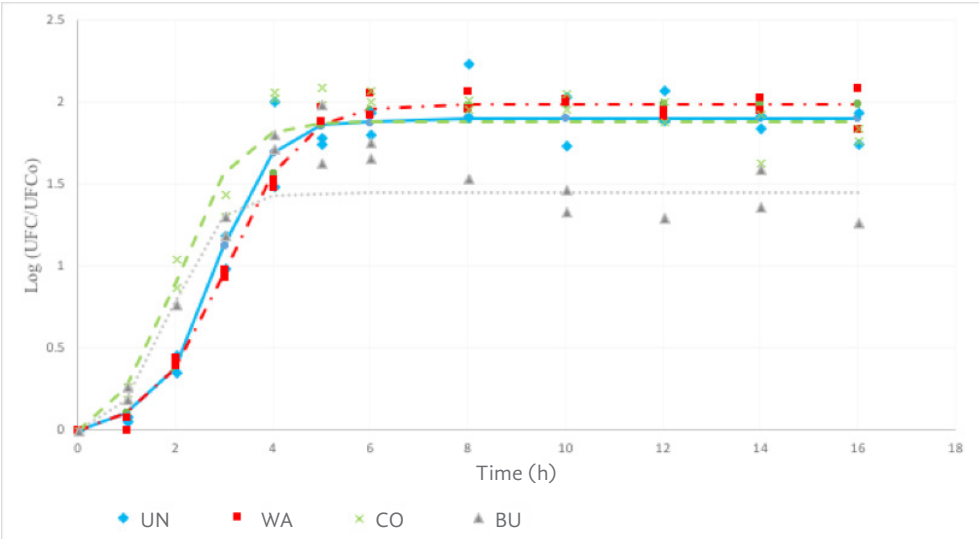
Note: The results are presented as the mean ± standard deviation. ($p \leq 0.05$)

The protein contents of UN and WA agree with that reported by Soteras (2011) for amaranth extracts. According to the nutrient content of the extracts reported in Table 1, the extract obtained from untreated seeds had the highest content of reducing sugars, a good content of protein and total sugars so it was selected to adapt to L. plantarum to the new substrate during the standardization of the inoculum.

Evaluation of the L. plantarum growth in amaranth extracts

The growth of *L. plantarum* in the four extracts (untreated amaranth extract (UN), washed amaranth extract (WA), cooked amaranth extract (CO) and burst amaranth extract (BU)) is shown in Image 1, where is appreciated that the maximum growth is reached around 4 and 5 hours of cultivation; in the burst amaranth extract, the maximum growth was lower (1.4 log cycles) compared to the maximum growth reached in the other extracts (1.9 log cycles), it is worth mentioning that a maximum growth of only 1.9 logarithmic cycles is observed because the initial cell concentration was 10^7 UFC/mL and a final concentration of 10^9 UFC/mL was reached. Table 2 shows the growth parameters obtained by adjusting the logistic model to the growth data.

Image 1. Growth of Lactobacillus plantarum NRRL B-4496 in untreated amaranth extract (UN), washed amaranth (WA), cooked amaranth (CO) and burst amaranth (BU)



In Table 2 it can be seen that, as far as maximum growth is concerned, the only treatment that was significantly different was that of the burst amaranth extract. Regarding the growth rate, no significant difference was found; and regarding the latency time, it was observed that it was similar in the untreated and washed amaranth extracts, but it was different from that obtained in the extracts of burst and cooked amaranth, which, in turn, did not showed significant differences between them. The latency time was shorter in the extracts made with burst and cooked amaranth.

Table 2. Effect of the type of amaranth extract on the Lactobacillus plantarum growth parameters NRRL-B4496

Extract	Maximum growth (Log (UFC/UFCo)	Growth maximum rate (h^{-1})	Latency period (h)
UN	1.90 ± 0.03^{a}	0.81 ± 0.12^{a}	1.63 ± 0.20^{a}
WA	1.99 ± 0.02^{a}	0.69 ± 0.04^{a}	1.63 ± 0.10^{a}
BU	1.44 ± 0.04^{b}	0.75 ± 0.24^{a}	0.97 ± 0.34^{c}
CO	1.88 ± 0.03^{a}	0.81 ± 0.14^{a}	0.91 ± 0.23^{c}

Note: UN: untreated amaranth extract; WA: washed amaranth extract; CO: cooked amaranth extract; and BU: burst amaranth extract. ($p \leq 0.05$)

Optimization of the operation variables

During the growth of *L. plantarum* in the extracts, the evolution of the pH, the titratable acidity and the reducing sugars were evaluated. Image 2 shows the evolution of pH, which decreased from approximately 6.6 to approximately 3.8 in 6 h, at which time the microorganism was already in a stationary phase. The pH in the untreated and washed amaranth extracts remained below 4 after 12 hours, while in the burst and cooked amaranth extracts was very close to the minimum values of 4.4 - 4.5 indicated in standards NOM-243-SSA1-2010 and NOM-185-SSA1-2002 for fermented milk products.

Image 2. pH evolution during the growth of *Lactobacillus plantarum* NRRL B-4496 in untreated amaranth extract (UN), washed amaranth (WA), cooked amaranth (CO) and burst amaranth (BU)

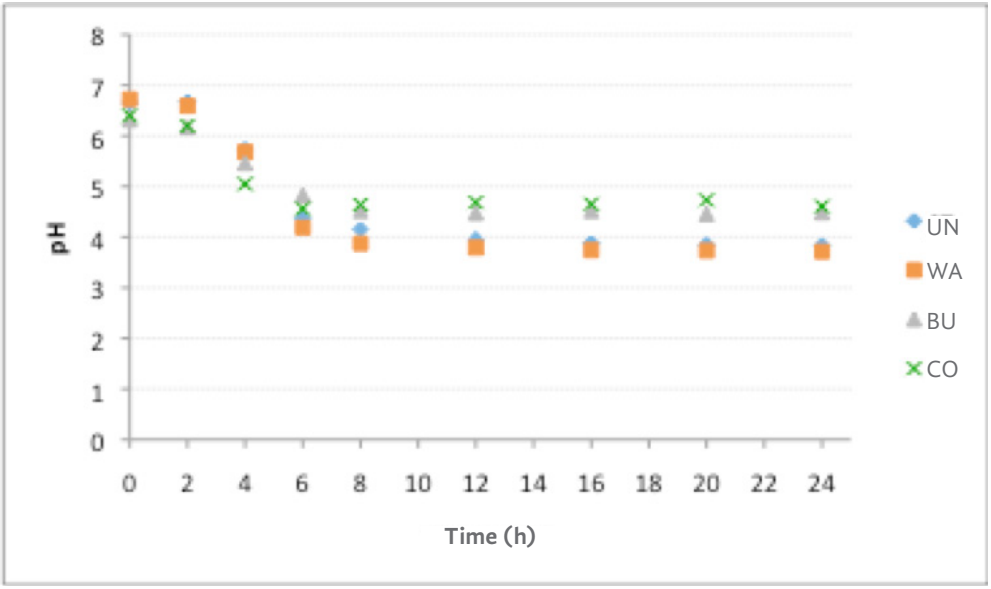
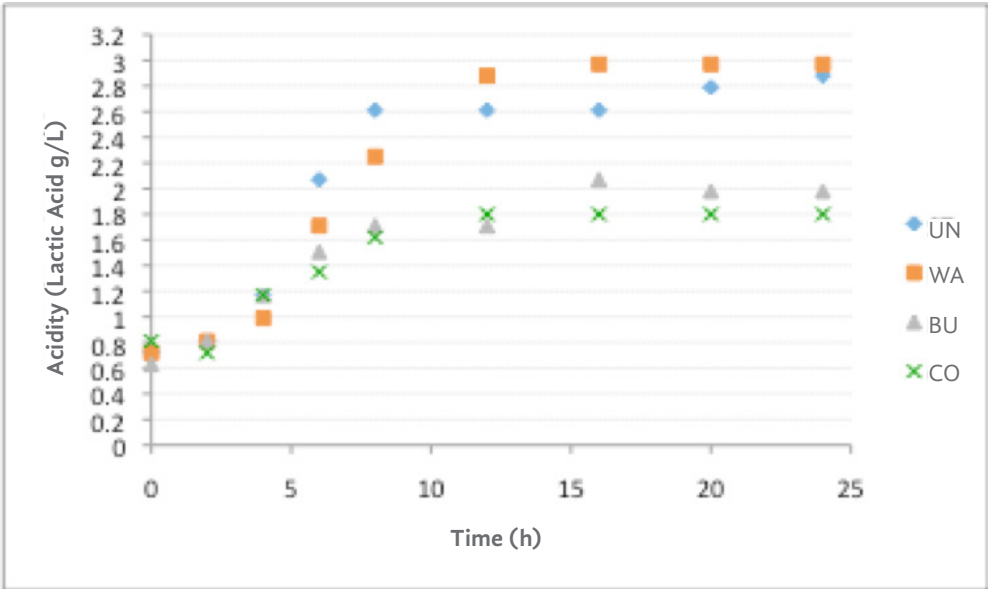


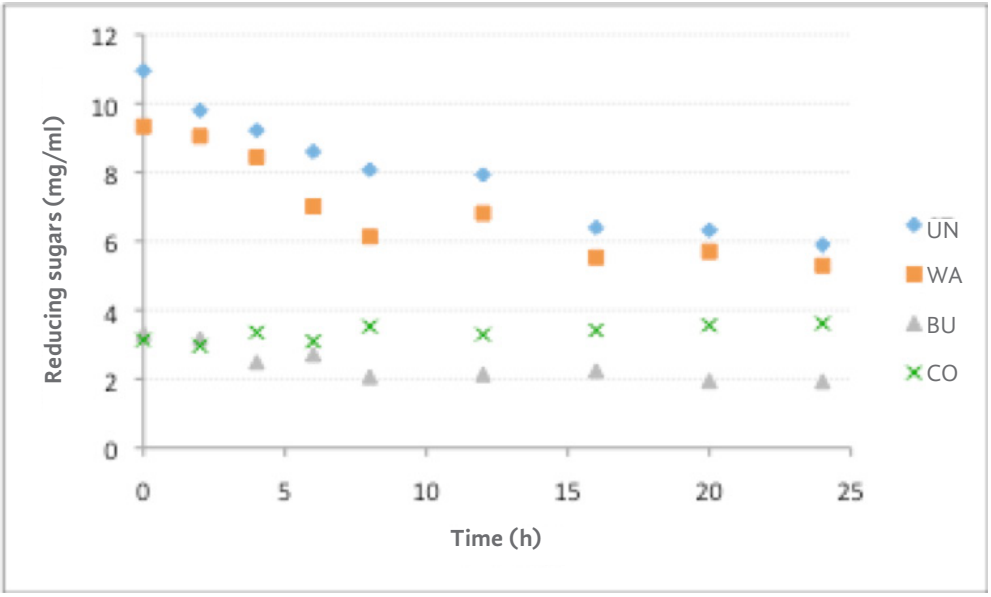
Image 3 shows the evolution of titratable acidity during the growth of *L. plantarum* in the four extracts. Image 3 shows that the production of acidity as lactic acid begins to be detected after 4 h, and seems to stabilize at 12 h. The titratable acidity of the untreated and washed amaranth extracts was similar and approximately 1g greater than that obtained in the extracts of burst and cooked amaranth. The titratable acidity expressed as lactic acid, in all extracts, was below the minimum value of 0.5% indicated in standards NOM-243-SSA1-2010 and NOM-185-SSA1-2002 for fermented milk products.

Image 3. Evolution of titratable acidity during the *Lactobacillus plantarum* growth NRRL B-4496 in untreated amaranth extract (UN), washed amaranth (WA), cooked amaranth (CO) and burst amaranth (BU)



As can be seen in Image 4, the content of reducing sugars in the untreated and washed amaranth extracts presented a constant decrease until around the 8 hours of cultivation, this period corresponds approximately to the start of the stationary phase, afterwards the content of reducing sugars remained constant.

Image 4. Evolution of reducing sugars during the *Lactobacillus plantarum* growth NRRL B-4496 in untreated amaranth extract (UN), washed amaranth (WA), cooked amaranth (CO) and burst amaranth (BU)



CONCLUSIONS

The results show that all the amaranth extracts can be functional for the growth of lactic acid bacteria, since no statistically significant difference ($p \leq 0.05$) was observed between the maximum growth rates and a maximum growth of around 2 logarithmic cycles was observed at the beginning of the stationary phase, although a slightly lower cell concentration was observed in the extract obtained from burst amaranth seeds as the stationary phase advanced. These results are the starting point for the development of symbiotic products (prebiotics + probiotics) from amaranth, with good quality protein content as an alternative to the consumption of probiotics for people who cannot consume milk-based products, either due to sensitivity to lactose or allergies to milk protein, as well as an alternative for celiacs.

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