AMARANTH PROTEIN HYDROLYZATES CAN BE USED TO FORM DOPACROME

Frida Monserrat Hosanna Paredes Ruiz, Edith Ponce Alquicira, María José Serrano Maldonado, Jorge Soriano Santos. Universidad Autónoma Metropolitana Iztapalapa (Biotechnology department), Av. San Rafael Atlixco 186. Col Vicentina, México, D.F., CP. 09340;
fridamoho@gmail.com; jss@xanum.uam.mx

Key words:Tyrosinase, dopachrome, protein hydrolyzates amaranth

Introduction. Melanin is the pigment responsible for the color of skin in humans. Tyrosinase is known to be the key enzyme in melanin biosynthesis. This enzyme catalyzes two distinct reactions: the hydroxylation of a monophenol (tyrosine) and the conversion of an o-diphenol (dopa) to the corresponding o-quinone (dopaquinone). Subsequently, dopachrome is formed, which is an intermediate in the biosynthesis pathway of melanin. Over-activity of this enzyme leads to overproduction of melanin leading to hyper-pigmentation of the skin. Overproduction of melanin can be prevented by avoiding excessive UV light exposure and can be treated with skin-lightening agents such as bleaching hydroquinone, kojic acid and retinoids. Good tyrosinase-inhibiting peptides derived from industrial proteins usually contain a hydrophobic, aliphatic amino acid residue as Val, Ala, Leu, Met and/or Ile.
The objective of this study was to assess amaranth protein hydrolyzates as a tyrosinase substrate to yield dopachrome.

Methods. Grain flour was obtained from A. hypochondriacus L. (0.25 mm) and was defatted with acetone (5 mL / g). Albumin 1, globulin and glutelin were extracted with Na2SO4 (5%) (3).The hydrolysis is carried out using Alcalase (0.8 AU / g) at pH 7.4 and 50°C. The degree of hydrolysis was determined by quantifying the free amino groups with TNBS (4). Tyrosinase Inhibition was assessed using the modified method of Ubeid et al., Tyrosinase activity was evaluated using L-tyrosine (1mg/ml), protein hydrolyzates to different concentrations and tyrosinase (96U/ml) at 37 ° C. The amount of dopachrome formed in the reaction mixture was determined at 475nm every minute during 10min [1].
Then, the hydrolyzates were separated by gel filtration (Sephadex G-15). Also, each of the fractions were used to evaluate the dopachrome obtained.

Results. Amaranth protein hydrolyzates (Albumin 1, globulin and glutelin) as well as ovalbumin and casein hydrolyzates showed enhanced activity of tyrosinase. Later amaranth protein hydrolyzates were separated by gel filtration, which yielded only a peptide fraction of albumin 0.8 kDa that managed to inhibit tyrosinase activity just by 25 ± 2.29%.

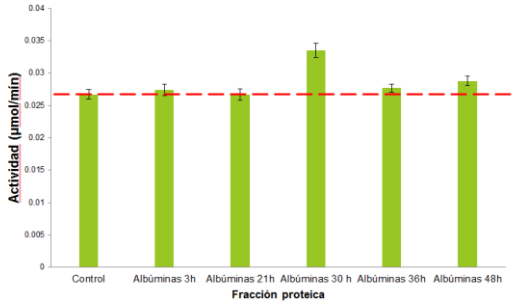

Fig.1 Evaluation of the inhibitory activity of tyrosinase by albumin hydrolyzates.

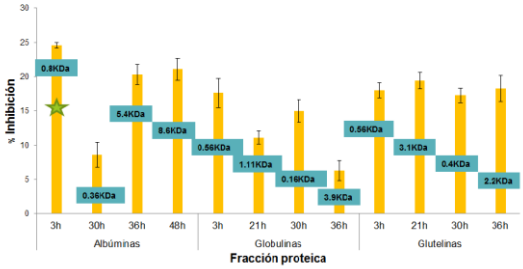

Fig.2 Peptide fractions of hydrolyzed proteins of amaranth with over tyrosinase inhibitory activity.

Conclusions. Amaranth protein hydrolyzates did not show a significant inhibitory tyrosinase activity. Fractionations yielded only a 0.8kDa peptide, as obtained of albumin 1, which could inhibit 25 ± 2.29% tyrosinase activity. On the other hand, hydrolyzates improved the enzymatic reaction to afford dopachrome.

References.

1. Ubeid, A.A. y Zhao, L. *Journal of Investigative Dermatology advance online publication*. 10.1038/jid.1-8.2009.
2. Schurink, S. y Van Berkel, W.. *El SEVIER*. 28:3.485-495.2007.
3. Padhye, V.W. y Salunke, D.K. *Journal Food Biochemistry*. 1: 111-129. 1977.
4. Adler-Nissen, J.. *Journal Agriculture Food Chemistry*. 27: 1256-1262.1979.
5. Chen, M.J. y Liu, J.R.. *Asian-Aust. J. Anim. Sci.*19:6.905-908.2006.