Summary for the final report

Amaranth as source of Omega fatty acids and gluten free grains: Marker-assisted breeding and cultivar selection to obtain functional healthy oils and raw materials for food-processing (AMOR)

Along with Quinoa, Peruvian Amaranth (*kiwicha*, *Amaranthus caudatus*) was a staple crop for the Incas. Since it was suppressed during Spanish colonialism, *kiwicha* was reinstalled only in the 1970ies by the efforts of the Peruvian scientist Luis Kalinowski. While the Amaranth species from Mexico, *A. hypochondriacus* and *A. cruentus*, have experienced a boom in Europe and are traded, mostly in popped form, as component of Muesli, bars, or even chocolates, the potential of *kiwicha* as functional food has remained unexplored. The project was motivated by the high content of polyunsaturated fatty acids in Amaranth oil that has high potential for vegan diet providing alternatives for fish oil capsules. At the same time, science-based quality standards have to be developed to safeguard *kiwicha* against the overwhelming dominance of Mexican Amaranth.

The project was using the strategy to valorise natural biodiversity based on a joint germplasm collection established by both, UHOH and KIT. The dominating species, *A. hypochondriacus* and *A. cruentus* were domesticated under the warm climate of Mexico, while the Peruvian *kiwicha* derived from the wild *A. quitensis* in the cool climate of the high Andes, and is, thus, endowed with cold tolerance. Cold tolerance in plants is often linked with a higher level of polyunsaturated fatty acids and a higher level of antioxidants to preserve membrane fluidity under low temperature and to buffer reactive oxygen species that are generated by respiration and photosynthesis under low temperature.

To safeguard and develop these valuable traits, it is crucial to preserve the authenticity of seed material. Unfortunately, many Amaranth growers, in Peru and elsewhere, are not aware of the differences between Amaranth species. As a result, growers often use imported seeds. This could pose a serious threat to the existence of *kiwicha*, not only, because it is more rarely planted, but also, because there will be introgression from imported varieties, such that its authenticity is on stake. Based on a detailed molecular study based on genetic barcoding, we could develop a PCR-based fingerprinting assay that allows to authenticate *kiwicha* in a rapid and robust manner, also in commercial samples. Using this assay, we could show that even in Peru, a significant part of *kiwicha* turned out to be, in fact, imported Mexican Amaranth, demonstrating how quality and authenticity of Amaranth seeds in Peru can be preserved.

By comparing the oil profile of *kiwicha* and Mexican Amaranth, we could show that *kiwicha* contains higher levels of polyunsaturated fatty acids, such as α-Linolenic Acid. This was accompanied by a more vigorous activation of metabolic genes involved in the synthesis of highly unsaturated fatty acids. In a few cases, albeit not consistently, we were even able to detect the valuable Docosahexaeonic Acid (DHA). We could further show that *kiwicha* indicates a lower expression of lipoxygenation and other markers for cold stress, consistent with our prediction that it is more cold-tolerant.

Amaranth oil is a new product, which means that the technology to make it available has to be developed. At harvest, Amaranth grains are still containing up to 30% of humidity. If they would be pressed in this stage, they would clog the machines and also be prone to fungal contamination. So, in a first step, the seeds have to be dried, until their moisture is lower than 12%. To preserve the precious components, the oil has to be extracted in a way that oxidation is prevented. Oils rich in unsaturated fatty acids tend to form epoxide compounds

that later decay, partially also to free fatty acids. This will not only cause a bad, rancid, taste, but it can also destroy the biological activity of these oils. Therefore, various extraction technologies like cold pressing, supercritical fluid extraction, and a combined process using pulsed electrical fields as a pre-treatment for cold extraction were tested. In terms of oil extraction yield, the oil extraction by supercritical fluid extraction showed the best results. Furthermore, various oil formulations including natural antioxidants such as Oregano, Rosemary, and traditional Peruvian crops, such as Muña (*Minthostachys mollis*) and Cañihua (*Chenopodium pallidicatule*) were evaluated in order to increase the shelf life of the final product. The more stable oils were obtained using Rosemary as a natural stabilizer. Finally, a sensory test was performed for evaluating the consumer acceptance of the amaranth oil being stabilized with rosemary, and the results indicated a medium score in the hedonic assessment, being suggested that as antioxidants are necessary for preserving the highly unsaturated fatty acids from oxidation, they should also be chosen regarding their taste profile and not only for their effectiveness. Therefore, formulations with other plant extracts with a proven antioxidant activity and in combination with a good taste profile should be further investigated.

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