

Effect of heat treatment of sardines and sprats on immunomodulatory activity *in vitro*

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Fish, rich in polyunsaturated fatty acids, is among the foods that may have a positive impact on human health. These include sardines and sprats, which are rich sources of PUFA. It is known that they can also play a role in inflammatory reactions in the body, where their long-term administration reduces the levels of several cytokines, such as interleukins, TNF- α , and others.

Sprats were caught in the Baltic Sea, and sardines in the Mediterranean Sea. After removing inedible parts, washing fish were thermally treated using the following processes: cooking, steaming, baking, or deep frying. In the next part, the samples were digested using the *in vitro* digestion model INFOGEST, simulating digestion in oral, gastric, and intestinal phases. At the end of intestinal digestion, samples were centrifuged, filtered through a 0.22 μ l filter, and then frozen at -80 °C until further testing. Samples were tested for inhibiting nitric oxide (NO) production on the RAW264.7 cell line when tested at a concentration of 20-0.009% digestion at 24 h incubation along with 1 μ g LPS. After 24h incubation, 50 μ l of supernatant was taken, mixed with 50 μ l Griess reagent, and measured at 540 nm. MTT was added to the cells, and after 2h incubation, the IC₉₅ value was determined. Subsequently, TNF- α was determined using the commercial ENZO kit.

The IC₉₅ was determined at 0.04% of the grass except for Steamed Sardines and sprats, where even a dose of 20% was not toxic with this treatment. Steamed Sardines and Sprats resulted in a 100% increase in NO production at a 20% grass concentration. Conversely, RAW Sardines led to a 30% decrease in NO production, as did Steamed Sardines and Baking Sprats, when there was a 10% decrease. On the other hand, increased production occurred in Cooking Sardines. TNF- α production was noted to be higher in all samples, and Steamed, Baked, and Raw Sprat Sardines showed almost double the production compared to the LPS-treated control ($p < 0.001$).

Heat treatment has a positive effect on increasing TNF- α production and thus enhancing anti-inflammatory activity.

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