

# Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale



**95<sup>th</sup> National Congress of the  
Italian Society for Experimental Biology**

**Trieste, Italy, 12-15 April 2023**

ABSTRACT BOOK

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or tris(2,4-di-tert-butylphenyl)phosphite, an antioxidant IAS commonly used to improve the stability of polyolefins (POs) and approved for food contact. During processing, IRGAFOS 168 can degrade by oxidation and hydrolysis forming two NIAS: tris (2,4-ditertbutylphenyl) phosphate (I168-Ox) and 2,4-di-tert-butylphenol (2,4 DTB) respectively; the extrusion conditions can affect the extent of IRGAFOS degradation and subsequent NIAS formation. By gas chromatography/mass spectrometry analysis we found that F48 contained 2,4 DTB and 168-ox at the concentration of 220 µg/g and 66 µg/g, while F56 showed a higher content of 2,4 DTB and I168-ox (342 µg/g and 150 µg/g respectively). To study *in vitro* cytotoxicity, hPBMCs from five unrelated human healthy donors were stimulated or not with phytohemagglutinin (PHA) and seeded in 96-well plates containing increasing concentrations (from 37 mg/ml to 296 mg/ml) of conditioned culture media previously exposed to F48 and F56 for 48 hours or 18 days to mimic a condition of NIAS production and release from POs. Cells were treated with these conditioned media and then MTT assay was used to evaluate the cytotoxic activity after 24 and 48 hours. Results showed that the treatment did not affect cell viability in both stimulated and unstimulated hPBMCs. However, an increase of cell viability at lower doses tested was observed at both 24 and 48 hours of treatment. In order to verify if this increase could be due to a general metabolic activation or was the consequence of the induction of a proliferative activity, a BrdU-based proliferation assay was carried out on PHA stimulated hPBMCs treated as in the MTT assay. Results have shown a marked (about 30 %) increase of cell proliferation compared to controls at lower concentrations. In summary, these preliminary results show that NIAS produced during F48 and F56 manufacturing do not induce any cytotoxic effects in differentiated human lymphocytes while improving cell metabolism. However, tested NIAS may alter the normal control of proliferation in lymphocyte precursors that are physiologically present in lymphoid organs. This might suggest that a chronic exposure to these substances, alone or in combination with other environmental pollutants could interfere with normal activity of human lymphocytes potentially representing a human health risk.

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#### BROILER CHICKENS AND THEIR HEALTH STATUS IN FARM: WINE WASTE, AN ADDED VALUE ON FEEDING

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Various key economic sectors of the National and Sicilian economy, in addition to contributing to the economic growth also cause a considerable production of waste which today are a problem both for companies then for the environment. For this reason, there is considerable interest in this waste and in the possibility of their reuse to extract bioactive molecules with added value, all in accordance with the objectives of the Blue Economy. In this regard, it has already been demonstrated that from the production waste of various economic sectors it is pos-

sible to obtain bioactive molecules with anticancer, antimicrobial, cosmetic, antioxidant and nutraceutical properties (Mauro *et al.*, 2022). If we focus on farm animals and the feed used, it is known that the latter can be a source of antioxidants which help the body to prevent the harmful effects of free radicals and metabolic products. In this context, polyphenolic compounds (*e.g.* flavonoids) showed important antioxidant activities *in vitro*, although it seems that these are poorly absorbed in the intestine and their tissue concentrations are too low to contribute to the antioxidant defense (Surai, 2013). In light of all this, within the SMILING Project a study concerned the possibility of reusing wine production waste (pomace and grape seeds, the cause of the production of considerable quantities of waste) rich in fatty acids, triglycerides and polyphenols (Di Stefano *et al.*, 2021; 2022) in the feeding of broiler chickens by evaluating the possible effects of these diets on the animal's health status also in terms of antioxidant capacity. Three experimental sets were carried out and concerned the administration of three different types of diets: Grape marc (0%, 3% and 6%), Grape seeds (0%, 3% and 6%) and a mix of grape marc and grape seed (0 and 3%). At the end of the experimental times, biomarkers typical of the evaluation of the state of health of the organisms were used for the preliminary analyzes of the blood and meat samples. Significant effects were observed in all diets depending on the concentration administered. Probably the phenolic content of the flours used contributes to the improvement of the health of the farmed animals.

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#### ANTITUMORAL ACTIVITY OF *Asparagus officinalis* EXTRACTS AGAINST BREAST CANCER

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Breast cancer (BC) is the most frequent cancer among women and high rate of resistance and loss of response to treatment enhance the attention in novel drug research, mainly from natural origins. *Asparagus officinalis* (Asp) is reported to possess therapeutic properties, both from edible and inedible portions. We characterized the chemical content of new aqueous extracts



derived from the non-edible portion of the plant and we assayed the biocompatibility and bioactivity *in vitro* on normal fibroblasts and on cellular models of BC. Results showed no interference with fibroblast viability, while a significant reduction in the proliferation rate associated with significant G1/S cell cycle arrest and low levels of apoptosis, was specifically observed in breast cancer cells. Asp extracts were also shown to significantly inhibit cell migration after 24 hours treatment in BC cells, in a dose-dependent manner. Additional investigation showed that Asp extracts exert specific pro-oxidant activity against tumoral cells, and, notably, that their combination with menadione resulted in a significant increasing of oxidants production compared with menadione alone only in breast cancer cells (but not in normal cells). Our results make the aqueous Asp extracts very attractive for further investigation in breast cancer research. The selectivity of action on tumoral cells and the easiness of their preparation make them good candidates to test role as co-adjuvant agents of clinical drug therapies.

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#### BIOFORTIFIED VEGETABLES AND METABOLIC SYNDROME: A POSSIBLE TOOLS TO PREVENT UNHEALTHY MICRONUTRIENTS REDUCTION IN LIFE

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Metabolic syndrome is defined as a pathological condition characterized by abdominal obesity, insulin resistance, hypertension and hyperlipidemia. This pathology has become the main danger to global health since can influence the develop of diseases as diabetes, cardiovascular, neurodegenerative disease and also cancer. Micronutrients as iodine and molybdenum are known to be key element in hepatic and lipid pathway and therefore playing a role in metabolic syndrome. The present study aims to evaluate, on healthy population, the effect of nutritional intervention using biofortified vegetables with Iodine and Molybdenum under the form of potassium iodate and sodium molybdate. A cohort of 69 volunteer subjects were recruited and divided into four experimental groups who ate fortified vegetables for 12 days: IODIUM GROUP (biofortified lettuce with iodine), MOLIBDENUM GROUP (biofortified lettuce with molybdenum), IODIUM+MOLYBDENUM GROUP (biofortified lettuce with iodine) and molybdenum), CONTROL GROUP (non-biofortified lettuce). We looked at blood and urine analysis at baseline (Time 0) and after 12 days (Time 1) of fortified vegetables administration. We analyzed, among others: aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), insulin (INS), LDL cholesterol, HDL cholesterol, LDL/HDL ratio, total cholesterol/LDL ratio; and the incretins GLP-1 (Glucagon-like peptide-1) and GIP (Gastric Inhibitory Peptide), GLP-2 (Glucagon-like peptide-2), PYY (Peptide Tyrosine Tyrosine). The results showed that after 12 days of biofortified vegetables the IODIUM+MOLYBDENUM GROUP showed a significative reduction of AST and ALT levels and increased HDL and PYY levels. This might shed light on a possible use of biofortified vegetables supplementation in order to prevent unhealthy micronutrients reduction occurring along different stage of life especially those related to metabolic syndrome.