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Healthy novel extruded gluten-free snacks based on legumes and rice: bioactivity evaluation

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Keywords: Bean/rice/carob fruit; extrusion; bioactivity; antitumoral and antimicrobial activity

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Extrusion-cooking technology is a high-temperature and short-time process, necessary to cause structural, physico-chemical and nutritional changes of raw materials, forcing the material to flow under different conditions (temperature, moisture, screw speed, and feed). Rice and legumes have a great potential in the development of healthier gluten-free products than traditional snacks [1].

Rice flour (*Oryza Sativa* L.) has been reported as a good raw material to obtain expanded food products [1]. Dry beans (*Phaseolus vulgaris* L.) are rich in proteins, dietary fibre, complex carbohydrates (leading to low glycaemic index), minerals and numerous phytochemicals endowed with useful biological activities [2]. Carob fruit (*Ceratonia siliqua* L.*)* is a tree leguminous native to the Mediterranean region. The fortification with carob fruit would provide adequate fibre content and a good amount of bioactive compounds. In previous studies, carob evidenced therapeutical properties against several diseases, such us, regulatory effect in blood glucose level, reduction in low-density-lipoprotein cholesterol in hypercholesteraemic patients, body weight benefits, and improved digestion, and lipid utilization [3]which were associated with endothelial dysfunction, inflammation, and fibrosis. Furthermore, sirtuin-1 (SIRT1.

In this study, novel gluten-free extruded foods (composed by rice: 50-80%, bean: 20-40%, and carob: 0-10%) were performed using a twin-screw extruded at CARTIF (Valladolid, Spain) and the effects of extrusion were evaluated regarding bioactive properties. Commercial extruded rice was used as external control. Raw materials, non-extruded, and extruded samples were extracted with ethanol/water (80:20, v:v) and purified using C18 SepPak® ***Vac 3 cc cartridge*** [4,5], in order to obtain an extract rich in bioactive compounds. The cytotoxicity, anti-inflammatory, and antimicrobial activity were evaluated following previously reported procedures [6, 7].

Carob, commercial extruded rice and most of the extruded samples, showed cytotoxicity in the majority of the tumour cell lines tested (HeLa - cervical carcinoma, HepG2 - hepatocellular carcinoma, MCF-7 - breast adenocarcinoma, and NCI-H460 - non-small cell lung cancer). While, bean, rice, and most of the non-extruded samples presented no toxicity (GI_{50} value >400 $\mu g/mL$) using a non-tumour porcine liver cell culture (PLP2). In general, it was observed that extrusion process improves the cytotoxic potential in the rice-legumes sample mixtures, revealing lower GI_{50} concentrations (ranging between 115 and 362 $\mu g/mL$). The anti-inflammatory activity revealed a high heterogeneity, presenting bean and carob samples, the highest activity in comparison to the extruded samples. Concerning the antimicrobial activity, which was tested using a panel of multi-resistant isolated clinical strains, a low potential was observed, with non-extruded and extruded samples revealing higher values of minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC).

In conclusion, rice-legumes flours, rice blends, beans, and carob fruits are a great alternative for the development of new gluten-free snacks products, in a market dominated mainly by cereals, due to the presence of different bioactive compounds, such as phenolic compounds, which can give healthier benefits to the consumers.

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