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Impact of manufacture and digestion process of foods enriched with sesame flour on the antioxidant response of human hepatocyte in vitro.

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Abstract: Bioactive compounds such as polyphenols are ubiquitous in many foods. However, their presence varies in structure, concentration, and action along food manufacturing and digestion. The simulated digestion assays allow estimating variations in bioaccessibility and bioeffectiveness of polyphenols within foods. In this study, a polyphenols enriched food model was used: sweet cookies with 10% defatted sesame flour (DSF). Polyphenolic extracts were obtained from DSF, a base cookie formulation and cookies enriched with DSF. In addition, extracts of potentially bioaccessible polyphenols from digested cookies in vitro were collected. The HepG2 liver cell line was incubated with all these mentioned extracts and then injured with H2O2. The following parameters were analyzed: intracellular oxidative state and viability by flow cytometry, antioxidant enzymes and glutathione content, and oxidation of proteins and lipids by spectrophotometry. Results show that DSF may be considered for functional incorporation in foods, since it may promote antioxidant response, providing preventive benefits and protective action in an oxidative damage context. This study also demonstrates that DSF actions on redox state vary depending on the food matrix and its degree of digestion. Cookies with DSF presented a pro-oxidant effect that could enhance the antioxidant response. Furthermore, after digestion, these cookies continue to show biological activity, evidencing the possibility of advantages from the consumption of these foods. This data remarks the need to improve the knowledge of the biological actions carried out by certain metabolites present in food and the impact of their digestion over their effective role in health.

Keywords: Antioxidant response; Cell Culture; Sesame; Digestion process; Functional ingredients

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BACKGROUND

Bioactive compounds in food such as polyphenols (PF) vary in structure, concentration, and action since the moment they are ingested until they reach the target organs (Arfaoui 2021; Di Lorenzo, et al. 2021). Even more, when immersed in a food matrix, their activity can be affected by the interactions with the remain components of food (Arfaoui 2021). Therefore, regarding polyphenols incorporation into functional food formulation, it result relevant to define the repercussion on the biological activities not only of food matrix interaction and manufacturing processes, but also the impact of each digestion steps involved in ingestion. Simulated gastric, intestinal and colonic digestion allows the estimation of changes in the effective PF activity present in food. Here, a sweet wheat cookie formulation snack made with 10% substitution of defatted sesame flour (DSF) was used as a model of plausible functional food enriched in PF (Lucini Mas, et al. 2022) and it bioactivity was evaluated in a cellular system.

The aim of the present study was to evaluate the impact of both manufacturing and digestion procedures of foods enriched with defatted sesame flour over the effective biological action on the cellular redox state.

MAT AND METHDS

A model snack food enriched in polyphenols was used: sweet cookie with the addition of sesame flour (DSF) (Lucini Mas et al. 2022). Polyphenolic extracts (methanol:water) were obtained from DSF, base cookies (no additives, CC), DSF-enriched cookie (SC). The cookies were processed by gastric/intestinal digestion (SID) and colonic fermentation (LID) in vitro, obtaining polyphenolic extracts for each digestion step. The HepG2 liver cell line was incubated with the aforementioned extracts (5ug/mL, 24h) and injured with H2O2 (10mM, 1h) to produce oxidative stress. To evaluate the effect of enriching foods with DSF and the impact of the digestion process on their biological action on the cellular redox state, the following were determined: the intracellular oxidative state and cell death by flow cytometry, the activity of antioxidant enzymes (Catalase (CAT), Glutathione Peroxidase (GPx) and Reductase (GR)) and glutathione (GSH) content, as well as protein and lipid oxidation by spectrophotometry. Statistics: ANOVA or MLGM, followed by Fisher Test ($p>0.05$). Different letters indicate statistical significance.

RESULTS AND DISCUSSION

Among the actions observed it stands out that the incubation with DSF extracts decreased the basal oxidative state and lipid oxidation, associated with an increase in the antioxidant response through the activity of CAT and GR and the content of GSH. In this condition, a slight increase in cell death was also observed. In a context of oxidative injury, cells exposed to DSF showed an accentuation of the general oxidation and protein damage already caused by the stimulus, accompanied by an increase in the antioxidant response through CAT, GPx and GSH, which resulted in the reduction of lipid oxidation. Based on these data, it is suggested that DSF is capable of inducing antioxidant biological activity, providing a preventive benefit at the basal level and even manifesting a protective role against oxidative injury.

Regarding the use of DSF as an ingredient in a formulation of an enriched cookie (SC), the tests show that the exposure of the cells to the extracts of the base cookie (CC) induced an increase in the basal oxidative state, concomitantly with the oxidation of macromolecules and the increase in the anti-oxidant response of the CAT, GPx and GR enzymes. In this same sense, enrichment of SC further increases these effects observed in CC, implying an increase in cell death. These findings suggest that the bioactive compounds both from the chosen food matrix and its combination with DSF would act in a pro-oxidant manner. Furthermore, in the face of an oxidative stimulus, cells incubated with the extracts of both cookies also showed increased overall oxidation and macromolecule damage. However, in this context of oxidation, a differential effect is observed for the incorporation of DSF, since exposure to SC extracts amplified the antioxidant response through GSH content and GR activity with respect to CC, resulting in attenuation of protein oxidation and cell death caused by CC. These results make it possible to infer that the incorporation of HS in a cookie presents biological actions with a potential pro-oxidant effect, which hermetically (Forman, et al. 2014), would collaborate in the stimulation of the antioxidant response that would protect against cell death by oxidation, pointing out the possibility of a preventive benefit from the consumption of these foods.

With the objective of estimating the repercussion of the digestion process on the effectiveness of the compounds incorporated with DSF, extracts were obtained by simulated digestion for CC and SC. The biological assays presented highlighted that the potentially available fraction of CC digested in the small intestine caused an increase in the antioxidant response through the GPx and GR enzymes, which implied, on the one hand, the

increase in the oxidation of proteins, as well as decreased basal lipid oxidation. Remarkably, the dialyzed fraction of the digested enriched cockerel further increased the antioxidant response, determined through GSH content and GPx activity, resulting in a reduction in macromolecule oxidation with respect to CC, to which added to an increase in cell death. Under the effects of oxidative injury, incubation with extracts of digested CC increased overall oxidation and cell death, related to an impaired antioxidant response that included reduced CAT activity and increased GPx. On the other hand, the digested SC extracts showed the potential to reverse this effect described for CC on general oxidation and cell death. These effects were linked to an increase in the antioxidant response mediated by GSH content and GR activity, which were accompanied by decreased protein oxidation. The results would indicate that the enrichment of the cookie with DSF after being digested in the intestine shows bioactivity with a potential antioxidant preventive and protective effect against oxidation, even counteracting the oxidizing actions of CC.

In the development of the colonic fermentation model, cells incubated with the dialyzed fraction potentially available in the large intestine of the CC revealed a decrease in overall oxidation and lipid oxidation. While exposure to SC extracts increased general oxidation, modifying antioxidant response with respect to CC: with an increase in GSH content and a decrease in GR activity that resulted in the attenuation of the oxidation of macromolecules. Added to this, in a context of oxidative injury, CC decreased general oxidation and lipid oxidation, while affecting the antioxidant response with lower CAT activity and lower GSH, which led to greater cell death. . For its part, exposure to fermented SC fractions increased the general oxidation and antioxidant response of GPx with respect to CC, leading to lower lipid oxidation. These data show that the cookie with DSF, even after fermentation in the large intestine, could present bioactivity with a potential pro-oxidant effect that, acting hermetically (Forman et al. 2014), would provide benefits both at a preventive level and in terms of oxidation protection compared to the intake of the cookie base formulation.

In conclusion, the data obtained show that it is relevant to consider the functional incorporation of DSF in food, since this by-product is capable of promoting antioxidant biological activity, providing a preventive benefit at a basal level and even exhibiting a protective action in a context of oxidative damage. In addition, these studies emphasize that the biological actions of DSF polyphenol enrichment on the cellular redox state vary depending on the food matrix and its degree of digestion. In line with this, our findings suggest that the incorporation of DSF in a cookie presents bioactivity, evidencing the possibility of a preventive benefit from the consumption of these foods. A potential pro-oxidant effect was observed that could enhance the antioxidant response by acting in a hormetic manner, favoring protection from damage and cell death by oxidation (Forman et al. 2014; Kim, et al. 2014; Masella, et al. 2005). The results indicate that the enrichment of the cookie with DSF continues to show biological activity even after being digested in the small and large intestine, exerting a stimulation of the antioxidant response, even being able to counteract the oxidizing actions of the matrix of the chosen food as vehicle. The detected effects of the DSF extracts in all the evaluated conditions are mainly grouped around changes in GSH content and its cycling enzymes (Masella et al. 2005) and the decrease in lipid oxidation, suggesting that this would be one of the mechanisms preferentially affected by the active compounds present in the DSF, which is in agreement with the previous bibliography. Concluding, the need to improve the knowledge of the biological actions carried out by certain metabolites present in food and its digestion, as well as its effective role in well-being and health result a relevant matter to discern.

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