"The assay of food functional properties using cell cultures"

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THE AIM

To **show different methods** for the assay of food functional properties using cell cultures.

Do an **experimental study of the Cytotoxicity** of saccharin and sucrose in Caco-2 cell line.

CELL CULTURES

Definition

A set of techniques that allows the development of cells ***in vitro***, maintaining their physiological, biochemical and genetic properties.

Advantages

- ✓Control of the environment
- ✓Cheaper than other studies.
- ✓Does not involve the sacrifice of animals.
- ✓Used for a wide range of compounds.
- ✓Possible to evaluate the effects of complex mixtures to study the combined effects.

Disadvantages

- ➢Instability of cell lines
- ➢Differs from a tissue in having lost:
  - •Dimensional spatial organization.
  - •Interactions between different cell types and between cells and the extracellular matrix.
  - •Components involved in the regulation of homeostasis.

We can integrate different assays in one study

IN VITRO ASSAYS

Cytotoxicity/genotoxicity

Proliferation measure DAPI

Fixation and permeabilization of cells and visualization of DNA with DAPI.

Dye staining and counting cells under the microscope

Cellular exclusion or absortion of a substance capable to stain the cells.

MTT Assay

Mitochondrial enzymes of live cells reduce the MTT to formazan (purple color).

Anticancer activity

DNA fragmentation (Comet assay)

Determination of the oxidative damage followed by staining of DNA and measuring fluorescence.

Celullar proliferation

Reduction of MTT or MTS, and read the absorbance in cells.

Antioxidant activity

ROS detection by fluorescence

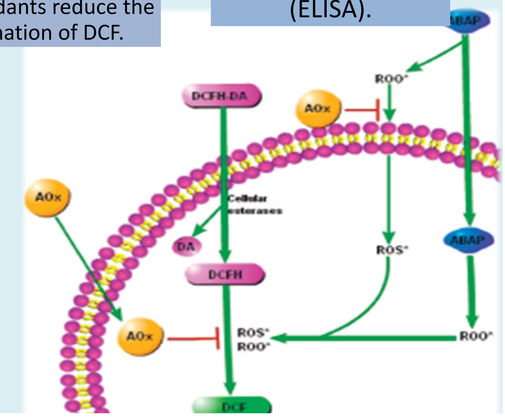
Evaluation of the ability of the compound to prevent oxidative damage and measurement of fluorescence.

CAA assay

DCFH-DA diffuses into the cell where is formed DCFH. Peroxyl radicals oxidize the DCFH to DCF. Antioxidants reduce the formation of DCF.

Anti-inflamatory activity

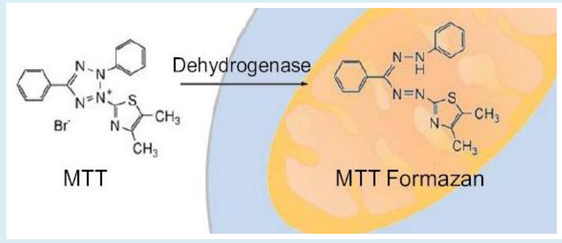
Measurement of proinflammatory cytokines (IL-6 and IL-8) gene expression (RT-PCR) and/or the cytokine concentration (ELISA).



General assays characteristics

- Several cell lines of human or other mammals origin are used
- The assays can be exposed in four basic stages:
- Always do a controlled assay under the same conditions

1. Characterization of cytotoxic doses of active compound or extract food.
2. Supplementation of cells with non-cytotoxic doses of the compound.
3. Induction specific damage on cells.
4. Determination of the protection from this harmful effect exerted by the compound.

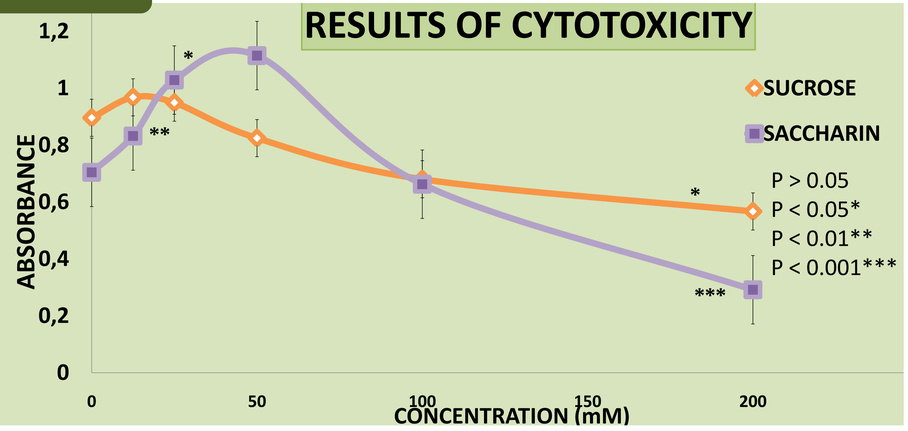


EXPERIMENTAL RESEARCH

OBJECTIVES

✓Establish *in vitro* cytotoxic concentrations of saccharin and sucrose on a cell line from human colon carcinoma (Caco-2), by the determination of the number of viable cells with Methylene Blue.

RESULTS OF CYTOTOXICITY



CONCLUSIONS

➢**saccharin :** Higher concentrations than 100mM exhibit cytotoxic effects.

➢**Sucrose**: No cytotoxic effect until 200mM concentrations.

CONCLUSIONS

- •Cell culture assays are better correlated with biological activity than chemical assays, but also more expensive and more difficult to carry out.
- •Nowadays cell culture is increasingly used.
- •During the interpretation of these assays we should consider that we are studying an isolated process from the complex system .
- •Some food companies have begun to use them to develop new products.