

# ***Capsicum annuum* L. cv Senise: from food to liposomes with high-value antioxidant potential**

**Immacolata Faraone<sup>1,2</sup>, Maria Ponticelli<sup>1</sup>, Ludovica Lela<sup>1</sup>, Luigi Milella<sup>1</sup>, Antonio Vassallo<sup>1,3</sup>**

<sup>1</sup> **University of Basilicata**, Department of Science, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy, [immacolata.faraone@unibas.it](mailto:immacolata.faraone@unibas.it), [maria.ponticelli@unibas.it](mailto:maria.ponticelli@unibas.it), [ludovica.lela@unibas.it](mailto:ludovica.lela@unibas.it), [luigi.milella@unibas.it](mailto:luigi.milella@unibas.it), [antonio.vassallo@unibas.it](mailto:antonio.vassallo@unibas.it);

<sup>2</sup> **Spinoff BioActiPlant s.r.l.**, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy;

<sup>3</sup> **Spinoff TNcKILLERS s.r.l.**, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy.

## **INTRODUCTION**

Different factors increase the production of free radicals in human body altering the homeostasis (Flora, S. 2007). The increase in free radicals resulting in the oxidative stress, is involved in the etiology of several major human diseases such as diabetes, cardiovascular diseases, cancer, and neuronal disorders. Specialized metabolites extracted from plant species are able to neutralize free radicals by carrying out numerous biological activities (Zhang, H. *et al.* 2016).

Among all, *Capsicum annuum* L. cultivar Senise, belonging to the Solanaceae family, is a precious source of health-promoting compounds. In fact, few studies have investigated the biological activity and chemical composition of this pepper cultivar (Loizzo, M.R. *et al.* 2013; Speranza, G. *et al.* 2019). The “red gold of the Basilicata region” is a sweet pepper traditionally sun-dried and eaten fried (“*cruschi peppers*”) or powdered and used as a spice in the Basilicata region (Italy).

As far as we are aware, this is the first study on the biological activity of *C. annuum* cv Senise in cells or incorporated in a vesicular carrier system. The purpose of this study was the evaluation of the antioxidant activity on HepG2 cell line used as cell model as well as the investigation of the phytochemical profile of *C. annuum*. Furthermore, the molecular signalling pathways involved in the antioxidant activity of the extract were assessed (Sinisgalli, C. *et al.* 2020).

## **METHODS**

*C. annuum* was collected in Senise, Basilicata, Italy during the autumn of 2016. Sun-dried red peppers without seeds and petiole were extracted by maceration with absolute ethanol.

Then, the dried extract was injected in positive and negative mode on LC-ESI/LTQOrbitrap/MS for the qualitative and quantitative determination analyses.

The extract of *C. annuum* was also incorporated in liposomes. For comparative purposes, empty liposomes were produced following the above procedure, but without including the extract.

The extract and the liposomes of extract were subjected to different assays as Oxygen Radical Absorbance Capacity (ORAC) assay, cell viability on HepG2 cells by the MTT assay, measurement of intracellular reactive oxygen species (ROS) and quantitative RT-PCR to evaluate the expression of some genes involved in antioxidant defence (Sinisgalli, C. *et al.* 2020).

## **RESULTS**

The phytochemical analyses of *C. annuum* extract showed 24 compounds identified based on the mass accuracy value, tandem mass experiments, literature data, and reference standards. The identified compounds belonging to a structurally different metabolic classes as phenols, capsinoids, carotenoids, sesquiterpenoids, flavones, flavonols, flavan-3-ols, vitamins and capsaicinoids.

The *C. annuum* extract had no effect on HepG2 cell viability, in fact the extract showed no cytotoxic effect after 24 and 48 h. Instead the pre-treatment with the extract for 24 h reduced ROS levels dramatically, restoring the basal level similar to that of cells treated with NAC, a known antioxidant.

The extract incorporated in liposomes was investigated in order to evaluate the effect of the formulation on its

biological activity. It is interesting to see that when the extract was incorporated in liposomes, the ROS levels decreased significantly even showing twice as potent activity in comparison with both the raw extract and NAC used as control.

Moreover, the extract did not affect the expression of genes, as compared to the control but it upregulated the expression of SOD-2 and GPx-1 after 48 h, as well as the nuclear factor erythroid 2-related factor 2 (Nrf2) and ATP-binding cassette transporter G2 (ABCG2).

## CONCLUSIONS

In this study, for the first time, was investigated the protective effect of *C. annuum* L. cv Senise raw extract and

of extract incorporated in liposomes against oxidative stress in cells.

Twenty-four compounds were identified by LC-MS and the results obtained demonstrated the better activity of the formulating the “*red gold*” extract into liposomes, which potentiate its antioxidant activity.

Furthermore, it has been demonstrated that several genes involved in the redox cell system are activated during treatment with the extract, with an evident impact on SOD-2 and GPX-1 as well as Nrf2 and ABCG2.

Overall, this study suggests that the incorporation in liposomes of a typical food of the Basilicata region could represent a new strategy in nutraceutical and pharmaceutical fields.

## REFERENCES

1. Flora, S. Role of free radicals and antioxidants in health and disease. *Cell. Mol. Biol.* 53, 1–2 (2007).
2. Loizzo, M.R.; Pugliese, A.; Bonesi, M.; De Luca, D.; Menichini, F.; Tundis, R. and O’Brien, N. Influence of drying and cooking process on the phytochemical content, antioxidant and hypoglycaemic properties of two bell *Capsicum annuum* L. cultivars. *Food Chem. Toxicol.* 53, 392–401 (2013).
3. Sinisgalli, C., Faraone, I., Vassallo, A., Caddeo, C., Bisaccia, F., Armentano, M.F., Milella, L. and Ostuni, A. Phytochemical profile of *Capsicum annuum* L. cv Senise,

incorporation into liposomes, and evaluation of cellular antioxidant activity. *Antioxidants*, 9(5), 428 (2020).

4. Speranza, G.; Scalzo, R.L.; Morelli, C.F.; Rabuetti, M. and Bianchi, G. Influence of drying techniques and growing location on the chemical composition of sweet pepper (*Capsicum annuum* L., var. Senise). *J. Food Biochem.* 43, e13031 (2019).

5. Zhang, H. and Tsao, R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Curr. Opin. Food Sci.* 8, 33–42 (2016).