

Le Corps professoral de
Gembloux Agro-Bio Tech - Université de Liège vous prie
de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Madame ZHU Yingying,

**Titulaire d'un *master's degree in engineering majoring in agricultural products processing
and storage engineering,***

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,
le 13 novembre 2018, à 14 heures précises (personne ne sera admis après cette heure),
en l'auditorium GR (Bât. 2),
Passage des Déportés, 2, à 5030 GEMBLoux.

Cette dissertation originale a pour titre :

**« Anticancer Effects of Soybean Bioactive Components and Anti-inflammatory
Activities of the Soybean Peptide Lunasin ».**

Le jury est composé comme suit :

Président : Prof. P. JACQUES, Professeur ordinaire,
Membres : Prof. N. EVERAERT (Promoteur), Prof. R. GUIXING (Copromoteur – CAAS, Chine)
Prof. F. DELVIGNE, Prof. N. PAQUOT, Prof. R. RAVALLEC (Université de Lille, France).

Summary

Soybean products have been demonstrated to have health-promoting benefits. In this study, we investigated the anticancer effect of 12 bioactive components from soybean on human breast cancer cells *in vitro*. Results showed that genistein, daidzein, glycitein, genistin and daidzin showed stronger anti-proliferative activity against MCF-7 cells with median effective concentration (EC_{50}) values of $66.98 \pm 4.87 \mu\text{M}$, $130.14 \pm 2.10 \mu\text{M}$, $190.67 \pm 5.65 \mu\text{M}$, $72.82 \pm 2.66 \mu\text{M}$ and $179.21 \pm 6.37 \mu\text{M}$, respectively. There was a synergistic effect of combination treatment of genistin plus daidzin in MCF-7 cells with combination index at inhibition of 50% (CI_{50}) of 0.89 ± 0.12 . Genistein, glycitein, genistin and β -sitosterol were demonstrated to have a stronger anti-proliferative activity against MDA-MB-231 cells with EC_{50} values of $93.75 \pm 5.15 \mu\text{M}$, $142.67 \pm 5.88 \mu\text{M}$, $127.82 \pm 4.70 \mu\text{M}$ and $196.28 \pm 4.45 \mu\text{M}$. The synergistic effect was observed in the mixture of genistein plus genistin, genistein plus β -sitosterol or β -sitosterol plus genistin with CI_{50} values of 0.56 ± 0.13 , 0.54 ± 0.20 and 0.45 ± 0.12 , respectively. These bioactive components were able to inhibit invasion and migration in breast cancer cells and the combination treatments enhanced the inhibitory effect. Regulation of PI3K/Akt/mTOR pathway seems to be the main mechanism involved in the anticancer activity.

Among all the tested components from soybean, lunasin attracted our interests because that it is a novel peptide and many problems about lunasin remained to be studied. About lunasin, we were concerned about its anti-inflammatory activity and involved signalling pathways, as well as its production using genetic engineering method. In this study, we isolated natural lunasin (N-lunasin) from soybean and expressed recombinant lunasin (R-lunasin) from *Pichia (P.) pastoris*. The expression level of R-lunasin reached 240 mg/L cell-free broth at the optimum condition (initial pH 7.0, 1.0% final methanol concentration and induction for 72 h at 26 °C). The anti-inflammatory activity of N-lunasin and R-lunasin was accessed and compared. Results showed that there was a comparable effect of N-lunasin and R-lunasin on inhibition of release of nitric oxide (NO), tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages in a dose-dependent manner. In addition, intracellular signalling array analysis demonstrated down-regulated levels of phosphorylated Akt, mechanistic target of rapamycin (mTOR) and p70 s6 kinase (p70s6k) and an up-regulated level of glycogen synthase kinase-3 β (GSK-3 β) after R-lunasin treatment. These results suggest that lunasin exerted anti-inflammatory activity in LPS-stimulated RAW264.7 cells partly via inhibiting the activation of Akt/mTOR/p70s6k signalling pathway.

Soybean is widely cultivated from north China to south China. We were also interested in the variation in lunasin content in soybean seeds. In this study, we determined the lunasin content in a total of 413 soybean samples that were collected from four major regions in China and harvested in 2014 and 2015 to reveal the regional distribution of soybean lunasin content in China and the effects of climate factors. The results showed that the cultivar Changmidou 30 collected from Jilin province and harvested in 2015 had the highest lunasin content (3.25 mg/g dry seeds). The data from both 2014 and 2015 showed that the lunasin content in soybean collected from north China was significantly higher ($p < 0.05$) than that from south China. There was a positive correlation ($p < 0.01$) between lunasin content and hours of sunshine (HS) as well as diurnal temperature range (DTR); however, there was a negative correlation ($p < 0.01$) between the lunasin content and rainfall (RF). In addition, the combined analysis of data from 2014 and 2015 demonstrated that DTR was the dominant factor that affects the lunasin content with a direct path-coefficient of 0.301.

These results generated from our study are anticipated to improve our knowledge of bioactive components in soybean as well as their bioactivities.