PP8C-37
Characterization of mendo (*Antriscus nemorosa*) and *Mentha* species polyphenol oxidase

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Introduction: Polyphenol oxidases (PPO) (EC 1.14.18.1) are very important enzymes in food industry for their involvement in the enzymatic browning of edible plants. Herby cheese, a semi-hard, salty and herb added, is manufactured in small family businesses for their needs and commercial purposes from raw sheep's and cow's milk between May and June in the Eastern and Southeastern of Turkey. A number of herbs such as *Allium* sp., *Thymus* sp., *Mentha* sp., *Antriscus* sp. and *Ferule* sp. are used in making herb cheese. Enzymatic browning damages nutritional quality and economic value of herbs and herby cheese.
Method: Polyphenol oxidase (PPO) was extracted from mendo (*Antriscus nemorosa*) and *Mentha* species added into herby cheese on Van region in Turkey.
Results: Mentha PPO showed activity to catechol and pregallol (K_m values were 25 mM and V_{max} values were 500 EU/ml/minute for catechol; K_m values were 2 mM and V_{max} values were 250 EU/ml/minute for pregallol). Mendo PPO activity was lower than Mentha PPO (K_m values were 2.5 mM and V_{max} values were 333 EU/ml/minute for catechol; K_m values were 0.25 mM and V_{max} values were 55 EU/ml/minute for pregallol). Its characteristics in terms of pH and optimum temperature, thermal inactivation, kinetic parameters were studied. Optimum pH and temperature for *Mentha and Mendo* PPO were found using catechol as substrate at pH 6.8 and 30°C, respectively. Heat inactivation studies showed temperature >40°C resulted in loss of enzyme activity.
Conclusions: Heat process can be used safely in herby cheese production. Thus enzymatic browning could be prevented.

PP8C-38
Improved stability of chemically modified α-chymotrypsin in aqueous organic media

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Introduction: In contrast to aqueous enzymology, biotransformations in water-organic solvent mixtures offer unique industrially attractive advantages. The removal of enzymes from their natural environs results in reduced reaction rates and low stabilities. The aim of our work was to modify the primary amino groups of α-chymotrypsin with different organic acid anhydrides (acetic, propionic, succinic, citraconic and phthalic) possessing different chemical structure to enhance the conformational stability of the enzyme and to compare the stabilities of the acylated enzyme forms.
Methods: For the measurement of α-chymotrypsin activity, *N*-acetyl-*L*-tyrosine ethyl ester was used and the decrease in absorbance at 237 nm was followed. Near-UV CD measurements were performed with the enzyme samples previously incubated for 30 minute. The band intensities were expressed as mean residue ellipticity $[\Theta]_{MR}$.
Results: Acylation of α-chymotrypsin with different anhydrides increased its stablity in aqueous organic solvents. In 60% ethanol, acetonitrile and 1,4-dioxane the modified enzyme forms preserved about 50% of their activities during a 2 hours incubation while the controll enzyme was inactivated within 20 minute. The modification of α-chymotrypsin by phthalic anhydride was accompanied by an enormous activation. The acylated enzyme forms did not show significant structural changes with the exception of phthalic anhydride modified one. The improvement of stabilities might be related to side chain reorientations of aromatics upon modifications especially caused by phthalic anhydride.
Conclusions: Our results demonstrate that the stability of α-chymotrypsin may be increased by acylation of amino groups thereby improving the efficiency of the biocatalyst in water organic solvent mixtures.

PP8C-39
Soy sauce inhibits protein glycation *in vitro*

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Introduction: Glycation and the subsequent formation of advanced glycation endproducts (AGEs) and free radicals produced during hyperglycaemia are major factors in the complications of diabetes and ageing. The development of clinically effective antiglycation compounds would be of obvious benefit in the management of such conditions. Soy and its products, soy sauces, have been used in East Asian countries since the 15th century. More recently, their anticarcinogenic, antioxidant, antimicrobial, antiplatelet, hypoallergenicity and antiallergic activities have been demonstrated.
Methods: The antioxidant properties of light and dark soy sauces were investigated using the ABTS method, by following the decolourization of the radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid). Their capacities to prevent the formation of AGEs were examined using polyacrylamide gel electrophoresis to determine the extent of protein cross-linking and polymerization.
Results: All the brands of soy sauces investigated exhibited antioxidant properties, with the dark having more potent actions than that of light. All soy sauces produced a dose dependent inhibition of protein cross-linking and AGE formation, although in this case, the light sauces were the more effective.
Conclusions: The antioxidant properties and antiglycation effects of soy sauces means some of their components may be of clinical value in the prevention of diabetic complications and age-related diseases, and therefore warrant further investigations.

PP8C-40
Anti-atherogenic activity of procyanidins is driven by inhibiting CD36 and inducing ABCA1 expression, thus reducing lipid accumulation in foam cells

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Introduction: Human and animal studies have demonstrated that procyanidin-rich diets reduce the risk of cardiovascular diseases and atherosclerosis. Some beneficial effects have been attributed to the well-known antioxidant activity of procyanidins. In this study, we investigated another potential role of procyanidins in cholesterol influx and efflux and inflammation in macrophage-derived foam cells.
Methods and Results: We cultured RAW 264.7 macrophages with highly oxidized LDL (oxLDL), moderately oxidized LDL (moxLDL) or with LPS (0.5 μg/ml) and oxLDL (LPS + oxLDL) to induce foam cell. Then, cells were treated with procyanidins derived from grape seed (PE, 45 μg/ml) for the last 12 hours of incubation with the different lipoproteins (25 μg/ml). After lipid extraction, we determined that total and esterified cholesterol and triglyceride accumulation in foam cells were increased by lipoprotein treatment but reduced by PE incubation in all the foam-cell models.
In order to asses the effect of PE on gene expression we determined the relative mRNA levels of CD36, ABCA1, iNOS and IkBα by RT-PCR. We show that PE reduced the oxLDL scavenger receptor expression (CD36), and enhanced ABCA1 (ATP-binding cassette A1) expression, a key regulator of macrophage cholesterol efflux, thus reducing lipid influx and inducing its efflux. PE also downregulated inflammatory related genes such as iNOS (inducible nitric oxide synthase) and IkBα (kappa beta inhibitor-alpha).
Conclusion: We provide evidence that procyanidins may attenuate the development of foam cell formation by reducing cholesterol accumulation through the modulation of key genes in cholesterol flux and inflammation.