ENZYME-ASSISTED SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ACTIVE PRINCIPLES OF BLACK PEPPER AND SMALL CARDAMOM AND APPLICATIONS OF THE EXTRACTS FOR DESIGN OF NUTRACEUTICAL FOODS AND SUPPLEMENTS

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Summary

The present work focused on enzyme-assisted supercritical carbon dioxide extraction of *spiceuticals* from black pepper and small cardamom to enhance the yields of bioactives from these spices. The extracts along with post-extraction sample matrices were employed in formulation of functional foods. The *spiceutical*-rich extracts were also encapsulated with the aim to enhance their shelf-lives.

The systems that were investigated in this work are:

Supercritical carbon dioxide extraction of piperine from black pepper

- Authentic Malabar black pepper was employed for SC-CO_2 extraction of its bioactive principle piperine. A 3^3 full factorial design of experiment was applied to optimize the extraction conditions.
- The optimum conditions of extraction which exhibited maximum yield of piperine along with the best combination of phytochemical properties were a pressure of 300 bar, extraction temperature of 60 °C, 45 min of extraction time and a flow rate of 2 L/min of gaseous CO_2.
- Statistical analyses revealed that extraction pressure (in quadratic and linear form) and temperature (in quadratic form) and the interdependence between them showed significant effects on yields of piperine.
- Solubility of piperine in SC-CO_2 at different extraction conditions were established by a correlated Chrastil equation, which can predict its solubility in SC-CO_2 under different extraction conditions.

- A regression equation was developed for prediction of yield of piperine in different SC-CO_2 conditions.
- The extraction of piperine from black pepper followed 'plug flow' model and its release was explained best by Higuchi model of first order kinetics.
- The empirical correlation, deduced from Reynolds, Schmidt and Sherwood numbers exhibited high correlation coefficient (r=0.96), suggesting that the extraction process was satisfactorily modeled.
- Enzyme-assisted SC-CO_2 extractions were conducted employing bacterial α-amylase (from *Bacillus licheniformis*) to enhance the yield of piperine by hydrolyzing the starch content (30.4±0.1% on dry weight basis) of black pepper.
- The SC-CO_2 extraction equipment was operated in two modes in this study: batch and continuous.
- The yield of piperine-rich extract was enhanced by 53% in the batch mode vis-à-vis 15% in the continuous mode. SC-CO_2 extracts obtained from enzyme-assisted extractions in either mode exhibited enhanced piperine content, Scoville heat unit (SHU) and *in vitro* phytochemical and antimicrobial potencies.
- SC-CO_2 treated (continuous mode) α-amylase exhibited 2.13 times enhanced specific activity compared to the untreated enzyme.
- ^{1}H NMR analysis revealed that the enhancement of specific activity of enzyme was due to an alteration in the conformational arrangement of α-amylase, possibly at its active site.
- The high pressure treatment of SC-CO_2 was found to be an alternative to the expensive skilled-technique based methods of genetic engineering to obtain enhanced specific activity

of enzyme. This would allow facile technology transfer to industries to produce cost effective products providing higher reaction rates in less time and thereby facilitating high production in reduced time.

- Piperine-rich extract of black pepper obtained from enzyme-assisted SC-CO_2 extraction in batch mode, *per se* can be treated as a food supplement. This extract along with post-extraction residual sample matrix was further employed in formulation of functional/nutraceutical cookies.
- The black pepper cookies possessed higher nutraceutical properties compared to the cookies without antioxidant (control).
- Electronic nose (e-nose) technology (having 8 MOS sensors) was successfully employed for detection of rancidity in cookies.
- SC-CO_2 extract of black pepper enhanced the shelf-lives of cookies by at least 120 days; while the post-extraction residual sample matrix enhanced the same by 80 days.
- Mahalanobis distances determined from e-nose responses of cookies were designated as the 'spoilage indices' to rapidly and accurately predict onset of rancidity and hence the shelf-lives of cookies.
- Regression equations were generated employing biochemical indices of spoilage (FFA content, PV value and MDA content for each type of cookie) and the spoilage indices (obtained by above method) , which would allow prediction of the former values, foregoing routine biochemical assays.

Supercritical carbon dioxide extraction of 1,8-cineole from small cardamom

- α-amylase-assisted SC-CO_2 extraction of 1,8-cineole from Alleppey green small cardamom was carried out at SC-CO_2 conditions of 200 bar pressure, 50 °C temperature with 2.25 h total extraction time, previously optimized and reported in thesis of my co-worker in green technology research laboratory of Department of Food technology and Biochemical Engineering, Jadavpur University.
- Enzyme-assisted SC-CO_2 extractions enhanced the yield of 1,8-cineole-rich extract in batch mode by 50.66% and in continuous mode by 11.21%.
- Extracts obtained from both batch and continuous modes exhibited enhanced 1,8-cineole content, *in vitro* phytochemical and antimicrobial potencies.
- SC-CO_2 conditions of 200 bar, 50 °C and 2.25 h in continuous mode exhibited 1.99 times higher specific activity of treated enzyme compared to the untreated enzyme.
- The 1,8-cineole-rich extract *per se* can be treated as a food supplement. This extract and post-extraction residual sample matrix of small cardamom were further employed in formulation of designer nutraceutical cookies.
- Small cardamom cookies exhibited enhanced nutraceutical properties compared to control set of cookies.
- E-nose technology and Mahalanobis distance method revealed that, SC-CO_2 extract and post-extraction sample matrix of small cardamom prevented rancidity and therefore enhanced the shelf-lives of cookies by at least 120 days and 40 days, respectively.
- Regression equations were generated for prediction of FFA contents, PV values and MDA values of small cardamom cookies from their e-nose responses, to circumvent the

requirement of conducting routine biochemical assays for estimation of rancidity in cookies.

Encapsulation of SC-CO_2 extracts of black pepper and small cardamom

- Small cardamom extract obtained from enzyme-assisted SC-CO_2 extraction, was microencapsulated by spray drying using maltodextrin and gum arabic as wall materials.
- The conditions of spray drying were optimized using a 3^2 experimental design to obtain an encapsulate having maximum content of 1,8-cineole, i.e., the highest microencapsulation efficiency. The optimized conditions of spray drying were 130 °C inlet air temperature and wall material composition of 70:30:: maltodextrin: gum arabic.
- The encapsulate obtained from the optimized conditions of spray drying was designated as E_{best} and can be termed as a 'finished herbal product' in accordance with WHO guidelines.
- E_{best} showed 6.12 times higher shelf stability at ambient temperature (23±2 °C) and 7.88 times higher shelf stability at accelerated temperature (70±2 °C), with respect to the SC-CO_2 extract.
- The mean particle diameter of E_{best} was determined to be 7.76 μm and the encapsulate was free from toxic metal compounds such as Cu, Pb, Cd, Hg and As. Therefore, the encapsulate was safe for consumption.
- E_{best}, at optimized concentration (4.5%) was further employed as a natural antioxidant in formulation of custard to protect 1,8-cineole from degradation during thermal processing.
- The newly formulated nutraceutical custard exhibited better rheological stability, higher phytochemical potency and lower microbial load than its control.

- Black pepper extract obtained by α-amylase-assisted SC-CO_2 extraction was encapsulated as nanoliposomes (29.75±0.84 nm) using probe sonication.
- The optimized conditions which exhibited the highest encapsulation efficiency (78.6%) of nanoliposomes were, soya phosphatidylcholine: Tween 80:: 1:1.2 and 2% (w/w) concentration of black pepper extract.
- The *in vitro* release profile exhibited 70% release of piperine within 8 h from nanoliposome of black pepper extract following Higuchi model of first order kinetics.
- After 3 months of storage at 4±1 °C, piperine in the nanoliposome of black pepper extract exhibited 92.9% stability. The half-life of the same at 4±1 °C was 2.48 times higher than that of the extract.