Antioxidant activity of a salt-spice-herbal mixture

The scientists at ALMPG Institute of Basic Medical Sciences, University of Madras, Chennai, India evaluated a combination of spices (Piper nigrum Linn., P. longum Linn. and Zingiber officinale Rosc.), herbs (Cyperus rotundus Linn. and Plumbago zeylanica Linn.) and salts make up Amrita Bindu for its possible antioxidant activity. The study was focused to evaluate the antioxidant property of individual ingredients in Amrita Bindu against the free radical 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid). The analysis revealed the antioxidant potential of the ingredients in the following order: P. nigrum > P. longum > C. rotundus > P. zeylanica > Z. officinale. Two different experiments were designed. In experiment I, rats were fed with normal diet, whereas in experiment II, rats were given feed mixed with Amrita Bindu for 3 weeks (4g/kg of feed). Rats from both experimental groups were challenged against a single intraperitonial injection of phenylhydrazine (PHZ) (7.5mg/kg body wt). At the end of 24 and 72 hours, blood was analysed for free radicals and antioxidant levels. It was interesting to note that rats with Amrita Bindu pretreatment showed significantly lower levels of free radicals, lipid peroxidation and protein carbonyls along with significantly higher levels of antioxidants when compared with rats without Amrita Bindu pretreatment on PHZ administration. These results revealed that Amrita Bindu, a salt–spice–herbal mixture exerts a promising antioxidant potential against free radical induced oxidative damage [Natarajan Kavithalakshmi S, Narasimhan Madhusudhanan, Shanmugasundaram K Radha and Shanmugasundaram ERB, Antioxidant activity of a salt–spice–herbal mixture against free radical induction, J Ethnopharmacol, 2006, 105(1-2), 76-83].

Aroma glycosides of nutmeg and their changes during radiation processing

Nutmeg (Myristica fragrans Houtt.) is an important spice of commerce, valued for its unique aroma and medicinal properties. Aroma glycosides are an important class of nonvolatile precursors that are currently gaining increased interest and attention for their role in imparting unique aroma to food. Aroma glycosides have been isolated in numerous foods and where their contribution to the characteristic aroma has been established. However, despite their important role, very few studies exist on the stability of these compounds during post-harvest processing and storage of agriculture and food commodities. Thus, scientists at Food Technology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India worked on isolation, identification and estimation of aroma glycosides in nutmeg and determining the effects of radiation processing on this important class of precursor molecules. Possibility of using post-irradiation changes in aroma glycosides as a method for detection of γ-irradiated nutmeg is also suggested. Glycosidically bound volatile compounds of nutmeg were identified as glyco-conjugates of p-cymene-7-ol, eugenol, methoxyeugenol and α-terpineol. Using phenyl-β-glucoside as external standard the contents of these glycosidic precursors were estimated based on the measurement of TLC spot density on a densitometer. p-Cymene-7-ol rutinoside was the major aroma glycoside (3.15g/100g), followed by glycosides of methoxyeugenol (0.61g/100g), eugenol (0.50g/100g) and α-terpineol (0.51g/100g). A dose-dependent breakdown of these glycosidic precursors was observed during γ-radiation processing. Among the four glycosides, α-terpineol glucoside was the most sensitive to radiation while p-cymene-7-ol rutinoside was the least sensitive. A reduction in the content of total glycosides by almost 50% was noted at a dose of 5kGy. Partitioning of aroma glycoside into n-butanol from aqueous extracts was found to result in rapid isolation of aroma glycosides, avoiding time consuming pre-purification on Amberlite XAD-2 column. A routine method based on extraction into n-butanol and subsequent quantification of post-irradiation changes in aroma glycosides on a TLC plate using a densitometer has been proposed by authors [Ananthakumar Arul, Variyar Prasad Sand Sharma Arun, Estimation of aroma glycosides of nutmeg and their changes during radiation processing, J Chromatogr, 2006, 1108(2), 252-257].<ref>Spices</ref>
Inhibition of $\text{CCl}_4$-induced liver fibrosis by *Piper longum* Linn.

The scientists at KM College of Pharmacy, Uthangudi, Madurai, Tamil Nadu, India carried studies to evaluate the antifibrotic effect of ethanol extract of the fruits of Indian Long Pepper, *Piper longum* Linn. Liver fibrosis was induced in rats by $\text{CCl}_4$ administration and the extent of liver fibrosis was assessed by measuring the level of liver hydroxy proline (HP) and serum enzyme levels. Following $\text{CCl}_4$ administration HP was significantly increased and serum enzyme levels were elevated. Treatment with the ethanol extract of this spice reduced the HP and also the serum enzymes. The liver weight that increased following $\text{CCl}_4$ administration due to the deposition of collagen was reduced by the ethanol extract. Hence, it is concluded that this extract inhibits liver fibrosis induced by $\text{CCl}_4$. The extract at a higher dose (100mg/kg) is more hepatoprotective and antifibrotic. Purification of the extract and identification of the active principle might yield a good hepatoprotective drug [Christina AJM, Saraswathy GR, Heison Robert SJ, Kothai R, Chidambaramanathan N, Nalini G and Therasal RL, Inhibition of $\text{CCl}_4$-induced liver fibrosis by *Piper longum* Linn., *Phytomedicine*, 2006, 13(3), 196-198].

Cardamom extract as inhibitor of human platelet aggregation

The inhibitory activity of cardamom (*Elettaria cardamomum* Maton) extract was studied by the researchers at Central Food Technological Research Institute, Mysore, India on human platelets. Platelet aggregation and lipid peroxidation were evaluated with platelet rich plasma (PRP) and platelet membranes, respectively, obtained from blood of healthy volunteers. Human platelets were subjected to stimulation with a variety of agonists including ADP (2.5 mm), epinephrine (2.5 mm), collagen (10 mm), calcium ionophore A 23187 (6 µm) and ristocetin (1.25 µg/mL). The IC$_{50}$ were 0.49, 0.21, 0.55 and 0.59 mg with ADP, epinephrine, collagen and calcium ionophore A 23187, respectively, and no inhibition with ristocetin. The inhibitory effect was dose dependent with concentrations varying between 0.14 and 0.70 mg and time dependent at IC$_{50}$. Lipid peroxidation induced by iron - ascorbic acid system in platelet membranes was analysed with malondialdehyde (MDA) as an index. An increase in concentration of cardamom has decreased the MDA formation significantly. Hence, it may be concluded that aqueous extract of cardamom contains component(s), which protect platelets from aggregation and lipid peroxidation [Suneetha W Jessie and Krishnakantha TP, Cardamom extract as inhibitor of human platelet aggregation, *Phytother Res*, 2005, 19(5), 437-440].
Use of mustard flour to inactivate *Escherichia coli* in ground beef

*Escherichia coli* O157:H7 causes life threatening haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopenic purpura in the young, old and immuno-compromised persons. *E. coli* O157:H7 outbreaks in a number of countries have been linked to undercooked or raw hamburgers eaten during the summer. Different options are being investigated for elimination of *E. coli* O157:H7 from ground beef, which might be acceptable to regulatory authorities, the food industry and consumers. One promising approach is the addition of natural antimicrobials of plant origin. Natural extracts and essential oils from plants were historically used to extend shelf-life by stabilizing or improving the sensory quality of food. Currently, a substantial amount of work is underway to determine whether natural antimicrobials from plants, animals or benign microorganisms can be used to kill pathogenic bacteria and improve the safety of packaged foods. Studies have demonstrated that allyl isothiocyanate (AIT), a naturally occurring non-phenolic volatile compound found in plants belonging to the Brassicaceae family, effectively inhibits a variety of pathogenic microorganisms when used at low concentrations.

A study was undertaken by researchers at Department of Food Science, University of Manitoba, Canada to determine whether the glucosinolates naturally present in non-deheated mustard (*Brassica* sp.) flour could serve as a source of allyl and other isothiocyanates in sufficient quantity to kill *E. coli* O157:H7 inoculated in ground beef at three different levels, during refrigerated storage of the meat under nitrogen. Mustard flour was mixed at 5, 10 or 20% (w/w) with freshly ground beef, then the beef was inoculated with a cocktail of five strains of *E. coli* O157:H7 at either 3, 6 or ≤1.6 log<sub>10</sub> cfu/g. The ground beef was formed into 100 g patties and each was placed in a bag of Nylon/EVOH/PE, which was back-flushed with 100% N<sub>2</sub> and heat-sealed and stored at 4°C for ≤21 days. During storage, the AIT levels in package headspaces were determined by gas liquid chromatography. By 21 days, the levels present in treatments were not significantly different. After 21 days storage, there were 0.5, 3 and 5.4 log<sub>10</sub> decreases in numbers of *E. coli* O157:H7 from the initial levels of 6 log<sub>10</sub> cfu/g in meat containing 5, 10 and 20% mustard flour, respectively. When inoculated at 3 log<sub>10</sub> cfu/g, *E. coli* O157:H7 was reduced to undetectable levels after 18, 12 and 3 days with 5, 10 and 20% mustard flour, respectively. When immunomagnetic separation (IMS) was used for *E. coli* recovery following its inoculation at ≤1.6 log<sub>10</sub> cfu/g, 5% mustard did not completely eliminate the pathogen from ground beef stored for 6 days. The natural microflora of the ground beef which developed in vacuum packages was unaffected by the addition of 5% mustard flour but some inhibition was found at higher concentrations. Sensory evaluation of the cooked ground beef showed that there were no significant differences in the acceptability of meat treated with 5 or 10% mustard flour. However, panelists could distinguish untreated controls from mustard treatments, but considered the mustard-treated meat to be acceptable. These results showed that it is possible to use mustard flour at levels of >5-10% to eliminate *E. coli* O157:H7 from fresh ground beef [Nadarajah D, Han JH and Holley RA, Use of mustard flour to inactivate *Escherichia coli* O157:H7 in ground beef under nitrogen flushed packaging, Int J Food Microbiol, 2005, 99 (3), 257-267].
Inactivation of peroxidase, polyphenoloxidase and lipoxygenase in paprika and chili powder

Paprika and chili powders are spices of considerable economic importance all over the world. Beside microbial contamination during drying, deteriorative enzymes such as lipoxygenase (LOX), peroxidase (POD) and polyphenoloxidase (PPO) are still active and may regain their activities, especially during storage of spices under humid conditions and after rehydration of dried foods. Since, endogenous as well as microbial enzymes may adversely affect colour, taste and aroma of spices and derived foods, their immediate and complete inactivation is a prerequisite for high quality spices. In Capsicum fruits these enzymes may cause quality deterioration in spice powders obtained thereof.

Residual activities of LOX, POD and PPO were determined by researchers at Institute of Food Technology, Hohenheim University, Stuttgart, Germany in paprika and chili powder after immediate thermal treatment of the fresh plant material. Heat treatments comprised water and steam blanching of the integral fruits and pasteurization of the comminuted tissues, respectively. PPO showed the lowest heat stability and was completely inactivated by heating at 80°C for 10 minutes. Inactivation of LOX was also largely accomplished by heating at 90° and 100°C for 5 min, whereas up to 3.5% (paprika) and 3.3% (chili) of the initial POD activities were retained even when applying rigorous time–temperature regimes. The results demonstrate that substantial inactivation of deteriorative enzymes was ensured by the recently suggested process, thus facilitating the production of high quality spice powders [Schweiggert Ute, Schieber Andreas and Carle Reinhold, Inactivation of peroxidase, polyphenoloxidase, and lipoxygenase in paprika and chili powder after immediate thermal treatment of the plant material, Innov Food Sci Emerg Technol, 2005, 6 (4), 403-411].

Antioxidant and radical scavenging proanthocyanidins from Nagkesar

The flower buds of Mammea longifolia Planch. & Triana (Hindi — Nagkesar) are extensively used in culinary preparations, especially as a minor spice in various spice blends, such as Garam Masala powder, used for flavouring foods. Dried buds are used as substitutes to cloves in making Pan Masala, which is a chewing product to improve digestion after heavy meal.

Currently, the use of some naturally occurring antioxidant molecules and their identicals in foods as well as preventive and therapeutic medicine is gaining popularity. It had been found that the methanol extract from the buds of M. longifolia showed a strong radical scavenging activity on DPPH radical scavenging assay and xanthine-xanthine oxidase system chemiluminescence assay. The polar fraction of methanol extract exhibited stronger antioxidative and radical scavenging activities. An attempt has been made by researchers at National Food Research Institute, Kannondai, Tsukuba, Ibaraki, Japan to isolate and identify the compounds responsible for the radical scavenging activity in these fractions. The most active fraction was subjected to spectral analysis and chemical degradation and found to contain proanthocyanidin oligomers with degree of polymerisation ranges from 2-10. Plant proanthocyanidins are known as the functional food factors that possess a variety of physiological activities such as antioxidant, antimicrobial, anti-allergy, hair-growth promotion, anti-caries, anti-hypertensive and inhibition against activities of some enzymes and receptors. Proanthocyanidins have attracted increasing attention recently, due to the rapidly growing body of evidence associating these compounds with a wide range of potential health benefits as mentioned above. Further, these groups of compounds are associated with potential cardiovascular benefits, including the reduction of platelet aggregation and reduction of tumour multiplicity in laboratory mice [Rao Lingamallu Jagan Mohan, Yada Hiroshi, Ono Hiroshi, Ohnishi-Kameyama Mayumi and Yoshida Mitsuru, Occurrence of antioxidant and radical scavenging proanthocyanidins from the Indian minor spice Nagkesar (Mammea longifolia Planch. and Triana), Bioorg Med Chem, 2004, 12 (1), 31-36].
The rhizomes of turmeric (*Curcuma longa* Linn., Hindi—*Haldi*) provide a yellow and flavourful powder when dried and ground. It has been valued for its colour and functional properties. However, it has a pungent flavour that resembles pepper and although this flavour is desirable in mustard and spices, the fact that it cannot be used in sweets or foods with a mild flavour, prevents its widespread use in the food industry. Researchers of Brazil and France investigated the influence of different hydro-distillation procedures on the deodorization of turmeric. Distillation of medium size grated turmeric using a Clevenger apparatus for 4 hours provided a powder with less residual turmeric flavour, without pigment loss and with similar colour characteristics compared to a control and to turmeric deodorized with the solvent hexane. The deodorized and non-deodorized turmeric samples were used as natural colours in orange-flavoured yoghurt and passion fruit-flavoured gelatin. No significant difference was observed for yoghurt samples by sensory evaluation. However, the gelatin prepared with deodorized turmeric was preferred over the control. The deodorization of turmeric using the Clevenger method would allow a more widespread use in the food industry, including products in which the characteristic turmeric flavour is undesirable [Silvaa LV, Nelsona DL, Drummondb MFB, Dufosséc L and Glória MBA, Comparison of hydrodistillation methods for the deodorization of turmeric, *Food Res Int*, 2005, 38(8-9), 1087-1096].

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**Therapeutic role of *Cuminum cyminum* Linn.**

Ethanol is one of the most widely used and abused drugs, increasing lipid levels in humans and experimental animals. Heating of oil rich in polyunsaturated fatty acids (PUFA) produces various lipid peroxidative end products that can aggravate the pathological changes produced by ethanol. The researchers at Faculty of Science, University of Annamalai, Annamalai Nagar, Tamil Nadu, India investigated the effect of Cumin, *Cuminum cyminum* Linn. (Hindi—*Jira*) on alcohol and thermally oxidized sunflower oil induced hyperlipidaemia. The results showed increased activity of aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) and increased levels of cholesterol, triglycerides and phospholipids in the plasma of rats given alcohol, thermally oxidized oil and alcohol + thermally oxidized oil when compared with the normal control group. The levels of tissue (liver and kidney) cholesterol and triglycerides were increased significantly in rats groups given alcohol, thermally oxidized oil and alcohol + thermally oxidized oil when compared with the normal control rats. The levels were decreased when cumin was given along with alcohol and thermally oxidized oil. The level of phospholipids decreased significantly in the liver and kidney of groups given alcohol, thermally oxidized oil and alcohol + thermally oxidized oil when compared with the normal control rats. The level increased when cumin was administered along with alcohol and thermally oxidized oil. The activity of phospholipase A and C increased significantly in the liver of groups given alcohol, thermally oxidized oil and alcohol + thermally oxidized oil when compared with the normal control rats, whereas the activity was decreased with the cumin treatment [Aruna K, Rukkumani R, Varma P Suresh and Menon Venugopal P, Therapeutic role of *Cuminum cyminum* on ethanol and thermally oxidized sunflower oil induced toxicity, *Phytother Res*, 2005, 19(5), 416 - 421].