

Nutraceutical/Functional Foods and their Assessment in Foods

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ABSTRACT

There is growing recognition of the potential role for nutraceuticals and dietary supplements in helping to reduce health risks and improve health quality. In the global marketplace nutraceuticals and functional foods have become a multi-billion dollar industry. In this paper we examine sources of nutraceutical and functional components in different foods and their assessment. Functional foods are found virtually in all food categories, however products are not homogeneously scattered over all segments of the growing market. Functional foods have nutritional and physiological benefits and are applicable in disease prevention and management.

Key words: Food, Phytosterols, Tannins, Fatty acids, Terpenoids, Saponins

INTRODUCTION

In the last decades consumer demands in the field of food production has changed considerably. Consumers more and more believe that foods contribute directly to their health^{31,47}. Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers^{29,42}. In this regard, functional foods play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years^{22,41,42}.

The term *nutraceutical* is a hybrid or contraction of *Nutrition* and *pharmaceutical* reportedly, it was coined in 1989 by DeFelice and the Foundation for Innovation in Medicine²¹. Restated and clarified in a press release in 1994, its definition was “any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary, supplements and diets to genetically engineered ‘designer’ foods, herbal products, and processed foods such as cereals, soups, and beverages”¹¹.

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According to the International Food Information Council (IFIC), functional foods are “foods or dietary components that may provide a health benefit beyond basic nutrition.” The International Life Sciences Institute of North America (ILSI) has defined functional foods as “foods that by virtue of physiologically active food components provide health benefits beyond basic nutrition.” Health Canada defines functional foods as “similar in appearance to a conventional food, consumed as part of the usual diet, with demonstrated physiological benefits, and/or to reduce the risk of chronic disease beyond basic nutritional functions.” The *Nutrition Business Journal* classified functional food as “food fortified with added or concentrated ingredients to functional levels, which improves health or performance. 6 Functional foods include enriched cereals, breads, sport drinks, bars, fortified snack foods, baby foods, prepared meals, and more.”

According to Dillard & German¹² the health promoting effects of phyto-chemicals and nutraceuticals and/or functional foods likely are due to a complex mix of biochemical and cellular interactions which together promote overall health of the individual. They suggest that these agents may function: as substrates in metabolic reactions or cofactors of key metabolic enzymes; as ligands that promote or compete with biochemical interactions at the cell surface or with intercellular receptors which can enhance absorption and assimilation of important macro and micro nutrients; and as agents which selectively promote the growth of bacteria with health benefits in the gastrointestinal system and compete with or partially eliminate the growth of harmful bacteria. In addition these agents may act as enzyme inhibitors, absorbents or toxicant scavengers that can associate with and help remove damaging substances or toxins from the body. Major chemical groups now recognized as having potential health promoting effects, at least under some circumstances are the phenolics, flavonoids, alkaloids, carotenoids, pre- and pro-biotics, phytosterols, tannins, fatty acids, terpenoids, saponins, and soluble and insoluble dietary

fibers^{6,8,9,34}. A considerable more work is required to support claims that often times have been made locally in support of herbal or other traditional medicines but cannot be supported globally due to biological variation in genotype and ecotypic responses³. While considerable research may have been done with individual biological isolates, this same research often fails to recognize the vast diversity of biological organisms and consumer products derived from them. Recognition of variation in functional food and nutraceutical composition will provide opportunity for the industry to give consumers a variety of new products that can be developed for niche or specialized markets. Development of new products with distinctive genetics, ecotypic response and reliable health benefits also could provide local producers with access to more stable and specialized markets similar to those already seen in the coffee and wine marketplace where regionally produced variants of these products have been successfully marketed based on their unique regional attributes. In any case, as scientific studies which reveal new discoveries with potential health benefits are identified by potential consumers and the media, more support, credibility and demand for functional foods and nutraceuticals is being generated. This is resulting in a marketplace with considerable potential for growth and many new opportunities within the industry both internationally and at a regional level.

Confusion exists about how to describe this newly evolving area of food and food technology because numerous interchangeable or related terms have been suggested or published in the United States, Europe, and Japan. These include terms such as pharma foods, functional foods, phyto-chemicals, chemo-preventive agents, and therapeutic foods.

Important definitions associated with the nutraceutical and functional food industry

Bioactive compounds:

Naturally occurring chemical compounds contained in, or derived from, a plant, animal or marine source, that exert the desired health/wellness benefit¹.

Functional ingredients:

Standardized and characterized preparations, fractions or extracts containing bioactive compounds of varying purity, that are used as ingredients, by manufacturers in the food (human and pet) and fractions or extracts containing bioactive compounds of varying purity, which are used as ingredients by manufacturers in the cosmetics and pharmaceutical sectors.

Industrial ingredients:

Standardized and characterized preparations, fractions or extracts of agri-commodities of varying purity that are used as ingredients by manufacturers of non-food products.

Natural Health Products (NHP):

Includes homeopathic preparations, substances used in traditional - medicines, minerals or trace elements; vitamins; amino acids; essential fatty acids; or other botanical, or animal or microorganism derived substances. These products are generally sold in medicinal or “dosage” form to diagnose, treat, or prevent disease; restore or correct function; or to maintain or promote health².

Traditional Food Ingredients (TFI):

Standardized and characterized preparations, fractions or extracts of agri-commodities of varying purity, that originate from plant, animal or marine sources and are used as ingredients, by manufacturers in the food (human and pet) and NHP sectors¹.

Traditional Processed Foods (TPF):

Conventional foods that have been manufactured by the traditional food processing industry and sold to the public through established distribution systems for generations.

Traditional Whole Foods (TWF):

Conventional foods that have been grown by agricultural producers for generations.

Designer Foods:

Foods that naturally contain or are enriched with cancer-preventing substances such as phyto-chemicals (Coined in 1989 by National Cancer Institute, USA).

Novel Foods:

Products that have never been used as food; foods that result from a processes that has not previously been used for food; or, foods that have been modified by genetic manipulation¹⁷.

Nutraceuticals:

A product isolated and purified from foods that is generally - sold in medicinal forms are

usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease (Coined originally by Stephen DeFelice in 1989, founder and chairman of the Foundation for Innovation in Medicine, USA).

Functional Foods:

A functional food is similar in appearance to. Or may be, a conventional food, is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond the basic nutritional functions

Phyto-chemicals / phyto-nutrients:

Plant components that have health-promoting properties; originally this term was limited to substances found in edible fruits and vegetables that appeared to be protective against cancer. Now the term is much broader and applies to any plant component that has health-enhancing benefits.

Medical Foods:

Special dietary food intended for use solely under medical supervision to meet nutritional requirements in specific medical conditions (Coined in 1992 by the Institute of Food Technologists Expert Panel on Food Safety and Nutrition.

Phyto-pharmaceuticals:

Chemicals in their natural form isolated from medicinal plants with potential benefits in human health and disease prevention (Association of Saskatchewan Home Economists, 2006).

Dietary Supplements:

A product that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, a herb or other botanical, an amino acid, a dietary substance, for use by man to supplement the diet by increasing the total daily intake, or concentrate. Metabolite, constituent, extract. Or combinations of these ingredient; intended for ingestion in pill, capsule, tablet, or liquid form; not represented for use as conventional food or as a sole item of a meal or diet; labelled as dietary supplement; includes products such as approved new drug, certified antibiotics, of licensed biologic that marketed as a dietary supplement or food before approval, certification, of license (unless a product is redefined through regulatory government agency³².

Important phyto-chemicals (nutraceuticals), their corresponding plant sources and medicinal properties

Chemical groups	Plant sources	Medicinal property
1.	Alkaloids	
Quinine	Cinchona	Anti-malarial
Tropane alkaloids	Solanaceous members : Deadly night shade, Datura	In treatment of heart ailments
Morphine	Opium poppy	Antidepressant, pain killer
Ergot alkaloids	Fungus: <i>Claviceps purpurea</i>	Abortifacients
Vincristine	Periwinkle	Antineoplastic
Vinblastine	Periwinkle	Antineoplastic
Coumarin,	Fenugreek	Hypoglycemic
Scopoletin	Fenugreek	Hypoglycemic
Fenugreekine	Fenugreek	Hypoglycemic
Trigonelline	Fenugreek	Hypoglycemic
2.	Carotenoid terpenoids/ Isoprenoids	
α -carotene	Carrots	Antioxidants, anti carcinogenic
β -carotene	Fruits & vegetables	Antioxidants
γ -cryptoxanthin	Oranges & tangerines	Antioxidants, anticancer
Lutein	Vegetables (kale, spinach, watercress, parsley)	Reduce risk of macular degeneration, protect against colon cancer
Zeaxanthin	Corn, avocado	Protects eye from macular degeneration and cataracts
Lycopene	Tomatoes, pink grapefruit, watermelon, guava, papaya	Reduces risk of prostate cancer in males
3.	Non-carotenoid terpenoids	
Perillyl alcohol	Cherries & mints	Anticancer
Saponins	Legumes (Chicks, peas, fenugreek, all pulse crops)	Reduces cholesterol levels in blood
Terpenol	Carrots	Anticancer
Terpene limonoids	Peels and membranes of citrus fruits	Anticarcinogenic
4.	Flavonoid polyphenolics	
Anthocyanins	Stawberries, raspberries, cherries, cranberries, pomegranate, apples, red grapes	Antioxidants
Betacyanins	Beet root	Antioxidants
Catechins	Tea	Antioxidants
Flavonones	Citrus fruits	Antioxidants
Flavones	Fruits & vegetables	Anticancer
Isoflavones	Soybean	Anticancer
Hesperetin	Citrus fruits	Antioxidants
Naringin	Grapefruit	Reduces cholesterol
Rutin	Asparagus, buckwheat & citrus fruits	Protects against cardio vascular ailments
Quercetin	Red onions, buckwheat, red grapes, green tea, apple skins	Anti-sitaminic, antioxidant
Silymarin	Artichoke & milk thistle	Anti-atherosclerotic
Tangeretin	tangerines	Anticancer
Tannins	Cranberries, pomegranate, cocoa & tea	Reduces blood cholesterol
	Phenolic acids	
Ellagic acids	Strawberries & raspberries	Prevents colon cancer
Chlorogenic acids	Blueberries, tomatoes, grapes & bell peppers	Antioxidant
p-coumaric acids	Red and green ball peppers, legumes	Antioxidant, anticancer
Phytic acids	Legumes and whole seed grains	Lowers blood glucos
Ferulic acids	Seeds of brown rice, whole wheat and oats, apple, artichoke, orange, peanut & pine apple	Antioxidant, anticancer
Vanillin	Vanilla bean	Antioxidant, anticancer
Cinnamic acid	Cinnamon, balsam tree resins	Antibacterial, antifungal
Hydroxycinnamic acid	Grapes, blueberries & blackberries	Antioxidant, anticancer
	Non-flavonoid polyphenolics	
Curcumin	Curcuma	Anti-microbial, anticancer, antioxidant
Resveratrol	Grapes	Anti-inflammatory, anticancer
Lignans	Plant cell walls	Reduces skin cancer
	Glucosinolates	
Isothiocyanates	Horseradish, radish & mustard	Anticancer
Phenethyl isothiocyanate	Watercress	Anticancer
Sulforaphene	Broccoli	Anticancer
Indoles	Broccoli	Anticancer
Thiosulfonates	Garlic & onions	Anticancer, antimicrobial, reduces blood pressure and cholesterol
Phytosterols (plant sterols)	Peanuts, cashews, almonds, peas, kidney beans & avocados	Anticancer, blocks cholesterol absorption
	Anthraquinones	
Senna	Legumes and pulses	Purgative,
Barbaloin	Aloe	Laxative, anti-helminthic
Hypericin	St. John's wort	Analgesic
Thiosulfonates	Garlic & onions	Anticancer, antimicrobial, reduces blood pressure and cholesterol
Phytosterols (plant sterols)	Peanuts, cashews, almonds, peas, kidney beans & avocados	Anticancer, blocks cholesterol absorption
Capsaicin	Capsicum (hot peppers)	Anticancer, anti-inflammatory, anti-apoptotic
Piperine	Black peppers, jalapeno peppers	Helps in digestion
	Terpenes	
Menthol (Monoterpene)	Plants of mint family	Topical pain reliever & anti-pyretic
Borneol (Monoterpene)	Pine oil	Disinfectant
Santonin (Sesquiterpene)	Wormwood	Photosensitizer
Gossypol (Sesquiterpene)	Cotton	Contraceptive

Determination of total phenolic content

The total phenolic content of the extracts was determined spectrophotometrically (Jasco V-530, USA) according to the Price and Butler method⁴⁵. Tannic acid was used as a standard (linear range 0.01–2.5 mM). The sample (250 µL) was added to 25 mL of deionized water and mixed. After that 3 mL of FeCl₃ was added and, additionally, after 3 min, 3 mL of K₃[Fe(CN)₆]₃ was added. The solution was mixed and incubated at room temperature for 18 min. The absorbance was measured at 720 nm spectrophotometrically.

Extraction and analysis of phenolic acids:

The white and colored varieties of seed coats were extracted with 80% aqueous methanol for 30 min at the ratio of 1:10 (w/v) in a shaking incubator. Solids were separated by centrifugation at 4000 rpm for 15 min and extracted twice longer with the same solution. The combined filtrates were evaporated under vacuum at 40°C to remove the organic solvent, and then the aqueous solutions were lyophilized. Phenolic acids (i.e. free and those liberated from soluble esters and from soluble glycosides) were isolated from methanol extract according to the previously described method^{23,46}. An aqueous suspension of the extract (0.5 g in 20 ml water) was adjusted to pH 2 (6M HCl), and free phenolic acids were extracted 3 times into 20 ml diethyl ether using a separating funnel. The ether extract was evaporated to dryness under vacuum at a room temperature. The dry residue was stored for the HPLC analysis of free phenolic acids. The aqueous solution left after extraction of free phenolic acids was neutralized and evaporated to dryness. The dry residue was dissolved in 20 ml of 2M NaOH and hydrolyzed for 4 h under nitrogen atmosphere at a room temperature. Phenolic acids released from soluble esters were extracted 3 times from the hydrolyzate into 30 ml diethyl ether after acidification to pH 2, using a separating funnel. The dry residue was stored for the HPLC analysis of phenolic acids liberated from esters. Aqueous solution was supplemented with 15 ml of 6M HCl and

subsequently placed under nitrogen atmosphere and hydrolyzed for 1 h in a water bath at 100°C. Phenolic acids released from soluble glycosides were separated from the hydrolyzate 3 times into 45 ml diethyl ether. After ether evaporation, the dry residue was stored for the HPLC analysis of phenolic acids liberated from glycosides. The samples obtained in the above-mentioned manner were redissolved in 10 ml methanol and filtered through a nylon filter (0.45 µm) before the HPLC analysis. Phenolic acids were analysed by the HPLC method using a Shimadzu system (Japan), including a 290 nm-fixed UV SPD-10 A detector, LC-10 AD pump and C-R 6A recorder. A 25 µl sample was injected into an HPLC system. Separation was performed with a LiChrospher 100 RP-18 column (5 µm, 4 × 250 mm) and a water/acetonitrile/ acetic acid mixture (88:10:2, v/v/v) as a mobile phase. Phenolic acids were identified and quantified by comparison with authentic compounds and expressed in µg/g dry matter.

Extraction and analysis of soluble tannins:

Condensed tannins were extracted from seed coats of white and coloured varieties with 70% aqueous acetone for 30 min at the ratio of 1:10 (w/v) in a shaking incubator. After centrifugation, the residues were extracted twice with the same solvent system. The combined filtrates were evaporated under the vacuum and the aqueous solutions were lyophilized. In the obtained samples, condensed tannins were assayed colorimetrically according to the modified vanillin method of Price *et al.*,³⁷ and the 4-(dimethylamino) cinnamaldehyde method as described by Naczki *et al.*,³³. These methods were also used for the determination of tannin content in extracts obtained with 80% aqueous methanol.

Determination of flavonoid content

The determination of the flavonoid content of plants was performed using the colorimetric assay²⁵. At first 50 µL of the skin extract was diluted with 0.5 mL of deionized distilled water and 0.03 mL of 5% NaNO₂ was added. Then 0.06 mL of 10% AlCl₃ and 0.2 mL of 1

M NaOH were added after 5 min and a further 6 min, respectively. Finally, 0.21 mL of deionized distilled water was added. The absorbance was recorded at 510 nm spectrophotometrically. Rutin was used as a standard.

DPPH radical scavenging capability

The free radical scavenging capability of the compounds under investigation was evaluated using a stable free radical DPPH for the decolorization assay^{44,27}. The assay is based on the reduction of DPPH by phenolic compounds and the adsorbance of DPPH radical at 515 nm. To the cuvette 3.9 mL of a DPPH methanolic solution (6.02×10^{-5} M) was transferred and 0.1 mL of the extract was added. The absorbance at 515 nm was recorded at certain time intervals until a steady state of the reaction was reached. The blank reference cuvette contained a 80 :20 mixture of methanol and water. The percentage of the DPPH radical remaining at the steady state was determined by the following equation:

$$\% \text{DPPH} = 1 - A_f/A_o \times 100$$

Where A_f and A_o correspond to the absorbance at 515 nm of the radical at the beginning of the reaction and at the steady state, respectively. The time needed to reach the steady state at an EC50 concentration of the compound (EC50 is the amount of an antioxidant needed to decrease the initial DPPH radical concentration by 50%) was calculated graphically^{44,27}. All the determinations were done in triplicate.

Carotenoids:

The determination of carotenoids was carried out by high-performance liquid chromatography (HPLC) analysis as previously described by Leonardi *et al.*,²⁶. Briefly, 0.1 g of lyophilized sample was extracted with tetrahydrofuran containing 0.01% BHT as the antioxidant agent, dried under nitrogen flow in dark tubes, resuspended in dichloromethane, and analyzed using a HPLC (Shimadzu LC10, Japan) controlled by Class VP software (Shimadzu, Japan) with a diode array detector (SPD-M10A Shimadzu, Japan) and a Prodigy column (5 μm ODS3

100A, 250 X 4.6 mm; Phenomenex, Torrance, CA). The injection volume was 20 μL , and the carotenoids were eluted with a flow of 0.8 mL/min, following this linear gradient: starting condition, 82% A and 18% B; at 20 min, 76% A and 24% B; at 30 min, 58% A and 42% B; at 40 min, 40% A and 60% B; and at 45 min, 82% A and 18% B. Phase A was a mixture of acetonitrile, *n*-hexane, methanol, and dichloromethane (2:1:1:1, v/v/v/v), while phase B was acetonitrile. Identification of the peaks in the HPLC chromatogram of the carotenoid extract was carried out by a comparison of UV-vis spectra or with retention times of eluted compounds with pure standards at 450 nm for R- and β -carotene, β -cryptoxanthin, and lutein, at 350 nm for phytofluene and at 290 nm for phytoene. To quantify phytofluene, phytoene, β -cryptoxanthin, and R-carotene, their respective peak areas were compared to the ones of standard β -carotene at known concentrations, established by the molar extinction coefficient in acetone reported in the literature and corrected by the molar extinction coefficient relative at each compound. The identification of *cis*-carotene isomers was based on spectral characteristics as described by Chen *et al.*,⁷. Because no standards for *cis* isomers are available; the quantification was carried out using the calibration curve of all *trans* isomers.

TAC Determination:

The TAC values were determined as previously described in Pellegrini *et al.*,³⁵. Briefly, raw and cooked samples were homogenized under nitrogen flow in a high-speed blender (Brawn Multimix MX32). A weighed amount (1 g) was extracted with 4 mL of water under agitation for 15 min at room temperature and centrifuged at 1000g for 10 min, and the supernatant was collected. The extraction was repeated with 2 mL of water, and the two supernatants were combined. The pulp residue was re-extracted by the addition of 4 mL of acetone under agitation for 15 min at room temperature and centrifuged at 1000g for 10 min, and the supernatant was collected.

The extraction was repeated with 2 mL of acetone, and the two supernatants were combined. All food extracts were adequately diluted in the appropriate solvent (depending upon their activity) and immediately analyzed in triplicate for their antioxidant capacity by three different TAC assays: Trolox equivalent antioxidant capacity (TEAC) assay^{35,36}, total radical-trapping antioxidant parameter (TRAP) assay¹⁴, and ferric reducing antioxidant power (FRAP) assay^{4,5}. The TEAC and TRAP values were expressed as millimoles of Trolox per 100 g of sample. FRAP values were expressed as millimoles of Fe²⁺ equivalents per 100 g of sample.

Normal phase high performance liquid chromatography (NP-HPLC) separation, identification and quantification of tocopherols:

Procedure: NP-HPLC was selected to avoid extra sample treatment (e.g., saponification) according to Ramadan *et al.*^{39,40}. The analysis was performed with a solvent delivery LC-9A HPLC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250 × 4 mm *i.d.* LiChrospher-Si 60, 5 µm, column (Knauer, Berlin, Germany). The separation of tocopherols was based on isocratic elution when the solvent flow rate was maintained at 1 ml/min at a column back-pressure of about 65–70 bar. The solvent system selected for tocopherols elution was isooctane/ethyl acetate (96:4, v/v) with the detection at 295 nm. Twenty µl of the diluted solution of TL in the selected mobile phase were directly injected into the HPLC column. Tocopherols were identified by comparing their retention times with those of authentic standards.

Preparation of standard curves:

Standard solutions were prepared by serial dilution to the concentration of approximately 5 mg/ml of vitamin E. Standard solutions were prepared daily from the stock solution which was stored in the dark at –20°C. Among of 20

µl was injected and the peaks areas were determined to generate standard curve data.

Quantification:

All quantitation was made by means of the peak areas using Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from six concentration levels by linear regression. Based on the established chromatographic conditions, repeated injections of different concentrations of the tocopherols were made 3-times onto the HPLC system. Injections in triplicates were made at each concentration of both standards and the sample. All work was carried out under subdued light conditions. All the experiments were repeated at least thrice when the variation in any one was routinely less than 5%.

Radical scavenging activity (RSA) of total lipids toward DPPH radical

Different solvents were used to assay the RSA of TL, the best results having been achieved with toluene which was able to dissolve completely the hydrophobic and the hydrophilic compounds^{38,39}. Therefore, the RSA of total lipids and lipid classes was assayed with DPPH radical previously dissolved

in toluene. Toluenic solution of DPPH radicals was freshly prepared at a concentration of 10–4M. For the evaluation, 10 mg of total lipids (in 100 µl toluene) were mixed with 390 µl toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. The decrease in absorption at 515 nm was measured against a blank of pure toluene without DPPH in 1-cm quartz cells after 30, 60 and 120 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control) and the inhibition percent was calculated according to Ramadan and Moersel³⁹ from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample}) / \text{absorbance control}] \times 100$$

Antimicrobial activity

The antimicrobial activities were determined out according to the conventional agar diffusion test¹⁵ using cultures of *Bacillus subtilis* NRRL B-94, *E. coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL, *Staphylococcus aureus* NRRL, *Aspergillus niger* NRRL 313, *Aspergillus flavus* NRC, *Saccharomyces cerevisiae* NRC, and *Candida albicans* NRRL 477. The bacterial strains were cultured on nutrient medium, while the fungi and yeast strains were cultured on malt medium and yeast medium containing 1% Tween 20, respectively. Broth media included the same contents except for agar. For bacteria and yeast, the broth media were incubated for 24 hours. For fungi, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation. For the preparation of the plate and inoculation, 0.5 ml of inocula were added to 50 ml of agar media (50°C) and mixed by simple inversion. Agar was poured into 120 mm Petri dishes and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using paper sterile tubes, then fill wells were filled up to the surface of agar with the tested lipids (20, 40, 60, 80 and 100 µl/well). The microbial growth inhibition zone was measured after incubation at 30°C by the appearance of clear microbial free inhibition zones, beginning within 24 h for yeast, 24–48 h for bacteria and 48–72 h for fungus. Antimicrobial activities were calculated as means of three replicates.

CONCLUSIONS

Scientific evidence has prompted consumers to increasingly opt for low calorie and low fat foods, as well as other foods that hold out the promise of health benefits. Food processors are eagerly adding value to their products based on nutritional information to meet the current consumer demand for healthier food products. These added values include removing or reducing anti-nutritive

components that are present naturally in the food matrix; reducing food components such as fat, caffeine or calories; adding bioactive ingredients that offer health benefits; and increasing the amount of essential nutrients present in food. Various food technologies must work together to achieve the goal of manufacturing healthy foods while at the same time maintaining their sensory qualities. With continuing advances in food technology, coupled with the seemingly unending stream of newly discovered functional ingredients, the sky is the limit for the development of novel food products for health benefits.

There is no doubt that functional foods generate one of the most promising and dynamically developing segments of food industry. There are several factors supporting the inflow of functional products like the increasing consumer awareness in combination with new advances in various scientific domains. The development and commerce of functional food products is rather complex, expensive and risky, as special requirements should be answered. In the case of a successful product development attention should be paid both to consumer demands and technical conditions, furthermore, the legislation background should not be neglected. Especially multinational companies could meet the special requirements occurring during the development and marketing of functional foods. They possess the adequate R&D activities, the know-how and economic potential due to their well known products that give them the opportunity to introduce a brand new product to the market.

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