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Chapter 2

Capsaicinoids and Vitamins in Hot Pepper and Their Role in Disease Therapy

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Abstract

Members of the genus Capsicum (Family: Solanaceae), which belongs to a dicotyledonous group of flowering plants, show fluctuating degrees of spiciness that mirror the relative concentrations of capsaicin, dihydrocapsaicin, and other analogs (nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin) collectively known as capsaicinoids present in the fruit placentas. Pungent Chili varieties are grown for their food value, health-promoting properties and as a source of capsaicinoids that have a variety of medicinal uses. Accessions of the cultivated species (Capsicum annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens) have not all been analyzed for their capsaicinoids content. Identifying Capsicum species and accessions (genotypes) within species with high levels of antioxidants and bioactive compounds (capsaicin, dihydrocapsaicin, vitamin C, vitamin E, phenols, and ß-carotene) that contribute to human disease therapy is the focus of this investigation. The main objectives of this chapter are to compile an overview of most recent achievements of the pharmacological properties of hot pepper compounds and provide a rationale for their use as analgesics and to present evidence that supports the use of capsaicinoids in the treatment of neuropathic pain and other top leading death of worldwide human diseases.

Keywords: capsaicin, dihydrocapsaicin, pungency, cancer, anti-obesity, diabetes, osteoarthritis, pharmacology

1. Introduction

Understanding the nutritional content in human diet could aid in prevention of diseases and malnutrition. Nutritional deficiencies, and their appearing diseases, remain widespread in both the developed and developing world. The enhancement of compounds in foods that...
have health promoting attributes, such as antioxidant properties is the current focus of agricultural practices and the search for healthy food. In consideration of the enormous worldwide consumption of fruits of various Capsicum spp. and the utilization of capsaicinoids as food additives and their current medicinal application in humans warrant a world-wide screening of hot pepper fruits. Identifying Capsicum spp. and accessions (genotypes) within species with high levels of antioxidants (capsaicin, dihydrocapsaicin, vitamin C, vitamin E, phenols, and β-carotene) is a unique way to explore phytochemicals in medicinal plants such as hot peppers. Capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin) are a group of phenolic alkaloids specific to the genus Capsicum [1] and are comprised of a vanillylamine head and a fatty acid tail. Capsaicin is the active ingredient in Chili peppers and the most abundant irritant compound in hot pepper that cause a burning sensation in humans. Dihydrocapsaicin constitutes 22% of total capsaicinoids and is almost similar to capsaicin pungency. Capsaicin (N-vanillyl-8-methyl-6-nonanamide) and dihydrocapsaicin (Figure 1) accounted for about 80–90% of the naturally occurring capsaicinoids in hot peppers [2]. Nordihydrocapsaicin is about 7% of the total capsaicinoids mixture. About 1% of total capsaicinoids is homocapsaicin that has about half the capsaicin pungency. Homodihydrocapsaicin represents about 1% of total capsaicinoids and its pungency is about half of capsaicin pungency. The ratio of capsaicin/dihydrocapsaicin can be 1:1 or 2:1 [3]. The chemical structure of each individual capsaicinoid contains a vanilloid group (an aromatic ring with a hydroxyl and a methyl group), attached a long hydrocarbon chain and a polar amide group [3, 4]. Capsaicinoids show antioxidant properties, potent antimutagenic and anticarcinogenic possessions [5].

The cultivation practices of Capsicum spp., for food production with nutritional composition cover a wide range of natural sciences (physiology, pharmacology, nutrition, agriculture, food industry, and medicine) that support both healthy food and human existence. Among the various plant metabolites that can help protect against free radical damage are phenols (including flavonoids and capsaicinoids), ascorbic acid (vitamin C), carotenoids such as β-carotene (vitamin A), and tocopherol (vitamin E) that are the major antioxidants produced in Capsicum spp. The field of pepper metabolites is rapidly expanding as interest in enhancing plant quality and nutritional composition rises. Several research studies have elucidated how levels of these compounds vary among pepper genotypes and species [6, 7]. The vanilloid group is common among other natural compounds of the so-called vanilloid family, such as vanillin, eugenol, and zingerone that determines the biological activity [3]. Capsaicin and dihydrocapsaicin are the predominant capsaicinoids in the crude pepper fruit extracts, although concentrations of each varied among genotypes. Nordihydrocapsaicin is always present at very low concentrations when compared to capsaicin and dihydrocapsaicin. Concentrations of nordihydrocapsaicin in fruits of C. frutescens averaged 0.1 μg g⁻¹ fresh fruit. Because of this low concentration, few studies and efforts were made by many investigators to quantify nordihydrocapsaicin and other capsaicin analogs in pepper fruit extracts which, in turns directed the screening of pepper genotypes to the two noticeable capsaicinoids, capsaicin and dihydrocapsaicin.
Pepper fruits have antioxidant activity. Antioxidant compounds protect macromolecules from dangerous free radicals, such as reactive oxygen species (ROS). Free radicals are unstable, highly reactive compounds created as the result of normal aerobic metabolism. Reactive species can damage nucleic acids, proteins and lipids, which can hasten aging and the onset of diseases including cancer, heart disease, atherosclerosis, and cataracts [8], if not deactivated. Pepper is an excellent source of antioxidants including flavonoids, capsaicinoids, vitamin C, vitamin E, and carotenoids such as β-carotene. When peppers compared with other vegetables, pepper ranks high for antioxidant activity. Using the ferric reducing antioxidant power assay, researchers found that Chili and red peppers ranked in the top when compared with other common

Figure 1. Molecular structures of five capsaicinoids detected in hot pepper fruit extracts. Note that a double bond (two hydrogen atoms) differentiates capsaicin from dihydrocapsaicin molecule.
vegetables [9, 10]. When antioxidant activity was measured in terms of total radical-trapping antioxidant parameter, Chili and red pepper were two of the top ten sources of antioxidant capacity [10]. Antioxidant capacity variation was also apparent at the genotypic level [11–13]. Pepper usually ranks first or second in terms of phenolic content with levels greater than other high-phenolic vegetables including spinach, broccoli and garlic [14, 15]. Pungent types of peppers have more phenolic compounds than sweet types, an expected result given that pungency is due to capsaicinoids, important phenolic compounds in pepper [13]. Hot pepper can be successfully grown in Kentucky. On a trial basis, several field studies were conducted at Kentucky State University (KSU) College of Agriculture and at the University of Kentucky (Figure 2) to produce hot pepper for industrial uses and as a cash crop for limited resource farmers. Results revealed that yield was sufficient so that we (KSU) are confident that we can produce and develop a hot pepper niche market in Kentucky. *Capsicum* spp. can provide an entrepreneurial niche market for small farmers because these species can be explored as a cash crop and also as a new industry in Kentucky and may provide an opportunity to collaborate with various well known food and pharmaceutical companies for producing hot pepper and extracting capsaicin at low costs.

There is a direct correlation between total capsaicinoids level and pepper pungency. Five levels of pungency are classified using the Scoville Heat Units (SHU). The SHU scale is a measurement of the pungency (spicy heat) of Chili peppers, or other spicy foods, as a function of capsaicin and dihydrocapsaicin concentrations. The SHU can be categorized into: (1) non-pungent (0–700 SHU), (2) mildly pungent (700–3000 SHU), (3) moderately pungent (3000–25,000 SHU), (4) highly pungent (25,000–70,000 SHU) and (5) very highly pungent (>80,000 SHU) [16]. Nordihydrocapsaicin which is a lipophilic colorless odorless crystalline to waxy compound has 9,100,000 SHU (Scoville heat units). Today, the SHU organoleptic test has been replaced by chromatographic methods which are found to be more consistent and accurate compared to the SHU scale that depends on subjective bases (sensory organs). Capsaicinoids content is a major quality factor in spice (Chile and paprika) peppers. Accordingly, variability in the content of capsaicinoids greatly impacts papper pungency and other quality peppers characteristics of interest such as yield, fruit size, fruit color, and shape [17]. The public interest and consumption of pepper is increasing [18]. In addition, growers and food producers have become more interested in developing new crops to meet the increasing demands of trades perceiving food with health promoting properties. There are thousands of different pepper varieties around the world, making the documentation of their variability in composition, plant, and fruit variations challenging.

1.1. Role of capsaicin in disease therapy

Pepper has been described for centuries as a source of compounds with therapeutic properties. In the past decade, many articles reported that capsaicin and dihydrocapsaicin exhibit considerable antioxxygenic activity [19]. Studies carried out using mixtures of 64.5% capsaicin and 32.6% dihydrocapsaicin revealed that capsaicinoids are not carcinogenic in mice experiments [20]. Capsaicin is exempt from the requirement of a tolerance in or on all food commodities when used in accordance with approved label rates and good agricultural practice (USEPA) [21]. The evidence of painkilling properties of capsaicin has led to the discovery of its pharmacological target, the transient receptor potential cation channel subfamily V member 1 (TrpV1), also known as the capsaicin receptor or polymodal receptor of pain. TrpV1, also
known as and the vanilloid receptor 1. Capsaicin has been shown, in vitro and in vivo, to have different biological effects, in addition to its analgesic ones, including anticancer, antiobesity, cardiovascular, urinary, and gastrointestinal effects, due to the large distribution of the target receptors; that is currently representing an active field of research [22].

1.2. Capsaicin and Parkinson’s disease

Parkinson’s disease (PD) is described by the progressive degeneration of nigrostriatal dopamine (DA) neurons, which is associated with motor dysfunctions such as slowness of movement, resting tremor and rigidity. Stimulations by capsaicin rescued nigrostriatal DA neurons, enhanced striatal DA functions and improved behavioral recovery in treated mice.
Capsaicin neuroprotection was associated with reduced expression of proinflammatory cytokines (signaling proteins) and reactive oxygen species/reactive nitrogen species from activated microglia-derived nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. These results suggest that capsaicin and its analogs may be beneficial therapeutic agents for the treatment of PD and other neurodegenerative disorders that are associated with neuroinflammation and glial activation-derived oxidative damage [23].

1.3. Capsaicin and renal disease pain relief

Effective pain relief can be difficult to achieve in patients with end stage renal disease (ESRD), which is the last stage of kidney disease. The active metabolites of most opiates are renal excreted and side effects, such as confusion and drowsiness, are common in patients with renal disease. Qutenza® (8% capsaicin patch) treatment has been presented to be effective and well-tolerated to treat neuropathic pain from critical ischemia (restriction of blood flow to tissues) in patients with ESRD. Qutenza® is an advanced dermal application system designed for rapid delivery of capsaicin through the patient skin. The high concentration of capsaicin results in reversible desensitization of the transient receptor potential channel subfamily V member 1 (TRPV-1), which is involved in detection and regulation of body temperature, expressing cutaneous sensory nerve endings and reduction in nerve fiber density in the epidermis. The resulting pain relief is long-lasting (12 weeks after a single application) [24].

Capsaicin topical route consists of the direct application to the skin, mainly in the form of creams and patches applied on the affected area. After application capsaicin is rapidly absorbed into the epidermis and derma in humans. Management of acute and chronic pain has been recognized and currently constitutes a promising approach for the peripheral neuropathic pain. The oral consumption of capsaicin with food is also safe, but it does not have pharmacologic effects on pain. For these reasons, the majority of studies on the pharmacokinetics of capsaicin in humans have been concerned with topical administration [4].

1.4. Capsaicin and heart disease

Heart disease is the top cause of death in the U.S. due in some cases to blood cholesterol level. HDL stands for high-density lipoproteins known as the “good” cholesterol because it carries cholesterol from all parts of human body back to your liver. The liver then removes the cholesterol from human body. Hart disease inflammation is related to high-density lipoprotein (HDL) cholesterol metabolism. Low levels of HDL cholesterol is associated with an increased risk of coronary heart disease (CHD). Taking 4 mg of capsaicin capsules orally considerably increased fasting serum HDL cholesterol levels and reasonably decreased levels of triglycerides and cholesterol-reactive protein and phospholipid transfer protein activity. In addition, other lipids like apolipoproteins, glucose levels, and other parameters did not significantly change in the human body cycle. In conclusion, capsaicin improved risk factors of CHD in individuals with low HDL-cholesterol and may contribute to the prevention and treatment of CHD [25].
1.5. Capsaicin and cancer

Cancer is still the second leading cause of death in the U.S. and a major cause of illness and mortality worldwide. Cancer cells acquire unique capabilities that most healthy cells do not possess. Cancer cells become resistant to growth-inhibitory signals, proliferate without dependence on growth-stimulatory factors, replicate without limit, escape apoptosis (breakdown of cells) and acquire invasive and angiogenic (creation of new blood vessels) properties. Capsaicin has been shown to alter the expression of several genes involved in cancer cell survival, growth arrest, angiogenesis, and metastasis (spread throughout the body). Recently, it was found that capsaicin targets multiple signaling pathways, oncogenes (genes that contribute to cancerous changes in cells), and tumor suppressor genes in various types of cancer models. Anticancer mechanisms of capsaicin include activation of apoptosis in many different cancer cell lines, while leaving normal cells unharmed. Capsaicin interacts with other cancer preventive agents synergistically, providing the possibility for the potential use of capsaicin in cancer therapy with other chemotherapeutic agents [26].

1.6. Anti-obesity of capsaicin

For individuals suffering from obesity, ingestion of capsaicin or other capsaicinoids increased energy expenditure and decreased respiratory quotient, indicating a rise in fat oxidation. Studies with mean body mass indexes (BMI) gives an indication of person’s nutritional status. While BMI of individuals below 25 kg m$^{-2}$ failed to report any effect of capsaicin or capsaicinoids on the energy expenditure or on the respiratory quotient, studies with mean BMI patients exceeding 25 kg m$^{-2}$ demonstrated an increase in energy expenditure and a marked decrease in respiratory quotient. Data clearly suggest that capsaicin or capsiate could be a new therapeutic approach in obesity promoting an increased fat oxidation [27]. Recent laboratory studies support a role of capsaicin as an anti-obesity agent. Studies in obese/diabetic mice revealed that dietary capsaicin reduced mice metabolic dysregulation by enhancing expression of adiponectin and its receptor. In addition, the effects of capsaicin in fat and liver tissues are related to its dual action on peroxisome proliferator-activated receptor alpha and transient receptor potential vanilloid-1 expression/activation. Capsaicin encourages apoptosis and prevents adipogenesis in adipocytes. Gastrointestinal exposure to capsaicin reduces energy and fat intake. This effect is stronger with oral exposure to capsaicin which indicates a sensory effect of capsaicin. Diets containing capsaicin enhance body weight maintenance following a high caloric diet. Consumption of capsaicin before low intensity exercise has been recommended as a valuable supplement for the treatment of patients with hyperlipidemia and/or obesity due to improvements in lipolysis [28].

1.7. Anti-diabetic effect of capsaicin

Diabetes is a condition in which the body is either impaired or unable to regulate blood glucose levels and involves either improper or an inappropriate reaction to the hormone insulin. Capsaicin in human diets reduces glucose levels and increases insulin levels following its oral administration in glucose tolerance test. Studies determined that capsaicin could be detected in...
the blood as early as 10 min after ingestion at levels maintained for up to 90 min. It was found that capsaicin levels is related to the lower blood glucose levels and preservation of the high insulin levels [29]. Gestational diabetes mellitus (GDM), is a case when a hormone made by the placenta prevents the body from using insulin successfully. This cause glucose level to build up in the blood instead of being absorbed by the cells. GDM may increase the future health risks of women and their offspring. Many women with GDM experience pregnancy-related complications including high blood pressure, large birth weight babies and obstructed labor. The effect of capsaicin supplementation on blood glucose, lipid metabolism and pregnancy outcomes in women with GDM was investigated. Capsaicin-containing Chili supplementation regularly improved postprandial hyperglycemia and hyperinsulinemia as well as fasting lipid metabolic disorders in women with GDM, and decreased the incidence of large-for-gestational-age newborns [30]. Capsaicin may inhibit glucose tolerance by inhibiting adipose tissue (body fat) inflammatory responses in obesity. Ingestion of capsaicin in human diets may reduce obesity due to induced glucose intolerance and enhance fatty acid oxidation in adipose and liver tissues that are important peripheral tissues affecting insulin resistance [31].

1.8. Use of vanilloids in urologic disorders

Urologic disorders are diseases of the kidneys and urinary tract. Neurogenic bladder (NGB) or bladder dysfunction can result from any neurological insult that interferes with the normal functioning of the lower urinary tract which requires intact pathways involving the central and peripheral neurological systems. There are many causes of NGB, but most commonly include spinal cord injury, multiple sclerosis, spina bifida, degenerative spinal disease, cerebrovascular accident and as a result of surgical extirpative procedures that affect peripheral bladder innervation. Vanilloids are compounds that contain vanillyl group such as vanillyl alcohol, vanillin, vanillic acid, homovanillic acid, capsaicin, and capsaicinoids (Figure 1), etc. Vanilloids bind to the transient receptor potential vanilloid type 1 (TRPV1) receptor (an ion channel which respond naturally to noxious stimuli such as high temperature and acidic pH). Capsaicin reduced the number of daily urinary incontinence episodes by 3.8 episodes when compared to a placebo. A noticeable reduction in the use of pads was achieved daily from 10 to 4. Studies conducted to compare capsaicin to a chemical called resiniferatoxin or RTX (the best-known vanilloid from Euphorbia resinifera) showed no difference between treatments with capsaicin and RTX and both compounds decreased the urinary incontinence episodes. The human pelvis include bowel, bladder, womb (uterus) and ovaries. When a person suffers from pelvic pain it is usually means pain that starts from one of the pelvis organs. MacDonald et al. [32] and Foster and Lake [33] reported that, while the use of capsaicin is promising at this time, it appears that RTX is a better treatment.

Urinary frequency or overactive bladder (OAB) is caused by assemblage of symptoms that can be summarized into urinary tract infection, bladder outlet obstruction, bladder cancer, or disorder of the neurologic system (i.e., cerebrovascular accident, spina bifida, Parkinson’s disease, etc.) which can result in a similar clinical presentation. Soontrapa et al. [34] published a report on 25 patients with either OAB or what they described as either a hypersensitive bladder or primary detrusor instability, who were treated with 1 mM of capsaicin diluted in 100 mL of 30% ethanol solution. In those with OAB, it was found that daytime urinary frequency
(16.5–8.6), incontinence episodes per day (9.7–2.4), bladder capacity (160.1–236.9 mL), and detrusor contraction pressure (71.1–57.3 cm H₂O) improved following treatment with capsaicin. Vanilloids have been found to be useful in patients with OAB for reasoning similar to that of neurogenic bladder. Although efficacy has been shown in some studies, currently the use of vanilloids cannot be recommended for routine use in patients with overactive bladder (OAB) [33]. Bladder pain syndrome (BPS) is a condition that falls under a larger category of genitourinary pain syndromes, hypersensitivity, or sensory disorders of the urinary tract. Vanilloid receptors in the bladder modulate activity of sensory neurons. RTX and capsaicin act at this receptor and may potentially offer a viable treatment modality for BPS, though at this time more research is required in this area.

1.9. Capsaicin for osteoarthritis pain

Osteoarthritis (OA) sometimes called degenerative joint disease is the most common joint disorder. It is commonly due to aging. Topical capsaicin treatment four times daily is moderately effective in reducing pain intensity up to 20 weeks regardless of site of application and dose inpatients with at least moderate pain. Capsaicin treatment is also generally well tolerated, suggesting that capsaicin should be used early in the OA treatment process, especially for superficial joints such as the hand and knee that is well tolerated [35].

Research suggests OA is a complex collection of heterogeneous pathologies which result in a common outcome [36], rather than a single disease entity. Most existing treatments focus on relieving pain, with a few examples of improving function. Capsaicin is found to be active in reducing osteoarthritis pain. However, it is unclear whether this activity has a dose response consistent across joints, or this effect changes over time. The most common adverse effect is contained burning sensations of slight to modest intensity associated with capsaicin use, but this burning weakens with ongoing use.

1.10. Research conducted on separation and identification of capsaicinoids in hot pepper genotypes at Kentucky State University (KSU), College of Agriculture (Franklin County, Kentucky, USA)

Studies on screening hot pepper, Capsicum spp. genotypes were started in summer 2006 at Kentucky State University Research and Demonstration Farm. Seeds of 63 previously uncharacterized genotypes of hot pepper were selected from the USDA/Agricultural Research Service (Griffin, GA, USA) to represent the main cultivated species of pepper (Capsicum annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens) from a world-genotypes collection. Seeds were germinated in the greenhouse and transplanted into the field. At harvest, pepper fruits were collected and transported from the field experiment, in coolers, to the KSU Environmental Toxicology Laboratories. Fruits were analyzed and screened for their composition of capsaicin and dihydrocapsaicin using spectrophotometric methods [37]. After 2 years of growing hot pepper, top twenty-nine genotypes (Figure 3) were selected based on their disease tolerance, fruit yield, and fruit size characteristics under Kentucky environmental conditions for further investigation on quantification of capsaicinoids and other phytochemicals having antioxidant properties.
1.11. Quantification of capsaicin, dihydrocapsaicin, ascorbic acid, β-carotene, and phenols in hot pepper

As described earlier, pepper pungency is measured in Scoville units, a subjective scale based on the ratio and capsaicinoids content. It is about 200,000–300,000 Scoville units for habanero and 16 million units for pure capsaicin. The content and the hotness of capsaicin increase during the process of fruit ripening. In general, dried forms of Chili pepper are spicier than fresh fruits, because the lower water content concentrates capsaicinoids. Capsaicinoids were extracted by blending 10 fresh fruit of comparable size in methanol for 1 min. The extracts were then decanted through 55 mm Whatman 934-AH glass microfiber filter discs (Fisher Scientific) and concentrated in a rotary vacuum evaporator (Buchi Rotavapor) at 35°C, chased with nitrogen gas (N₂), and reconstituted in 10 mL of methanol. Each extract of the genotypes tested was subsequently passed through a 0.45 μm GD/X disposable syringe filter. One μL of this filtrate was injected into a gas chromatograph (GC) equipped with a nitrogen phosphorus detector (NPD). GC separations were accomplished using a 25 m × 0.20 mm ID capillary column with 0.33 μm film thickness (HP-1). Operating conditions were 230, 250, and 280°C for injector, oven, and detector, respectively, and a carrier gas (He) flow rate at 5.2 mL min⁻¹. Peak areas were determined using a Hewlett-Packard (HP) model 3396 series II integrator. Quantifications were based on average peak areas of 1 μL injections obtained from external standard solutions of capsaicinoids (capsaicin and dihydrocapsaicin) prepared in methanol. Peak identities were confirmed by consistent retention time and co-elution with standards under the conditions described above. A HP gas chromatograph model 5890A equipped with a mass chromatograph operated in total ion monitoring (GC/MS) with electron impact ionization (EI) mode and

Figure 3. Hot pepper genotypes grown at Kentucky State University HR Benson Research and Demonstration Farm (Franklin County, Kentucky, USA).
70-eV electron energy was also used for identification and confirmation of individual peaks. Standards of capsaicin (N-vanillyl-8-methyl-6-nonenamide) and dihydrocapsaicin were purchased from Sigma-Aldrich Inc. in Saint Louis, MO, USA and used to prepare standard curves using regression lines. To determine the recovery of the extraction, cleanup, and quantification procedure, concentrations of capsaicin and dihydrocapsaicin in the range of 20–200 μg g⁻¹ fresh fruit were added to 20 g of non-pungent bell pepper (C. annuum) fruits. Linearity over the range of concentrations were determined using regression analysis.

Mass spectrometry of the fruit crude extracts (Figure 4) indicated that the molecular ions at m/z 305, 307, and 293 that correspond to capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, respectively, have a common benzyl fragment at m/z 137 that is a fingerprint for monitoring capsaicinoids in pepper fruit extracts. The two capsaicinoids, capsaicin and dihydrocapsaicin, were the main capsaicinoids found in the fruit extracts. However, their concentrations varied among pepper genotypes tested. Nordihydrocapsaicin was present at very low concentrations when compared to capsaicin and dihydrocapsaicin. Capsaicin concentrations were typically greater than dihydrocapsaicin. Concentrations of total capsaicinoids in the 29 genotypes tested varied from not detectable to 11.2 mg fruit⁻¹. Statistical analysis revealed that accession PI-441624 (Capsicum chinense) had the highest capsaicin content (2.9 mg g⁻¹ fresh fruit) and accession PI-497984 (C. frutescens) had the highest dihydrocapsaicin content (2.3 mg g⁻¹ fresh fruit). Other characteristic fragment ion peaks were consistent with the assignment of the molecular formulae of capsaicin (C₁₈H₂₇NO₃), dihydrocapsaicin (C₁₈H₂₉NO₃), and nordihydrocapsaicin (C₁₇H₂₇NO₃), respectively. These capsaicinoids had a common benzyl cation fragment (C₈H₉O₂, m/z 137) that was observed in all hot pepper extracts. The retention time and mass spectra of capsaicinoids isolated from the fruits of Capsicum genotypes matched those of their standards.

### 1.12. Quantification of other antioxidants in hot pepper fruits

Capsicum spp. also contain other medicinal agents, such as vitamins and antioxidants (flavonoids, carotenoids, vitamin C, and vitamin E), that have biological activity as well, such as the role of vitamin C and β-carotene in inflammation and cancer prevention, respectively. Pepper fruits of the twenty-nine genotypes selected, after 2 years of field screening, were cut into small pieces and 30 g representative subsamples were blended in a household blender at high speed with 100 mL of acetone for 2 min in dim light to extract β-carotene [38]. The homogenate were filtered with suction through a Buchner funnel containing Whatman filter paper No.1 (Fisher Scientific, Pittsburg, PA). The resulting thick paste were extracted twice with acetone until the extract is colorless. The filtrates were combined, transferred to separatory funnel containing 50 mL of 4% aqueous NaCl and 100 mL of petroleum ether (BP 40–60°C). Absorption of the petroleum ether layer were measured at 450 nm in dim light. β-carotene was quantified using a high performance liquid chromatograph (HPLC) for confirmation purposes. A calibration curve was also prepared using 99% pure β-carotene in the range of 10–100 μg mL⁻¹. Representative fruit samples (20 g) were also blended with 150 mL of ethanol to extract phenols. Homogenates were filtered through Whatman No. 1 filter paper and 1 mL aliquots of filtrate were used for determination of total phenols [39, 40] using a standard calibration curve (1–16 μg mL⁻¹) of chlorogenic acid. Ascorbic acid (vitamin C) was extracted by blending 20 g of fruit with 100 mL of 0.4% (w/v) oxalic acid solution and determined by the
dichlorophenolindo-phenol method [41]. Purified standards of β-carotene, ascorbic acid, and chlorogenic acid were purchased from Sigma-Aldrich Inc. (Saint Louis, MO, USA) and used to obtain calibration curves. Concentrations of each compound, expressed on a fresh weight basis, was calculated and statistically analyzed using ANOVA procedure. Means were compared using Duncan’s multiple range test [42]. Quantification of the level of capsaicinoids and other antioxidants in the selected genotypes allowed the identification of accessions having high levels of health-promoting phytochemicals and provided a mass balance information for each accession tested in relation to countries of seeds origin (Figure 5). Results of fruit analysis revealed that the concentrations of individual capsaicinoid and the proportion of capsaicin/dihydrocapsaicin fluctuated within and among species and even among genotypes of the same species. Absolute capsaicinoids concentrations are subject to a variety of environmental,
The greatest concentrations of capsaicinoids (1405 μg/g fresh fruits) were found in pepper seeds obtained from Mexico [43]. Among the 63 accessions analyzed, concentrations of total phenols were significantly higher in PI 438648, PI 159248, and PI 360900. Seeds of these accessions are originated in Mexico and the US, respectively. While, total phenols concentration was generally low in fruits of genotypes from Belize.

Statistical analyses of 63 genotypes obtained from the USDA/ARS (Griffin, GA) revealed that the greatest concentrations of β-carotene were found in fruits of accessions obtained from Ecuador. Fruits of _C. chinense_ accessions PI-152452 (Brazil) and PI-360726 (Ecuador) contained the greatest concentrations of vitamin C (1.2 and 1.1 mg g⁻¹ fresh fruit, respectively), while PI-438648 (Mexico) contained the greatest concentration of total phenols content (349 μg g⁻¹ fresh fruit) among the other 63 accessions tested. Accession PI-355817 from Ecuador contained the greatest concentrations of β-carotene (8 mg g⁻¹ fresh fruit). These accessions were identified as potential candidates for mass production of antioxidants with health-promoting properties. When different colored pepper fruits (green, yellow, orange, and red) were analyzed, orange and red contained the greatest β-carotene and sugar contents; whereas, green fruits contained the greatest concentrations of total phenols and vitamin C. Capsaicinoids (capsaicin plus dihydrocapsaicin) were higher in orange and red compared to green and yellow colors [17].

Figure 5. Eight top hot pepper (_Capsicum_ spp.) generating countries of the world indicating 1404 μg capsaicinoids g⁻¹ fresh fruits in fruits of seeds obtained from Mexico and 135 μg g⁻¹ fresh fruit in fruits of plants grown from seeds obtained from Puerto Rico.
In a similar study carried out by Antonious 2017 [44] at the University of Kentucky South Farm (Fayette County, KY), seeds of 29 genotypes were obtained from the USDA/ARS (Griffin, GA, USA). The selected genotypes were: from *Capsicum annuum* (PI 123474, PI 127442, PI 138565, PI 159256, PI 164271; PI 169129, PI 200725, PI 210980, PI 215743, PI 241670, PI 246331, PI 257048); *Capsicum baccatum* (PI 260539, PI 260571, PI 439409); *Capsicum chinense* (PI 209028, PI 224443, PI 238047, PI 238051, PI 257136, PI 439464, PI 594139, PI 485593, PI 439420, PI 281443); and *Capsicum frutescens* (PI 631144). These selected genotypes represented cultivars originally acquired from world-wide different locations. Results of capsaicinoids analysis revealed that PI 631144 of *C. frutescens* contained the greatest concentrations of capsaicin compared to other genotypes tested (Table 1). Concentration of capsaicin and dihydrocapsaicin varied among genotypes and no one genotype contained the greatest concentration of both. Total capsaicinoids varied from 1 μg g\(^{-1}\) (PI 169129; *C. annuum*) to 465 μg g\(^{-1}\) fresh fruit (PI 631144; *C. frutescens*), the greatest capsaicinoids content in the 2017 study. PI 631144 (*C. frutescens* from Guatemala) was identified as potential candidate for the mass production of total capsaicinoids. Concentration of dihydrocapsaicin also varied among genotypes and was lowest in PI 169129 and greatest in PI 123474 and PI 127442 (Table 1 and Figure 6). In most cases, capsaicin concentrations were greater than dihydrocapsaicin as shown in the chromatogram (Figure 4). Concentration of total capsaicinoids (capsaicin and dihydrocapsaicin) was greatest (465 μg g\(^{-1}\) fresh fruits) in PI 631144 and lowest in (1.2 μg g\(^{-1}\) fresh fruits) in PI 169129 compared to all genotypes analyzed. Accordingly, integration of nutrient rich pepper types into diets could help combat nutrient deficiencies by providing a significant portion of recommended daily nutrients [45].

### 1.13. Other pharmacological properties of chili pepper, *Capsicum* spp. fruits

The enhancement of compounds in foods, such as antioxidants in hot pepper (*Capsicum* spp.) is due to the presence of capsaicin, dihydrocapsaicin, vitamin C, vitamin A, vitamin E, and total phenols content. Peppers, an important component of the human diet in many regions of the world, protect macromolecules from dangerous free radicals, i.e., reactive oxygen species (ROS). Free radicals are unstable, highly reactive compounds produced as the result of normal aerobic metabolism. They can damage nucleic acids, proteins and lipids, which can hasten aging and the onset of diseases including cancer, heart disease, atherosclerosis, and cataracts. When antioxidant activity was measured in terms of total radical-trapping antioxidant parameter, Chili and red pepper were two of the top ten sources of antioxidant capacity [10]. Pepper usually ranks first or second in terms of phenolic content with levels greater than other high-phenolic vegetables including spinach, broccoli and garlic [15, 46]. Pungent types had more phenolic compounds than sweet types, an expected result given that pungency is due to capsaicinoids, important phenolic compounds in pepper.

Chili pepper constitutes one of most consumed spices in the world. Capsaicin and vitamin C content in hot pepper determine chili pepper quality on the international market [47] as two important antioxidants in food. Antioxidants could be organic or inorganic compounds, either present naturally or synthesized industrially. When antioxidants added to a formulation even in minute amounts, they tend to neutralize free radicals, preventing the cells from potential damage which in turn cure numerous diseases [48]. Antioxidants are also useful as dietary supplements for sustaining health, prevention of diseases reducing the adverse effects of chemo-and radio-therapy [49]. Antioxidants can be classified on the basis of their
origin, solubility (i.e., aqueous or lipid). Ascorbic acid (vitamin C), α-tocopherol (vitamin E), β-carotene (vitamin A) and capsaicin have been documented in hot pepper as natural antioxidants [50, 51]. Recent findings have demonstrated the antibacterial, anti-inflammatory, anticancer, antimutagenic properties, and blood glucose regulation [52] of various natural antioxidants. In addition to their natural resources, antioxidants can be synthesized and

<table>
<thead>
<tr>
<th>PI</th>
<th>Capsaicin (A)</th>
<th>Dihydrocapsaicin (B)</th>
<th>Total (A + B)</th>
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<tbody>
<tr>
<td>PI123474</td>
<td>170.61 ± 23</td>
<td>203.41 ± 25</td>
<td>374.02 ± 45</td>
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<td>172.73 ± 15</td>
<td>68.1 ± 11</td>
<td>240.83 ± 22</td>
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<td>39.1 ± 9.2</td>
<td>134.63 ± 33</td>
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<td>PI200725</td>
<td>29.31 ± 5</td>
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<td>46.38 ± 12</td>
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<td>17.03 ± 2.5</td>
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<td>10.62 ± 2.4</td>
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</tbody>
</table>

*Each value in the table is an average of three replicates ± std. error.

Table 1. Capsaicin and dihydrocapsaicin in hot pepper genotypes of twenty-nine Capsicum spp. grown at Kentucky State University South Farm (Fayette County, Kentucky).
added to food and pharmaceutical products to improve the shelf-life of therapeutic agents that are susceptible to chemical degradation by oxidation. However, preclinical and clinical reports suggest that synthetic antioxidants cannot afford appropriate protection against oxidative stress [53].

1.14. Role of β-carotene (vitamin A) in hot pepper in disease therapy

Vitamin A deficiency is a serious and widespread public health problem and the leading cause of preventable blindness in young children. Deficiency of vitamin A can lead to a series of ocular symptoms, anemia, and weak resistance to infection, which can increase the severity of infectious diseases and the risk of death [54]. Deficiency of vitamin-A impacts hundreds of millions of pre-school age children in low income countries. Fruits of pepper (C. annuum L.) can be a major dietary source of precursors to vitamin A biosynthesis, such as β-carotene [55]. Antioxidants such as β-carotene are suggested to decrease risk of type 2 diabetes by preventing progressive impairment of pancreatic β-cell and endothelial function. A study was conducted to investigate the relationship between dietary antioxidants and risk of type 2 diabetes in 24,377 adults (19–74 years old) [56]. Men in the highest quartile of β-carotene intake showed lower risk of type 2 diabetes. Peppers are an important source of nutrients in human diet. C. annuum

Figure 6. Fluctuations in total concentrations of capsaicinoids (capsaicin and dihydrocapsaicin) in fresh fruits of Capsicum spp. grown at Kentucky State University HR Benson Research and Demonstration Farm (Franklin County, Kentucky).
is characterized by its high levels of vitamin C (ascorbic acid), and provitamin A (carotene). Ingestion of 50–100 g fresh pepper fruits can provide about 100 and 60% of the recommended daily amounts of vitamin C and A, respectively. Ripe fruits of pepper are also rich in compounds with antioxidant and anticancer action [57]. In addition, vitamin A deficiency was associated with a 10-fold increase in risk of tuberculosis [58].

1.15. Role of ascorbic acid (vitamin C) in disease therapy

The presence of pus-forming bacteria or their toxins in the blood or tissues is known as “sepsis” due to the body response to an infection by harmful bacteria and their toxins that enter the body through a wound. Without appropriate treatment, sepsis can rapidly damage human tissue, cause organ failure, and sometimes death. Sepsis is affecting approximately 26 million people worldwide every year [59]. Vitamin C is found effective in mediating inflammation through its antioxidant properties and is also important in the synthesis of cortisol, catecholamines, and vasopressin, that are all key mediators in the disease development. Investigators provided data that revealed the effect of vitamin C therapy to ameliorate the effects of inflammation and improve hemodynamic stability in patients with sepsis. However, further evidence is needed to support this practice. According to Teng et al. [59], vitamin C is a cost-effective therapy that can be used to ameliorate the effects of inflammation and oxidative stress in sepsis. Although classical vitamin C deficiency marked by scurvy is rare in most parts of the world, some research has shown variable heart disease risks on plasma vitamin C concentration, even within the normal range. Studies have also proposed possible heart-related assistances when vitamin C is taken. It is well established that vitamin C inhibits oxidation of LDL-protein, thereby reducing atherosclerosis [60]. Vitamin C has been found effective in enhancements in lipid contours such as, arterial stiffness and endothelial function. Research findings indicated vitamin C deficiency is linked with a higher risk of cardiovascular diseases and mortality and that vitamin C may slightly improve endothelial function and lipid profiles in some patients, particularly patients with low plasma vitamin C levels.

At Kentucky State University Environmental Toxicology Laboratories, screening 63 hot pepper genotypes of worldwide collection revealed that hot pepper is a rich source of vitamin C. Concentrations of the analyzed phytochemicals in hot pepper genotypes varied significantly among accessions from the same country of origin, and between countries of origin. Concentrations of vitamin C in two accessions, PI 152452 (Brazil) and PI 360726 (Ecuador), were significantly higher (1224 and 1139 μg g\(^{-1}\) fresh fruit, respectively) compared to other genotypes analyzed. These two genotypes may be useful as parents in hybridization programs to produce high vitamin C containing varieties. On the contrary, PI 281424 from Peru contained the lowest vitamin C concentration (266 μg g\(^{-1}\) fresh fruit) [43].

1.16. Role of tocopherols (vitamin E) in hot pepper in disease therapy

Tocopherols are among the most important lipid-soluble antioxidants in food and in human and animal tissues. There are different types of vitamin E (tocopherol), the most found are α, β, γ, δ, ζ, and η tocopherols. α-Tocopherol in Capsicum spp. is mainly found in the pericarp and γ-tocopherol is a specific constituent in the pepper seeds [18]. All studied
C. pubescens pepper accessions contained α-tocopherol at levels between 6.8 and 18.4 mg 100 g\(^{-1}\). β-Tocopherol was found in only a few pepper accessions at trace levels reaching 0.2 mg 100 g\(^{-1}\) at maximum [18]. Peppers are rich in antioxidants, including carotenoids, capsaicinoids, and tocopherols [61].

Waniek et al. [62] reported that vitamin E levels in foods might protect from gallstone disease (gallstone disease is defined as gallbladder stones visualized at the ultrasound examination). Participants with gallstones had lower circulating α-tocopherol and α-tocopherol/cholesterol ratio levels compared to participants without gallstones. A total of 44 participants (7.6\%) of a study were taking vitamin E supplements. The results revealed that higher levels of the antioxidant α-tocopherol may protect against gallstone disease. More investigation in this important disease is needed to prevent the incidence of gallstone. Nonalcoholic fatty liver disease (NAFLD) is defined as the accumulation of excessive fat in the liver. NAFLD is strongly associated with obesity and related metabolic disorders such as insulin resistance and dyslipidemia. Like other fat-soluble vitamins, the bioavailability of vitamin E depends on pancreatic function, biliary secretion, micellar formation, and penetration across intestinal membranes. In the last decades, adult and childhood obesity has reached epidemic levels, and as a consequence the global incidence of NAFLD has increased significantly. According to a recent report, prevalence estimates in the general population of Europe and the Middle East are 20–30\%, with higher prevalence in Western countries’ populations with obesity or diabetes (75\%) and with morbid obesity (90–95\%) [63]. Oxidative stress is defined as the imbalance between the generation of reactive species and antioxidant defense, and leads to the damage of DNA and disturbances in cellular biology. The antioxidant property of vitamin E is attributed to the hydroxyl group from the aromatic ring of tocochromanols, which donates hydrogen to neutralize free radicals or reactive oxygen species (ROS). Vitamin E is widely accepted as one of the most potent antioxidants in nature [64]. Vitamin E biological activity is not limited to antioxidant properties. In fact, vitamin E forms are involved in the regulation of inflammatory response, gene expression, membrane-bound enzymes, modulation of cellular signaling, and cell proliferation.

1.17. Role of total phenols in hot pepper in disease therapy

About 8000 different classes of polyphenols are found as secondary products in plants. The most important are flavonols, flavones, flavan-3-ols, flavanones and anthocyanins and the most commonly occurring polyphenols in food include flavonoids and phenolic acids. Dietary polyphenols have shown a substantial evidence in vitro that they can affect numerous cellular processes like, gene expression, apoptosis, platelet aggregation, intercellular signaling, causing anticarcinogenic and antiatherogenic implication [65]. Polyphenols in Capsicum spp. also possess antioxidant, anti-inflammatory, anti-microbial, cardioprotective activities and play a role in the prevention of neurodegenerative diseases and diabetes mellitus [66]. Polyphenols are mostly acknowledged for their antioxidant activities on the basis of their structural chemistry. They have been recognized as more effective antioxidants in vitro than vitamins E and C on a molar basis. Recent research reveals that dietary spices in minute quantities has an immense influence on the human health by their antioxidative, chemopreventive,
antimutagenic, anti-inflammatory, immune modulatory effects on cells and a wide range of beneficial effects on human health by the action of gastrointestinal, cardiovascular, respiratory, metabolic, reproductive, neural and other systems [67].

1.18. FDA regulations regarding capsaicin in human therapeutic application and development

The repurposing of existing drugs that have been permitted by the Food and Drug Administration (FDA) for human therapy is a continuous strategy for drug development [68]. Drugs that are likely to have low risks and known mechanisms of action after methodically and extensively screened for safety, are usually FDA approved. This is because many pharmaceuticals have much broader ranges of action and application than their certificates recommend. Accordingly, chemicals in the FDA inventory of approved drugs can be used again and again in different purposes when the safety of these drugs in humans is well-known. Phytochemicals found in various plants are frequently included in the human diet that are commonly assumed to be safe for consumption since they are created naturally. However, there are some exceptions and in fact many natural compounds found in several commonly consumed plants can be toxic and may cause cancer or can be tumor promoters and should be evaded. In the United States most phytochemicals are not under regulation by the FDA and their possible toxicity is understudied [69]. One of the most controversial phytochemical is capsaicin. In spite of being a well-studied phytochemical, capsaicin epidemiologic studies revealed that ingesting of hot peppers, which contain variable levels of capsaicin, might be connected with cancer, mostly of the gallbladder [70]. Thus, a complete consensus as to whether the primary effect of capsaicin is cancer anticipation or elevation has not yet been reached with complete evidence.

Conflicting epidemiologic and basic research studies propose that capsaicin could have a role in either preventing cancer or causing cancer. Hundreds of basic research studies show that capsaicin suppresses growth of numerous types of cancer cell, suggesting that it has chemopreventive activities and these studies have been well reviewed [69]. Capsaicin formulations in the form of creams have been in use for many years to relieve painful conditions such as arthritis, osteoarthritis, and diabetic neuropathy. Results of the effectiveness and safety of capsaicin use in pain relief is controversial. Prolonged use of capsaicin in topical treatment in patients exposed to sunlight and its ultraviolet radiation should be more investigated. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage [71]. There are two common types of pain, acute pain and chronic pain. Basically, acute pain is crucial in alerting an individual to withdraw from a harmful situation while chronic pain could constitute of serious, separate disease entity. Formulations of capsaicin in the form of patches containing capsaicin in the range of 0.025–0.1% by weight are currently available in many countries around the world in drug stores out of the counter, without the requirement of a prescription, for the controlling neuropathic and musculoskeletal pain. Clinical studies involving 3–5 topical skin applications per day for periods of 2–6 weeks have been recommended for use against various pain syndromes, including osteoarthritis, postherpetic neuralgia, and painful diabetic neuropathy [72]. Accordingly, with the FDA and
European Medicines Agency (EMA) approvals of Qutenza® , a single-use high concentration topical capsaicin formulation for the management of peripheral neuropathic pain is approved on the basis of safety and effectiveness by FDA under sections 505 of the Federal Food, Drug, and Cosmetic Act in 2016 [73]. However, published articles from case studies revealed that a high dose of capsicum can cause tissue inflammation, which can trigger an inflammatory response such as anaphylactic shock defined as an allergic reaction to an antigen to which the body has become hypersensitive [74] and gastrointestinal tract irritation that occur when people overeat Chili peppers [74, 75]. Symptoms caused by ingesting too much capsaicin also include severe burning mouth sensation, mouth pain, itchiness, vomiting, nausea and diarrhea [76]. It could be concluded that customers of Chili pepper are at higher risk for gastric cancer, anticarcinogenic, and antimutagenic. Capsaicin in pepper fruits has dual effects. When consumed at minute amounts, capsaicin shows few or no poisonous effects, but substantial ingestion of capsaicin has been linked with necrosis, ulceration and carcinogenesis. This is why some scientists in 2011 [77] called the capsaicin molecule “two-faces-molecule” Capsaicin has elicited enormous interest for several centuries due to its conspicuous culinary and clinical applications. Despite its adverse effects, capsaicin is still being used as an active principle in several pharmaceutical formulations for treating various human ailments [78].

### 1.19. Pepper cultivation, pesticide application, and fruit storage

In Kentucky, peppers are grown primarily for the fresh market. Lying pepper fields close to creeks and rivers is subject to high humidity and moisture conditions that result in serious disease risks such as bacterial leaf-spot disease. Poorly drained soils and some herbicides such as atrazine that may have been used in previous seasons should be avoided. This is because herbicide carryover can cause serious injury to pepper plants. Soils high in N content should also be avoided to prevent pepper plants from producing excessive foliage at the expense of fruit production. Growers should also plow (rototill) the soil 8–10 inches (20.5–25.4 cm) deep several weeks in advance of the transplanting date. Seed should be treated with chlorine bleach by the grower to help reduce seed transmission of bacterial leaf spot. Two rows of peppers spaced 15 inches (38 cm) apart are planted on each bed; plants are spaced 12–15 inches (about 20–25 cm) apart within each row. The beds are usually 5–6 feet (1.5–1.8 m) from center to center (approximately 14,500 plants acre−1) considering that 1 acre is equal to 0.405 hectare) using trickle irrigation and plastic mulch cover. Several pesticides are permitted for use in growing peppers for commercial crop production in Kentucky agriculture. The most common are the insecticide dimethoate 4E formulation that requires 48 re-entry hours following spraying to reach safe residue levels, the fungicide chlorothalonil that requires 12 re-entry hours after spraying, and the herbicide command 3ME [79]. After harvest, pepper fruits should be stored at 45–50°F in cooler as soon as possible, cool rooms with forced-air equipment will extend fruit shelf life. Once fruits are precooled, growers hold them at 45–50°F with 90–95% relative humidity. When pepper fruits are stored at temperatures below 40°F, chilling fruit injury symptoms appear. Chilling injury are browning at the calyx end and surface pitting. Fungal and bacterial diseases are common in pepper production, and most spraying programs target bacterial leaf spot, anthracnose, and Phytophthora blight. Mefenoxam (the active ingredient in the fungicide Ridomil Gold®) should be repeated 30–60 days after transplanting [79, 80].
1.20. Capsaicin toxicological data

In environmental toxicology, a general measure of acute toxicity of a chemical is its lethal dose (LD$_{50}$) or lethal concentration (LC$_{50}$) of that chemical that causes significant toxicity or death to 50% of that living organism resulted from a single or limited exposure of the treated animals. LD$_{50}$ is generally expressed as the dose of a chemical in milligrams (mg) kilogram (kg) of body weight. Whereas, LC$_{50}$ is often expressed as mg of chemical per volume e.g. liter (L) of air or water that the living organism is exposed to. Toxicological studies revealed that capsaicin acute oral LD$_{50}$ values are in the range of 97.4 mg kg$^{-1}$ and 118.8 mg kg$^{-1}$ in female and male mice, respectively. LC$_{50}$ values of capsaicin are in the range of 148.1 mg kg$^{-1}$ and 161.2 mg kg$^{-1}$ in female and male rats, respectively [81]. Male mice exposed to capsaicin in their stomach, revealed an average lethal dose (LD$_{50}$) of 68 mg kg$^{-1}$ [82]. Exposure to capsaicin can cause several dose-dependent acute physical responses such as feeling of burning and pain, respiratory depression, and occasionally death [83]. Cytochrome P$_{450}$ is a group of enzymes found mainly in the liver cells and also in other cells in the animal body. These enzymes are responsible for drug metabolism. Metabolism of capsaicin by cytochrome P$_{450}$ decreased its toxicity to lung and liver cells. When the metabolism of two capsaicinoids, capsaicin and nonivamide (a capsaicin analogue) was investigated, the results demonstrated similar pathways in the cytochrome P$_{450}$ dependent metabolism. Cytotoxicity was enhanced 5 and 40% for both compounds by 1-ABT in BEAS-2B (human lung epithelial cell line) and HepG2 (human liver cancer cell line), respectively [83]. These observed results proposed that metabolism of capsaicinoids by cytochrome P$_{450}$ in cells denoted a detoxification mode of action. Generally, when the LD$_{50}$/LC$_{50}$ ratio is small, the toxicity of a chemical is high and when this ratio is high, it indicates that a chemical might be practically slightly toxic or non-toxic. It is also important to mention here that LD$_{50}$/LC$_{50}$ ratio could not be used to predict an organism long-term exposure to diseases, such as cancer [84].

2. Conclusion

Capsaicin, and its analogs, is an affordable inexpensive and effective therapeutic molecule present in fresh and dry fruits of Capsicum spp. The addition of this compound to human diet at minute amounts has the potential of curing several diseases. Natural capsaicinoids in hot peppers could be obtained by growing pepper in home gardens on small-scale and could be produced on large-scale (Figure 7) for industrial purposes. Some pepper fruit types were found to have high levels of antioxidants such as vitamin A, phenols, and vitamin C [50]. Information and correlation between pepper nutrient content, species, genotypes of the same species, cultivation practices, and geographic regions are limited. Selecting pepper genotypes for plant breeding programs provide a management tool to produce fruits with high levels of nutrient content. Incorporation of nutrient rich pepper genotypes that contain high levels on antioxidants into human diets could help combat nutrient deficiencies by providing the needs of recommended daily nutrients [45]. Capsaicin, the main pungent ingredient in ‘hot’ Chili peppers, causes a sensation of burning pain, mechanical or thermal stimuli by selectively activating sensory neurons in humans that transport information about harmful stimuli to
the central nervous system. When consuming peppers that contain capsaicin, capsaicin binds with the pain receptors in the mouth and throat releasing the pain sensation. In spite of all the outlined medicinal positive and negative properties of capsaicin, the efficacy of capsaicin in the treatment of chronic pain disorders is still indefinite. In topical capsaicin application, capsaicin employs its therapeutic action by the desensitization process and continued usage of topical capsaicin may lead to persistent desensitization [85]. According to the FDA Qutenza patch, a pure synthetic capsaicin-containing prescription drug, may cause a significant rise in blood pressure [86]. Capsaicin is associated with some severe side effects such as capsaicin-induced dermal pain and contact dermatitis [77]. In addition, there is a lack of information on the effectiveness of capsaicin on post herpetic neuralgia (PHN) that affects nerve fibers and skin, causing burning pain that lasts long after the rash and blisters of shingles disappear [85]. Capsaicin might be associated with an increased risk of cancer, especially gallbladder [70] and stomach cancer [87].

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