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Exemplified screening standardization of potent antioxidant nutraceuticals by principles of design of experiments

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ABSTRACT

Standardization is an essential step in the development of nutraceutical based formulations or products. The objective of the present study was to exemplify screening standardization of the potent antioxidant nutraceuticals (generic), namely curcumin, quercetin, hesperidin, gallic acid, citric acid, L-ascorbic acid, phytic acid and α-tocopherol based on ferric reducing antioxidant power (FRAP) value and also tried to justify the reliability of two different designs of experiments i.e. Plackett–Burman fold-over design and Taguchi L_{18} in screening related experiments. First screening of antioxidant nutraceutical was performed by Plackett–Burman fold-over design (resolution IV) and the outcome was analysed based on Pareto chart of standardized effect where the quercetin, gallic acid and curcumin have shown p value of <0.05 and the same factors were found to be positive outliner in half normal probability analysis. Further, the results were validated using factorial Taguchi orthogonal array, based on SN ratio (maximum the best response). Both statistical designs have given the same order of potency based on FRAP value as: quercetin > gallic acid > curcumin. Therefore it can be concluded that the statistical design of experiments is reliable in screening related experiments for development of nutraceutical formulations or products.

1. Introduction

Nutraceuticals and functional foods are important for health promotion and disease management. Functional foods resemble traditional foods but possess marked physiological benefits and safety (Shahidi, 2012). Nutraceuticals are diet supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods (Zeisel, 1999). Nutraceuticals can be grouped into different categories like (i) Nutrient food constitutes: carbohydrates, minerals, amino acids and vitamins; (ii) Dietary supplements that contain formulation or mixture of nutrient constitutes like minerals, amino acids, fats and vitamins, among others; (iii) Non-toxic secondary metabolites obtained from plants or herbs, which have proven health benefits and; (iv) Herbal extracts or traditional

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Chemical compounds: Curcumin (PubChem CID: 2889); Quercetin (PubChem CID: 5280459); Hesperidin (PubChem CID: 6419939); Gallic acid (PubChem CID: 370); L-Ascorbic acid (PubChem CID: 5176753); Phytic acid (PubChem CID: 890); 2, 4, 6-Tripyridyl-S-trizaine (PubChem CID: 77258); Sodium acetate trihydrate (PubChem CID: 23665404); α-Tocopherol (PubChem CID: 14985); Citric acid (PubChem CID: 22230).

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Chinese, Ayurvedic or other traditional medicines which are recognized by human civilization (Brower, 1998). Nutraceuticals that are present in the diet, and have been associated with health benefits, include glucosinolates, sulphur-containing (allicin) compounds, carotenoids, monoterpenes, phytosterols and various groups of polyphenols (anthocyanins, flavones, flavan-3-ols, isoflavones, stilbenoids, ellagic acid). Some of the plant derived nutrient and non-nutrient secondary metabolites have been shown to exhibit a wide range of biological activities. These metabolites when ingested regularly and in significant amounts as part of the diet may have a noticeable long-term physiological effect. Their bioactivity has been, to some extent, associated with their antioxidant properties (capacity to scavenge free-radicals). Free radicals are involved in the onset of many chronic degenerative diseases like atheroma plaque development, cancer, ageing and inflammation, among others (Espín, García-Conesa, & Tomás-Barberán, 2007).

According to FICCI and FROST reports (2010) the global nutraceutical market has seen maximum growth in the last decade. While, nutraceuticals as an industry emerged in the early 1990s, however, 2002–2010 has been the key growth period for this industry. From 1999 to 2002, the nutraceutical industry grew at an annual average growth rate (AAGR) of 7.3%, while from 2002 to 2010, the AAGR doubled to 14.7%. The industry is expected to maintain comparable growth till 2015 driven by growth from India, China and Brazil. In 2010, the US nutraceutical market stood at US $ 50.4 billion and was by far the largest nutraceutical market in the world. The total European nutraceutical industry was valued at US $ 35 billion. The Indian nutraceutical industry was estimated at US $ 2 billion, roughly 1.5 per cent of the global nutraceutical industry.

A large percentage of nutraceuticals and functional foods are herbal-based products. Nutraceutical development involves major steps as shown in Fig. 1, starting with pre-processing (Step 1) e.g., raw material harvesting, storage and evaluation for Herbal Pharmacopoeia standards. The pre-processing ensures that the active ingredients are maintained before processing. The processing is a critical aspect of nutraceutical production, especially for low yield of extracts (Aziz, Kumaresan, Taher, & Yee, 2003) in order to standardize percentage yield of nutraceuticals, the common extraction methods used include high pressure water extraction, two stage supercritical fluid extraction (Sánchez-Camargo et al., 2014), pressurized low polarity water extraction, membrane separation, distillation, dehydration, and other relevant bioprocessing techniques (Step 2). Further the nutraceutical products are purified by various chromatographic methods such as RP-HPLC, ion exchange chromatography, affinity chromatography, hydrophobic charge-induction chromatography (HCIC) (Soni, Trivedi, & Madamwar, 2008) and HPLC-tandem mass spectrometry (HPLC-DAD-ESI-MS), among others (Chandrasekara & Shahidi, 2011), to ensure a high degree of purity and potency of the product (Step 3). Improved formulation design enhances physicochemical and physiological properties of nutraceuticals (Braithwaite et al., 2014). Augmented physicochemical properties of formulation have extended shelf life in a variety of forms, such as tablets, capsules, bags and extracts. These nutraceuticals are used as is or as a formulation/fortified form (Step 4), followed by packaging (Step 5).

The design of experiments (DOE) refers to the process of planning, designing and analysing the experiment so that valid and objective conclusions can be drawn effectively and efficiently. In order to draw statistically sound conclusions from the experiment, it is necessary to integrate simple and powerful statistical methods into the experimental design methodology. DOE is a well-established and proven statistical method which has broad application across many disciplines and industries. Typically there are levels of designs which can

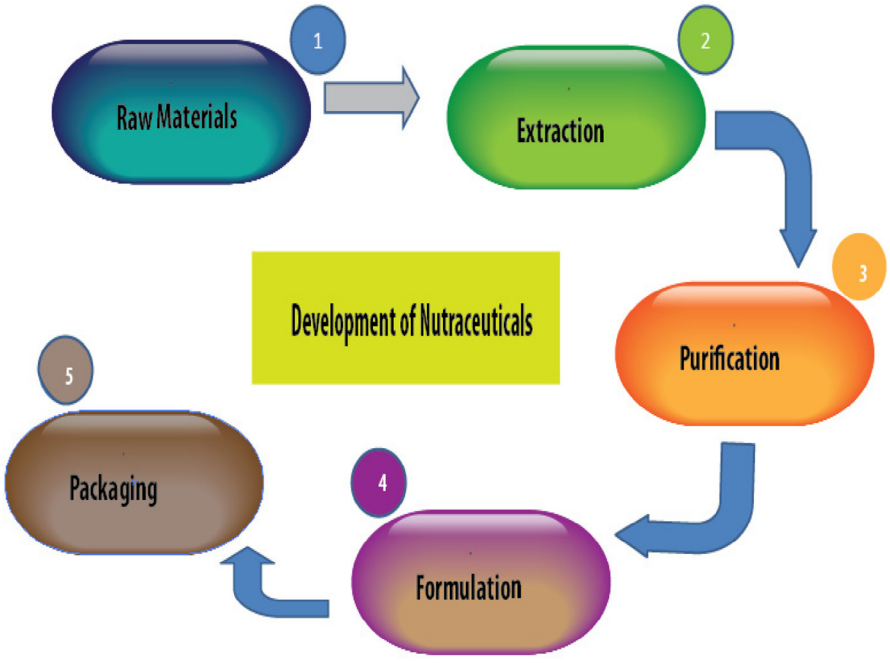

Fig. 1 – Critical processing stages of nutraceuticals.