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Shelf-Life Evaluation of a Novel Functional Product from a Blend of Powdered Vegetables

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ABSTRACT

Perishable fresh vegetables that do not meet cosmetic standards and other crop waste rich resources are presently wasted. *Mangifera indica* leaves, *Psidium guajava* leaves, *Petroselinum crispum* leaves and *Daucus carota* were selected as model vegetables to show that they can be converted into a shelf-stable novel functional powdered product. A novel functional product from a blend of these powdered vegetables (FPPV) was formulated. To evaluate the shelf-life of FPPV. The novel functional product from a blend of powdered vegetables (FPPV) was prepared in the Food Science and Nutrition laboratory, Sam Higginbottom University of Agriculture Technology and Sciences, India. The samples were subjected to accelerated stability study maintaining temperature and relative humidity 40°C ± 2°C and 75% ± 5% respectively. Organoleptic, physico-chemical and microbiological properties of FPPV was analyzed at an interval of 0, 1, 3 and 6 months to check the degradation levels in the formulation. Organoleptic characters showed no significant changes in accelerated stability condition. There were insignificant changes in physico-chemical profiles and product was free from microbial contamination at different intervals of analysis. On extrapolation of the observations the shelf-life of FPPV was found to be 51 months (4 years and 3 months) for climatic zone I & II countries and 34 months (2 years and 10 months) for climatic zone III & IV countries, respectively. The conversion of perishable vegetables and crop wastes into shelf-stable functional food products will reduce food loss and waste in the vegetable industry.

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Introduction

Shelf-life of a food product is the finite length of time, following manufacture and packaging during which it maintains the necessary degree of quality to be consumed. Shelf-life is a function of time, environmental factors and susceptibility of product to quality changes (Labuza et al., 2008). Physico-chemical and biological changes that take place in the food chain are involved in product deterioration. In general, the effect of these changes might compromise several aspects of foods such as nutritional, microbiological and sensory quality. The Food and Agriculture Organization of the United Nations (FAO) estimates that 32% of food produced worldwide is generally lost or wasted. Global quantitative food losses and waste per year are roughly 30% for cereals, 40–50% for root crops, fruits and vegetables, 20% for oil seeds, meat and dairy and 35% for fish (FAO, 2019). Furthermore, throwing food away implicates economic and environmental issues (Stuart, 2009). Therefore, the possibility of using these wastes to obtain functional ingredients for the food industry could represent a substantial step towards maintaining environmental balance (Papargyropoulou et al., 2014; Difonzo et al., 2019; Ying et al., 2021).

The loss and waste of fruit and vegetable is a serious problem. Fruits and vegetables are a rich source of micronutrients (vitamins, minerals), macronutrients (fibre, protein, carbohydrates, fats), and phytochemicals (carotenoids, polyphenols, flavonoids, etc.) (Li et al., 2021). Crop wastes and fresh produce that does not meet cosmetic standards and may be recovered and manufactured into a variety of value-added nutritional and functional food products. This allows the high content of desirable nutrients and phytonutrients in fruits and vegetables to be captured, allowing their useful return to

the human food supply (Augustin et al., 2020; Kowalska et al., 2017). Fruits and vegetables are highly perishable in nature, hence the conversion of produce that would otherwise be lost or wasted into shelf-stable functional ingredients and products is necessary. Their conversion into powders is a simple way to help preserve the nutritional components in fruits and vegetables (Jiang et al., 2013; Ying et al., 2021).

A healthy diet must include an adequate amount of fruits and vegetables to lower the risk of disease (Wallace et al., 2020). Mango leaves, guava leaves, parsley leaves and carrots were chosen as these vegetables were of interest to the Indian horticulture industry. Mango (*Mangifera indica*) leaves is an important source of mangiferin, phenolic, flavonoids, benzophenones and antioxidants with free radical scavenging activity. These bioactive compounds has been linked to biological activities including anti-inflammatory, antioxidant, antidiabetic and others (Medina Ramírez et al., 2016; Pan et al., 2018; Ribeiro and Schieber, 2010; Ribeiro et al., 2008; Zhang et al., 2011). Guava leaves have been used as a folk medicine or herbal tea to treat diarrhea (Morais-Braga et al., 2016) and diabetes (Oh et al., 2005; Ojewole, 2006) in India, China, Pakistan, Bangladesh, and Mexico for a long time owing to lower toxicity and good therapeutic function (Gutiérrez et al., 2008; Kaneko et al., 2013). Díaz-De-Cerio et al. confirmed that the hypoglycemic effects of guava-leaf ethanolic extract were associated with improving endothelial dysfunction in obesity mice (Elixabet et al., 2017). Shen et al., (2008) confirmed that aqueous soluble extract from guava leaves has antihyperglycemic function against type 2 diabetes. Previous studies were focused on flavonoids and phenolic compounds extracted from guava leaves (Luo et al., 2019). Carrots (*Daucus carota*) are high in fibre and contain phytonutrients including carotenoids and phenolic compounds (Arscott et al., 2010) and rich in other natural bioactive compounds, which are recognized for their nutraceutical effects and health benefits (Ahmad et al., 2019). Carrot is also rich in β-carotene, thiamine, vitamin B-complex, riboflavin, and minerals. Parsley (*Petroselinum crispum*) is widely used as a flavoring and aromatic food additive (Díaz-Maroto et al., 2002; Zhang et al., 2006). Additionally, it has been found that parsley's bioactive constituents exhibit a wide range of pharmacological properties, such as antioxidant, anti-diabetic, analgesic, anti-platelet, antibacterial and antifungal activity, brain protective, cytoprotective, diuretic, estrogenic, gastroprotective, hepatoprotective, hypotensive, immunosuppressant, laxative, spasmolytic (Farzaei et al., 2013). Therefore, the combination of powdered *Mangifera indica* leaves, *Psidium guajava* leaves, *Petroselinum crispum* leaves and *Daucus carota* in the formulation of a shelf-stable functional food product seems to be the right choice. Few studies Shamim et al., (2020); Bhagwat et al., (2017); Vidhya et al., (2016); Rani et al., (2015); Patgiri et al., (2014); Verma et al., (2014) reported shelf-life of some powdered formulations but for FPPV been a new product, the same is not available hence there is a need to evaluate its shelf-life. Therefore, this study aimed to evaluate the shelf-life of a novel functional product from a blend of powdered vegetables (FPPV) with the help of modern analytical techniques.

Materials and Methods

Procurement of Raw Materials

Fresh carrots (*Daucus carota*) and fresh healthy leaves of parsley (*Petroselinum crispum*) were purchased from a local market of Allahabad, India while fresh healthy mango (*Mangifera indica*) leaves and guava (*Psidium guajava*) leaves were collected from Sam Higginbottom University of Agriculture Technology and Sciences farm and these leaves were all authenticated and taxonomically identified.

Sample Collection and Preparation

The fresh leaves were separated from the roots and thoroughly washed under running tap water for the removal of dirt, debris and any foreign matters that adheres to it, followed by rinsing with distilled water and drained completely. Using a grating (kitchen grater) machine, carrots were sorted, cleaned, peeled and grated into thin slices of 2 mm thickness and 2–5 cm long. Prepared leaves (*Petroselinum crispum*, *Psidium guajava* and *Mangifera indica*) were blanched in boiling water at a temperature of 96–98 °C for 90 s as described by Raja et al., (2019), Lin et al., (2012) and James and Matemu (2018), Gamboa-Santos et al., (2013), Patras et al., (2011). Grated carrots were blanched into hot water at 94 °C for 2–3 min as described by James and Matemu (2018). Both samples were cooled immediately under running tap water after blanching. Blanching was performed aiming at inactivating enzymes to prevent enzymatic browning and micronutrients oxidation, sterilising vegetables, structural softening to facilitate moisture removal during drying and evaporating herb-like flavours (Chiewchan et al., 2010).

Drying of Vegetables

The blanched leaves and carrots were separately dried in a tray dryer for 12 hours. The tray dryer was allowed to reach required temperature (50 °C). After reaching the temperature the samples were placed on the trays and kept for 12 hours for moisture evaporation, as described by Reddy et al., (2020). All samples were finely powdered separately in the electric grinder and sieved through no. 100 mesh. 1:1:1:1 ratio of powdered *Daucus carota* and leaves of *Petroselinum crispum*, *Mangifera indica*, *Psidium guajava* was used for the formulation of FPPV.

Shelf-life Evaluation

Sample quantity and packing

For storage purpose, transparent polyethylene terephthalate containers with a capacity of 250 mL were procured from a local market. The formulation was filled into four different containers covered with aluminum foil and tightly closed with the cap. Each container contains 200 g of FPPV.

Storage conditions and frequency of withdrawal

The accelerated stability study was conducted as per ICH Guidelines Q1A (R2) (Anonymous, 2003), for the period of 6–months. Temperature was maintained at 40°C ± 2°C while relative humidity (RH) was maintained at 75% ± 5%.

The products were withdrawn from the container and analyzed initially (0), and at a gap of 1, 3 and 6 months, as described by Rani et al., (2015) and Shweta et al., (2020).

Analytical parameters

Organoleptic and physico-chemical analysis

Colour: 5 grams of FPPV was taken in watch glasses. A perfectly white background was used and the sample was observed for colour with naked eye in white tube light.

Odour: 2 gram of FPPV was smelled for odour test.

Taste: A pinchful of FPPV was tasted on taste buds of tongue.

Loss on drying: was determined by weighing about 2gm of FPPV in previously weighed dried petridish and dried in an oven at 105-110ºC, till two consecutive weights, which do not differ by more than 5mg. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as % w/w with reference to the dried sample (Anonymous, 2007a).

pH: was determined by mixing 1g of FPPV with 100 ml distilled water in a 100 ml volumetric flask. The solution was sonicated for about 10 minutes. The pH was measured using digital pH meter (Anonymous, 2006).

Total ash: was determined by incinerating about 5g of the sample, in a previously weighed crucible at gradually increasing heat up to 450 °C until it is carbon free. Then cooled in a desiccator and weighed. The percentage of total ash was calculated and expressed as % w/w of the dried material (Anonymous, 2007b).

Water soluble extractive: 5 g of accurately weighed FPPV was macerated in a glass-stopper conical flask. 100 ml chloroform water was added and macerated for 6 hours, shaking frequently and then allowed to stand for 18 hours then after 24 hours it was filtered rapidly and 20 ml of the filtrate was transferred in a tarred flat bottom evaporating dish with a pipette and evaporated to dryness on a boiling water bath. Then evaporating dish was dried at 105 °C for 6 hours and then cooled and weighed. From the weight of the residue the percentage of water soluble extractive was calculated and expressed as % w/w with reference to the dried sample (Anonymous, 2007c).

Microbial Analysis

Test for microbial contamination (total bacterial count, total fungal count, and the specific pathogens, i.e., *Escherichia coli*, *Salmonella spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) was done initially and at the completion of 6 months of storage by following standard guidelines (Anonymous, 2007d).

Results

FPPV was prepared as per classical guidelines and tested for organoleptic characters, various physico-chemical parameters at 0, 1, 3 and 6 months while microbial contamination was analyzed initially and at the completion of 6th month at accelerated stability conditions. Organoleptic and observations of Physico-chemical analysis of FPPV at initial (0), 1, 3, 6-month interval is shown in Table 1. Overall, insignificant difference in organoleptic characters at 0, 1, 3 and 6 months were observed. Percentage loss on drying was observed 4.6 at initial stage, 4.62 at one month interval, 4.67 at three months interval and 4.76 at six months interval. pH of the product was observed 4.5 at the initial stage, 4.51 at one month interval, 4.56 at three months interval and 4.64 at six months interval. The percentage total ash was observed 2.55, at initial stage, 3.1 at one month interval, 3.95 at three months interval and 4.94 at six months interval. Water soluble extractive value was observed 22.19 at initial stage, 21.6 at one month interval, 20.79 at three months interval and 19.3 at six months interval. Microbial growth was found below prescribed limits of World Health Organization guidelines initially and after 6th month (Table 2). Based on the physico-chemical values, intercept and slope were calculated followed by expected time for 10% degradation for individual parameters. 10% degradation was set as the acceptable point to extrapolate the accelerated stability data. Real time aging factor 5 and 3.3 was used for extrapolation of shelf-life for climatic Zone I & II countries and climatic Zone III & IV countries respectively. Nigeria comes under climatic zone III & IV. Number of months when 10% degradation was occurred was calculated using following formula:

$$M = \frac{[AV - \{(AV \times \frac{10}{100})\}] - \text{Intercept}}{\text{Slope}} \quad (1)$$

(Formula Adopted from: Shweta et al., (2020)):

M : Months when 10% degradation occurs
AV : 0 month Assay value

For current study, On extrapolation of these values; mean months for 10% degradation was found 10.3 and the shelf-life of FPPV was found to be 2 years and 8 months (Tables 3).

Table 1. Organoleptic and Physico-chemical parameters of FPPV

Organoleptic parameters				
Parameters	0 month (Initial)	1st month	3rd month	6th month
Colour	Light green	Light green	Light green	Light green
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Characteristic	Characteristic	Characteristic	Characteristic

Physico-chemical parameters						
Parameters	Initial Month (0 month)	1st Month	3rd Month	6th Month	Intercept	Slope
Loss on drying (%w/w)	4.6±0.03	4.62±0.01	4.67±0.02	4.76±0.01	4.59	0.03
Total Ash (%w/w)	4.5±0.01	4.51±0.02	4.56±0.01	4.64±0.02	4.49	0.02
pH value (% w/v)	2.55±0.00	3.1±0.00	3.95±0.00	4.94±0.00	2.65	0.39
Water soluble extractive (%w/w)	22.19±0.05	21.6±0.04	20.79±0.08	19.3±0.03	22.15	0.47

All values represent mean ± standard error

Table 2. Total microbial growth in FPPV

Organism	0 month (Initial)	6th month	WHO Permissible Limits
Total plate count (cfu/g)	<10 cfu/g	<10 cfu/g	10^5/g
Total yeast and mold count (cfu/g)	<10 cfu/g	<10 cfu/g	10^3/g
E. coli	Ab	Ab	Ab
Pseudomonas aeruginosa	Ab	Ab	Ab
Staphylococcus aureus	Ab	Ab	Ab
Salmonella Spp	Ab	Ab	Ab

cfu: Colony Forming Units, Ab: Absent, g: Grams

Table 3. Extrapolated shelf-life of FPPV from different physico-chemical parameters

Parameters	Initial (0 Month)	Result at 10% Degradation	Months when 10% degradation occurs
Loss on drying	4.6	4.14	17.31
pH value	4.5	4.05	18.33
Total ash	2.55	2.29	0.91
Water soluble extractive value	22.19	19.97	4.64
Mean Months at accelerated condition			10.3
Climatic zone I & II			51.5 (4.29 years)
Climatic zone III & IV			33.99 (2.83 years)

Discussion

Finished product of FPPV was a light green powder with characteristic odor and taste, and did not show any significant change in their organoleptic characteristics in accelerated thermal/humidity conditions. Color changes are mainly caused by pH changes or light exposure (Anonymous, 2008a). There was no change in color in this study, which correlates with an insignificant change in pH and confirms to the storage condition criterion.

However, only organoleptic characters are insufficient to prove the shelf-life in the modern era to meet the quality standards of food products, as chemical degradation is usually undetectable by the naked eye examination. Only excessive chemical degradation occasionally is accompanied by observable physical changes. In addition, some physical changes not necessarily related to chemical potency. As a result, commonly it should be assumed that a product that has undergone a physical change not explained on the labeling may also have undergone a chemical change, and such products shouldn't be dispensed (Ali and Ahsaani, 2006).

The World Health Organization and other food and drug regulatory agencies mentioned that the physico-chemical stability data are also important in determining food products shelf-life. Hence, further physico-chemical and microbial evaluations were carried out to confirm this product's shelf-life.

Insignificant differences were observed in basic physico-chemical profiles of the FPPV at different stages of analysis except water soluble extractive value. The moisture content of FPPV did not change significantly, because this formulation was subjected to a stability chamber in airtight containers of good standard quality that prevent moisture adsorption, the moisture content is unlikely to have changed.

Moisture is one of the most important factors that determines a product's shelf-life and is the primary cause of product deterioration. Moisture in a product is sufficient to activate different enzymes, which slowly decompose the product resulting in its degradation (Sharma et al., 2013).

Change in pH from 0–6 months was found to be as insignificant, as these changes were below 5%, to be considered as insignificant as per the ICH guidelines. One of the most important factors determining the quality of food products is the pH value. It regulates a wide range of chemical and microbiological reactions. Abba et al., (2008) found that when the pH value is low (presence of acidic substances), the bacterial count is low, whereas when the pH is neutral or higher, the level of contamination of the food products is higher. This shows that food products with a neutral or alkaline pH are more prone to contamination. As the pH of the functional food product was 4.64 or less and microbial count was also within the normal limit according to WHO guidelines, it is consistent with previous research findings Abba et al., (2008).

Water soluble extractive value plays an important role in evaluation of food products. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. A high ash value indicates contamination, substitution, adulteration or negligence in the preparation of the food products for marketing (Singh et al., 2011). As these changes were <5%, it confirms to the ICH guideline. However, the decreasing value of Water soluble extract and increasing value of Ash is indicates less efficacy of the product after a period.

Total bacterial count was found to be <10 cfu/g, Total fungal count was <10 cfu/g, while pathogenic bacteria that is, *E. coli*, *Salmonella*, *S. aureus*, and *P. aeruginosa* respectively were all absent at both initial (0 month) and 6th month in the present study samples. Thus, all packs confirm to the microbial standards set by WHO, (2007), and Ayurvedic pharmacopoeia of India (API), (Anonymous, 2008b), (Table 2). Hence, in the present study, microbial count within the prescribed limits indicates product safety and quality.

Micro-organisms require readily accessible water in appreciable quantities for growth (Kamil and Lupuliasa, 2011). Scott, (1953) established that only moisture does

not have a significant effect, but the water activity is the key to determine if microorganisms will grow or not. Drying at a specific temperature decreases the total microbial count in plant material as it lowers the water activity (Kulshrestha et al., 2008). Before preparing the functional product, raw vegetables were properly washed under running tap water for few minutes, followed by distilled water rinsing, blanched, dried at 40°C stored in airtight containers, and all precautions were taken to avoid contamination during processing in the current study. Thus, the finished samples had very low moisture (4.6–4.76%). Further, the pH of samples was ranging from 4.5 to 4.64. Probably these factors played an important role in keeping total microbial load in the prescribed limit.

Overall, it was assumed that the FPPV contained relatively less amount of water, therefore no significant physico-chemical changes or microbial growth occurred.

According to Grimm (1998), the predictive factor for zone IV was 3.3 of the accelerated study period. It indicates that if the product is stable for 6 months (temperature maintained at 40°C ± 2 °C while relative humidity was maintained at 75% ± 5%), its shelf-life will be 33.99 months at 30°C/70% RH (climatic zone IV).

Thus, in the view of above interpretations, it can be safely affirmed that FPPV has the shelf-life of 33.99 months at room temperature. However, according to the ICH Harmonized Tripartite Guideline for the evaluation of stability data, if no significant change is found at accelerated condition, the retest period or shelf-life would depend on the nature of the long-term data (ICH, 2003).

A few studies reported shelf-life of *Rasayana Churna* (Verma et al., 2014), *Hridya Yoga Churna* (Vidhya et al., 2016), *Sufoofe Sailan* (Rani et al., 2015), Herbal Digestive Powder (Shamim et al., 2020), but for FPPV, the same is not available. The findings of previous investigations support the findings of FPPV.

Conclusion

Shelf-life study of the novel functional product from a blend of powdered vegetables (FPPV) showed insignificant difference in the formulation at 1st, 3rd and 6th month when compared with 0 month sample in all the parameters tested. Organoleptic characters were acceptable. The changes at 6 months in physico-chemical parameters i.e. Loss on drying, pH, Total Ash value, Water soluble extractive value, were insignificant, and the total microbial count was within the limit set by WHO. Thus, FPPV confirms to the ICH Harmonized Tripartite Guideline for accelerated stability testing of the product. As per the Grimm's statement, the shelf-life of FPPV was calculated 51 months at room temperature for climatic zone I & II countries and 34 months at room temperature for climatic zone III & IV countries respectively. This observation is specific to FPPV, been it newly developed functional food product. Nevertheless, since the accelerated stability studies alone do not serve as the sole basis to calculate products shelf-life; it should be supported by long-term and real-time studies.

Conflicts of interest

There are no conflicts of interest.

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