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Organic solvent-free extraction of carotenoids from sweet potatoes and their utilization in fortification of table spread

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Abstract
The study was aimed to extraction of carotenoids from sweet potatoes using green solvent based ultrasonic–assisted extraction method and their fortification in a table spread. Four formulations were tried for preparation of table spread with varying composition of milk fat, carotenoid extract, stabilizer, emulsifier and water. On the basis of textural and sensory attributes, table spread prepared with 6% carotenoid extract were highly acceptable. The physio-chemical parameters of freshly produced spread were analysed as soon as after preparation. The same samples were stored at 5 ºC for 75 days and anlysed for oxidative detoriation after regular interval of 15 days. The proximate composition of developed table spread was observed to be 36.03 to 36.22% moisture, 56.01 to 54.98% fat, 5.2 to 5.3%, protein, 1.7 to 2.26%, carbohydrates, 1.06 to 1.19% minerals, 7.88 to 13.2 µg/100g total carotenoids and 20.71 to 34.4 (mg GAE/gm) total phenolic content. The antioxidant activity, peroxide value, free fatty acids and TBA values in spread were observed to be 7.4 to 14.40 %, 0.17 to 0.13 (meq/kg oil), 0.91 to 0.71% oleic acid and 0.428 to 0.368 OD, respectively. The carbohydrates, antioxidant activity, total carotenoids and total phenolic content was significantly (p<0.05) increased in table spread fortified with carotenoids compared to control sample, while fat content, free fatty acid, peroxide value and TBA content were slightly decreased. Non-significant changes were found in the protein and minerals value. During storage significant (p<0.05) increase in TBA, FFA and peroxide values as the storage period progressed. The carotenoid enrich spread was qualitatively stable for 75 days at 5 ºC, indicating a suitable shelf life. The table spread was successfully fortified with carotenoids and can be effectively used for enhancing health standard of the people.

Keywords: Table spread, sweet potato, carotenoid, green technique, sunflower oil

1. Introduction
There has been an inevitable marked inclination in present consumer's dietary pattern towards foods which have improvised health benefits. The demand for such functional food is being driven by the growing consumer understanding of the linkage between diet and disease and the interest in self-health maintenance, rising health care costs and advances in food and nutrition [1]. A range of dairy and non-dairy spreads have been developed to provide nutrition and consumer convenience [2]. Margarine and fat spreads are an interesting and effective food vehicle to be fortified with lipid soluble compounds. At the same time, it is a food item that is regularly consumed in small amounts. Many European Member States presently require the mandatory addition of vitamin A and D to margarine and fat spreads in order to help to improve the public health situation within the European Community [3].
Sweet potato (*Ipomoea batatas* Lam) is an important tuber crop grown in the tropics, sub-tropics and warm temperate regions of the world for its edible storage roots. It can be used as food supply to combat malnutrition in the developing nations, since the tuberous roots (tubers) are enriched with starch and dietary fiber, along with carotenoids, anthocyanin, ascorbic acid, potassium, calcium, iron, and other bioactive ingredients [4, 5]. For people of South-east Asia and Africa, this crop is the main source of β-carotene. Yellow and orange sweet potatoes have higher carotene than other vegetables, with 8.509 mg per 100 gm [6]. The carotenoids are a micronutrient that plays an important role in decreasing the risk of certain types of diseases like cardiovascular, chronic inflammation and cancer [7]. Moreover, carotene also acts as a precursor for production of vitamin A in body, an essential vitamin at any age, including for cellular health and proper vision. However, deficiency of carotene or vitamin A causes night blindness, conjunctivitis of the eye or inflammation of the cornea, disturbance in bone growth, retard normal growth and defects in teeth [8].

Several conventional solvent extraction methods are used for extraction of carotenoids, while these methods required a large amount of harmful solvents such as acetone, methanol, ethanol, ethyl acetate, isopropyl alcohol, petroleum ether, etc. in multiple extraction steps. These solvents are highly flammable, volatile, typically poisonous and cause environmental pollution and impacted on greenhouse gases [9, 10]. According to many reports, the principle failure in marketplace for that natural bioactive components or colorants extracted from various fruits and vegetables was mainly due to issue of residual solvent in isolated extract [11, 12]. Thus the solvent removal is important due to health risk association with their consumption [13]. On other hand, green bio refinery based concept is a new trend and need of the hour that focuses on using green solvent that have the potential to protect from adverse effects of solvents that are of petrochemical origin. Bioavailability of carotenoid is lower and during various treatments like heating they exhibit low solubility and stability. Apart from this problem, application of carotene in development of value added foods is relatively restricted because of its low chemical stability and water-dispensability [14]. Therefore, extraction of carotenoids by organic solvent free techniques and its utilization by encapsulation through emulsion-based delivery systems seems to have more scope as it increases its bioavailability and stability. The objectives of the present work to extraction of carotenoids through UAE assisted process using sunflower oil (eco-friendly solvent) and their fortification in table spread with suitable emulsion based delivery system. The novelties of this study were the use of a green technology for the extraction of carotenoids from sweet potatoes and the demonstration of real-world application in fortification in fat rich food products.

2. Material and Methods

2.1 Chemicals and raw materials: Milk fat, stabilizer, emulsifier, and sweet potatoes were procured from, local markets of Prayagraj. Whey Protein Concentrate (WPC) was purchased from Bhole Baba Milk Food Industries Ltd., New Delhi. All analytical grade chemicals were used during the experiment.

2.2 Extraction of carotenoids: Extraction of carotenoid using sunflower oil as green solvent was done by method adopted by [14] with little modification. Initially, the sweet potatoes were washed, cleaned and peeled off. It was then crushed and made into uniform dough. The prepared dough was mixed with sunflower oil in the ratio 1:1 further it was centrifuged (BT 36 R, Emtek Instruments, India) at 12000 rpm for 30 min. Followed the mixture was subjected to ultrasound assisted extraction with sunflower as solvent at 45% duty cycle and 750 W power. Final centrifugation was done after each treatment at 4000 rpm for 12 min. Later the supernatant was filtered and separated and stored in deep freeze (-20 °C).

2.3 Preparation of emulsion based delivery system: The emulsion based delivery system for carotenoid extract was prepared as per the method suggested by [15] with some modification. The 30 g whey protein concentrate and 60 mL water were mixed in magnetic stirrer for half an hour to get coarse emulsion. Further, 20 mL of carotenoid extract was added and mixture was centrifuged at 7000 rpm for 10 min. The obtained fine emulsion was used for development of table spread.

2.4 Experimental design for optimization of formulation of table spread: Table spread was prepared by blending ingredients at different ratios as shown in table 1. The formulated table spread contained both the fat phase and aqueous phase. The fat phase was prepared by thoroughly mixing and blending of fat soluble additives and fat sources accordingly with treatment table. Followed the mixture of fat phase gently warmed at 40-50 °C with continuous stirring for 5-10 min to obtained proper homogeneity and solubilization of ingredient. The aqueous phase was prepared separately; all water soluble ingredients were weighed, calculated and blended properly using electric mixture for 1-2 min with the addition of calculated amount of water as per treatment. Using an ice bath, aqueous phase was slowly mixed with fat phase with help of electric mixer for 10 min to obtained complete homogenization and solubilization. The spread was then filled in polypropylene containers and stored at refrigeration temperature.

Table 1: Formulation for preparation of table spread

Treatment	Milk fat (%)	Carotenoid emulsion extract (%)	Whey Protein Concentrate(%)	Stabilizer, Emulsifier(%)	D/W(%)
T0	75	0	7	2	16
T1	72	3	7	2	16
T2	69	6	7	2	16
T3	66	9	7	2	16
T4	63	12	7	2	16

2.5 Physico-chemical analysis of table spread: The physico-chemical parameter such as moisture, fat, protein, minerals, carbohydrates and acidity of table spread were determined as per the method described in [16] The TBA value was determined according to the method suggested by [17]. The antioxidant activity (2, 2-Diphenyl-1-picrylhydrazyl, DPPH) was measured according to the method given by [18]. The analysis of total phenolics content was done by using the method reported by [19]. The estimation of total carotenoid content was carried out by the protocol proposed by [20].

2.6 Sensory evaluation: Sensory analysis of table spread was carried out using 9- point hedonic scale by 20 semi-trained panelists comprising of 15 men and 5 women from the Department of Dairy Chemistry, WCDT, SHUATS, India.

2.7 Texture Profile Analysis: The prepared spread was carefully filled into a screw-capped plastic container, making sure there were no air spaces inside the samples. The samples were stored at 5 °C in the refrigerator, and analysis was carried out at the same temperature on next day. The samples were evaluated for its textural attributes i.e., firmness, stickiness, and work of shear using Texture Analyzer TA-XT plus (Stable Micro Systems, UK) fitted with a 50 kg load cell.

The product was subjected to application of force to a depth of 20.0 mm by a compression platen probe attached to the texture analyzer. Pre-test, test and posttest speeds were 5, 2 and 5 mm/s respectively. Other settings were; trigger force: 1 g, trigger type: Auto, data acquisition rate: 200 pps.

2.8 Statistical analysis: The data obtained by physico-chemical were analysed by using Prism Graph Pad (Prism version 7.01) through one-way ANOVA with Bonferroni Post-Tests and the results of oxidative changes during storage of UHT milk were compared using two-way ANOVA with Tukey Post-Tests. The *p*-value ≤0.05 was considered as significant figure for the results.

3. Results and Discussion

Assessment of the physico-chemical parameters of developed crotenoids rich table spread was required to understand any imapact on the nutritional properties, sensorial qualities and bioavailability of carotenoids on the spread.

3.1 Physico-chemical characteristics of fortified table spread: The compositional characteristics of freshly prepared table spread fortified with different concentration of carotenoids extract are shown in Table 2. The moisture, fat, protein, carbohydrate, minerals, titratable acidity (lactic acid), total carotenoids, total phenolic content and antioxidant activity of different treatment of table spread were in the range of 36.03–36.22%, 54.58–56.01%, 5.2–5.35%, 1.7–2.26%, 1.06–1.19%, 0.27-0.31% lactic acid, 7.88-13.2 mg/100g, 20.71-34.43 mg GAE/gm and 7.4–14.40% DPPH, respectively. The carbohydrates, antioxidant activity, total carotenoids and total phenolic content was observed significantly (p<0.05) increased as the incorporation of carotenoid emulsion, while fat content, free fatty acid, peroxide value and TBA content significantly (p<0.05) decreased. Howevere, protein and mineral content non-significant (p>0.05) change in among the all treatment. Comparing to T0 treatment (control), there was a non-significant (p>0.05) change in moisture content, peroxide value in treatment T_1. Accordance to our results [21–24] reported increased in content of carbohydrates, antioxidant activity, total carotenoids and total phenolic content, while slightly lesser value for fat, free fatty acid, peroxide value and TBA content in carotenoid fortified products. The increased in fat and carbohydrate content may be due to the increase the rate of fortification level of carotenoid emulsion in table spread [21, 23, 24]. Also the peroxide, TBA and FFA value slightly lesser than the control may be due to higher the antioxidant activity in carotenoid emulsion fortified table spread [25]. The antioxidant activity and total phenolic content were significantly high in carotenoids fortified spread compared to the control sample. Correlate to our results previously [26] reported that sweet potato powder contained the higher antioxidant activity and phenolic compound such as vanillic acid (64.81 mg·kg^{-1}), pyrogallol (10.49 mg·kg^{-1}), catechol (9.66 mg·kg^{-1}) and p-hydroxybenzoic acid (5.18 mg·kg^{-1};).

3.2 Sensory evaluation: The sensory attributes of the milk products may be affected by the addition of functional ingredients that could lead to a decrease in the consumer acceptability. Therefore, it is required to evaluate the changes in the sensory properties of developed spread when using the sweet potato carotenoids emulsion. The results in Table 3 demonstrate an improvement in the sensory attributes of the functional spread as a result of fortification with the sweet potato emulsion. The sensory evaluation of the table spread fortified with carotenoids emulsion revealed that all the treatments developed were organoleptically acceptable. Based on the results, it was found that significant differences (p < 0.05) were observed for the color, flavor, body and texture, and overall acceptability among all treatment samples. Compared to the treatment T0 (control), it was noted significant increase in the body and texture, flavour, colour and appearance and overall acceptability of table spread in other treatment. Howevere the table spread prepared from T_3 and T_4 treatment score minimum average overall acceptability than the T_1 and T_2 treatment. On the basis of sensory attributes, table spread prepared from T_2 formulation were highly acceptable among the all treatments. The spread prepared from a treatment T_2 formulation, that is substitution up to 69% of butter, 6% of carotenoid emulsion, 7% WPC, 2% stabilizer and emulsifier, 16% distilled water was found to be organoleptically acceptable. Accordanc to our results [27] reported that the addition of sweet potato purée to fermented milk contributed to an increase in firmness, yellow colour, flavour, and overall acceptability.

3.3 Textural characteristics: Textural and rheological properties of most of the fat rich products are based on firmness (N), Work of adhesion (N.S), work of shear (N.S) and stickiness (N). The changes in textural attributes of spread prepared fortified with emulsion based carotenoids extract are presented in Table 6. The data of firmness revealed hardness of product to spread over a food. From the table it is observed that Firmness ranged between 7.42±0.15 and 9.43±0.43 of spread. There was a significant difference (p<0.05) observed in the firmness of spread prepared among the treatments. The stickiness of semi-solid foods is one of the most important rheological attributes as a sticky feeling that can be perceived by tongue and palate. Stickiness of a spread can be defined as work required overcoming attractive forces between contact surface and the surface of the material [28]. The stickiness value was observed varied between -9.49±0.04 to -8.98±0.05 among the treatment. There was observed significant (p<0.05) increased in the value of stickiness as increasing the rate of carotenoids fortification. The work of shear is the resistance offered by table spread when probe penetrates in to sample during compression. In general term it is the work required in inserting spoon or scoop in to the table spread [29]. There was observed significant (p<0.05) increase in values of work of shear as increasing the rate of carotenoids fortification. The work of shear was observed highest in treatment T_4 as compared to the other treatments. Work of adhesion corresponds to area under penetration cycle in force distance curve represented the amount of energy required to remove probe from sample was referred to work of adhesion (N.s). The recorded values for work of adhesion varied from -8.48±0.6 to -8.21±0.04.

3.4 Assessment of oxidative deterioration during storage of fortified table spread: Assessment of oxidative deterioration is important because it influences the chemical, sensory, and nutritional properties of fat rich dairy products and thus plays a vital role in determining their use and shelf-life [30, 31]. The control and fortified spread samples were

stored at 5 ºC for 75 days and anlysed for oxidative detoriation after regular interval of 15 days. The obtained results are presented in figure.

Lipolysis of fat-rich products affects the consumer acceptability and has a negative impact on product shelf-life. FFAs are produced by hydrolysis by action of lipase and it mainly contributed to rancidity in fat-rich products [32]. The results of the FFA content value during storage of apread illustreted in The FFA value was significantly (p<0.001) increased during storage from 0.91±0.04 to 2.20±0.09 (T_0), 0.89±0.08 to 2.04 ±0.02 (T_1), 0.86±0.02 to 1.93±0.08 (T_2), 0.83±0.02 to 1.85±0.008 (T_3) and 0.79±0.012 to 1.79±0.04 (T4), respectively in spread samples. It was noted that steady increase in FFA during storage of all the spread samples. Comapring among all treatments, T0 (control) sample was observed highest FFA value followed by supplemented spread T_1, T_2, T_3 and T_4. Furthermore, there was a gradual increase in FFA as the storage period progressed from day 0 to day 75 of storage. However, primary lipid oxidation was significantly higher in the control compared to the carotenoids fortified spread. Previous studies have found a direct link between Total phenolic content and antioxidant potential, with Tunisian butter having the ability to reduced lipid oxidation and improved shelf life [33].

Peroxide value is one of the most commonly used quality assessment indicator in food and dairy industry, especially for fat based products, expressed as meg/kg oil. It is the measurement for evaluating complete status of lipid oxidation in fat based food corresponding to time, because peroxides are susceptible to break down with time [34]. The evolution of PV in the carotenoids supplemented table spread is presented in PV of control and supplemented butters increased in a classical manner during the storage period of 75 days at refrigeration temperature, but with varying magnitudes. During the first 30 days, the variation of PV was very slow and followed it was rapidely increased as the storage period progressed. In T0 (control) sample noted highest PV and its rate of incrsing during storage also very rapid compared to carotenoids supplemeted table spread. The continuous steady increase of peroxide value may be contributed due to level of oil and formation of primary oxidation substances during initial stages of lipid oxidation. Similar findings were found by Kamble and Sharma [23, 35] who reported that peroxide value exhibited increasing during storage of spread.

TBA is the another most important quality assessment factors of table spread, which measures oxidation product of fatty acids having three or more double bond i.e. monoaldehyde [36]. The evolution of PV in the carotenoids supplemented table spread is presented in The trend of results obtained by the TBA value was similar to rate of increse in peroxide value and FFA value. The TBA value of spreads samples significantly (p<0.001) increased during storage from 0.428±0.011 to 0.593±0.012 (T_0), 0.417±0.004 to 0.587±0.012 (T_1), 0.407±0.007 to 0.573±0.004 (T_2), 0.391±0.04 to 0.562±0.007 (T_3) and 0.358±0.01 to 0.558±0.009 (T_4), respectively. The Comapring among all treatments, T_0 (control) sample was observed highest TBA value followed by supplemented spread T_1, T_2, T_3 and T_4. Similar to our study Sengar *et al*. [37] who observed the similar trends of increasing TBA value in their respective spreads samples during storage.

4. Conclusion

The carotenoids were successfully extracted using organic solvent-free techniques and fortified in table spread. On the basis of sensory attributes and textural analysis, table spread prepared with (T_2) 6% carotenoid extract were highly acceptable among other treatments. Carotenoids fortified table spread showed a significant higher carbohydrates, antioxidant activity and total phenolic content and lower FFA, peroxide and TBA value. During storage, oxidative deterioration significantly lower was noted in carotenoids fortified table spread compared to the control. From the obtained results it could be concluded that fortification of carotenoids has great potential to increase nutritional as well as antioxidant activity which helps in extending the shelf-life of table spread.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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