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Development and Storage Study of Table Spread fortified with carotenoids extracted from Sweet Potato (*Lpomoea batatas* (L) Lam.) using Green Solvent extraction

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ABSTRACT: In this study, carotenoids were extracted from sweet potatoes using ultra-sonication and sunflower oil as a green solvent, and the extracted carotenoids were fortified in a spread for the table. Since concentration of carotenoids are much lower in dairy based products, enriching it using suitable delivery system helps to uplift existing vitamin A deficiency in our country. Carotenoid rich emulsion delivery system was developed using Whey Protein Concentrate as emulsifier. Stability of emulsion was analyzed. Four formulations of table spread were tried with varying composition of milk fat and carotenoid rich emulsion, stabilizer, emulsifier and water viz., T_0 – 75% butter, T_1-72% butter, 3% carotenoid emulsion, T_2 -69% butter, 6% carotenoid emulsion, T_3 -66% butter, 9% carotenoid emulsion and T_4 -63% butter, 12% carotenoid emulsion. The proximate composition of developed table spread was 36.25% moisture, 55.5% fat, 5.25% protein, 2% carbohydrates and 1% ash. Total carotenoid content and antioxidant activity in the formulated treatments were significantly ($p<0.05$) higher than control. The fortified table spread was stored at 37°C vis-à-vis 5°C and analyzed for physicochemical changes during storage for 75 days by 15 days of interval. During storage period, qualitative and bio-functional parameters like moisture content (%), titratable acidity (%), peroxide value(meq/kg oil), thiobarbituric acid value(OD) and Free fatty acids(%) were recorded at every 15 days interval. There was significant ($p<0.05$) increase in acidity, TBA, FFA and peroxide values were observed, whereas moisture content and sensory scores was found to be significantly ($p<0.05$) decreasing. The carotenoid enriched spread was qualitatively stable and indicated suitable shelf life. The table spread with 6% carotenoid rich emulsion (T_2) was found to be the best among all the treatments.

Keywords: Table spread, Carotenoid, ultrasonication, green technique, shelf-life.

INTRODUCTION

Dietary pattern of today's consumers has changed and they are going for foods having improved health benefits. The demand for such functional food is being driven by the growing public 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 (Kuster -Boluda and Vidal-Capilla 2017). The milk products especially ghee and butter are too expensive and high in calories owing to its high fat content. Therefore, the popularity of such products has decreased as the consumers become increasingly aware of their calorie intake. As a lower-calorie alternative to butter, spreads, which are similar to margarine, contain less than 80% fat (Kharb *et al.*, 2022). A range of dairy and non-dairy spreads have been developed to provide nutrition and consumer convenience (Feeney, 2017). Spreads have excellent spreadability at refrigeration temperatures and can withstand high ambient temperatures. 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. Since it is used as a replacement of butter naturally rich in vitamin A and D many European Member States currently 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 (Sioen, 2013).

Sweet potato (*Ipomoea batatas* (L) Lam) is an important tuber crop grown in the tropics, sub-tropics and warm temperate regions of the world for its edible storage roots (Wu *et al.*, 2008). 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. Sweet potato also possesses a potential for bioenergy production as it can

adapt to growth on marginal lands. For people of South-east Asia and Africa, this crop is the main source of β-carotene. Comparing with other vegetables, yellow and orange sweet potatoes contain more β-carotene (Kidmose *et al.,* 2007). Sweet potatoes mainly contain all-trans-β-carotene which is a potent antioxidant. Oil in water based emulsions was found to be effective delivery systems for lipophilic bioactive ingredients (Mao *et al.,* 2018). 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 (Maria *et al.,* 2015). 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 (Grune *et al.,* 2010). The carotene is a main public health concern in pregnant and lactating women and preschool-age children (Thorne-Lyman and Fawzi 2012). 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 (Saini and Keum 2018). 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 problem of residual solvent in them (Kumar *et al.,* 2017). Thus the solvent removal is important due to health risk association with their consumption and association. On other hand side, 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. 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 (Tiwari and Arora 2019). Therefore extraction of carotenoids by organic solvent free techniques and its utilization by encapsulation through emulsion-based delivery systems seems to be more scope.

Carotenoid contribution in bovine milk and milk products are very low and hence its bio availability to consumers is decreased. Thus aim of the study was to develop carotenoid fortified table spread and thereby increasing its bioavailability to consumers.

MATERIALS AND METHODS

The research was carried out in 2021-2022 at Warner College of Dairy Technology, Allahabad. A total of 4 treatments and 3 replications were carried out. Randomized design was used. Sweet potatoes were procured freshly from local market and carotenoids were extracted using appropriate technique.

Preparation of carotenoid extract:

Extraction of carotenoid using sunflower oil as green solvent was done by method adopted by Tiwari and Arora (2019). Sweet potatoes were washed, peeled and crushed and made into dough. The crushed potato dough was mixed with sunflower oil in the ratio 1:1 and was centrifuged at 12000 rpm for 30 min. The mixture was then subjected to ultrasound assisted extraction with sunflower as solvent at 45% duty cycle and 750W 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. Carotenoid rich extract was incorporated to table spread using suitable emulsion delivery system. Emulsion of beta-carotene was prepared by following the method of Chen (2017) with slight modification in concentration of solvents and extract. 30g Whey protein concentrate and 60 ml water were mixed in magnetic stirrer to get coarse emulsion for half an hour. 20ml of carotenoid extract was added and stirred at high speed at 7000 rpm for 10 min. Fine emulsion obtained was used for developing table spread.

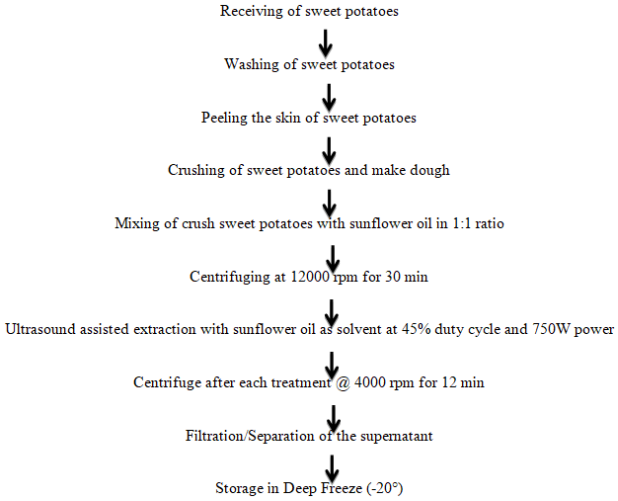

Fig. 1. Flow diagram for Extraction of carotenoids from sweet potato.

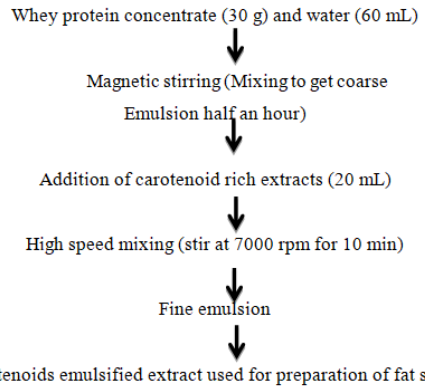

Fig. 2. Flow chart for the preparation of Emulsion for extracted Carotenoids.

Manufacturing of Table Spread. Table spread contains two different phases' viz., fat phase and aqueous phase. Fat soluble additives and fat source are taken accordingly with treatment table and mixed with proper

blending and gently warming at 40-50°C with continuous stirring for 5-10 min so that proper homogeneity and solubilization of ingredient occurs. The aqueous phase is prepared separately; all water soluble ingredients are weighed and calculated as per treatment table (Table 1) and blended properly using electric mixture for 1-2 min with the addition of calculated amount of water, so that proper mixing of ingredients takes place. Followed by mixing aqueous phase with fat phase slowly using ice bath, with continuous blending using electric mixer at low speed for 10 min, followed with high speed for 5 min so that complete homogenization and solubilization occurs and prepared spread showed the semi-solid texture. The spread was then filled in polypropylene containers and stored at refrigeration temperature. Qualitative parameters like moisture, fat, protein content, titratable acidity, antioxidant activity, total carotenoid content and sensory parameters like flavor, spread ability, colour, taste, appearance and overall acceptability at 15 days interval for 75 days were studied.

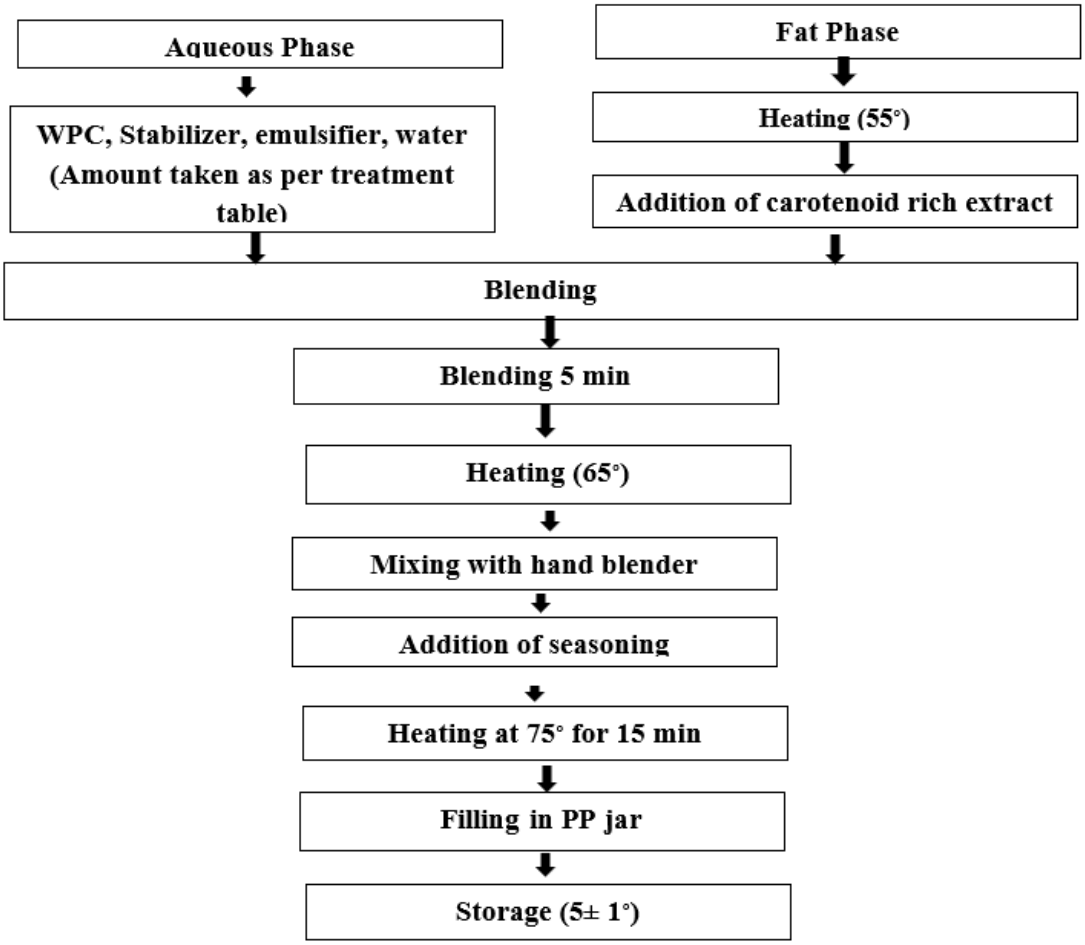

Fig. 3. Flow chart for the preparation of Table Spread.

Table 1: Standardization for development of Carotenoid-rich table spread

Treatment	Butter (%)	Emulsified Carotenoids Solution (%)	Whey Protein Concentrate (%)	Stabilizer, Emulsifier (%)	D/W(ml
T0	75	—	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

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 were compared using two-way ANOVA with Tukey Post-Tests. The p-value ≤0.05 was considered as significant figure for the results.

RESULTS AND DISCUSSION

The effect of carotenoid rich emulsion on developed table spread on different parameters like moisture content(%), titratable acidity(%), peroxide value(meq/kg oil), thiobarbituric acid value(OD), Free fatty acids(%) and overall acceptability of fat spread during storage were studied and the results obtained

were statistically analyzed and tabulated. Inthedevelopedtablespreadtheproximatecompositionshowedapproximately35.25%moisture, 55.5% fat, 5.25% protein, 2% carbohydrates and 1% ash.

Moisture content (%). It was observed that moisture content was decreasing gradually during storage significantly ($p<0.05$). The lowest moisture content was observed for 75th day for T0 (33.06±0.021%). The continuous steady decrease of moisture content may be due to evaporation during storage study. Results obtained are in accordance with Jung Min *et al.* (2014) who observed continuous steady decrease of moisture percentage of butter table spread due to evaporation. The moisture percentage decreased significantly from initial day to 75th day in control and treatment samples. Moreover, statistical analysis indicates that storage study had significant effect on moisture content. The decreasing rate was higher during storage study at 37°.

Titratable Acidity (%). The steady increase in acidity and decrease in pH was observed during storage study could be attributed to the production of acid by concomitant increase in total bacterial count and yeast and mold count. It can be observed that Titratable acidity was increasing gradually during storage significantly ($p<0.05$). The Titratable acidity content was highest for T_4 (0.31±0.028) in the initial day and increased to 0.39±0.028 and 0.49±0.014 when stored at 5°C and 37°C respectively for 75days.The continuous steady increase of titratable acidity may be contributed due to increased microbial load in table spread during storage study. Similar findings were done by Tiwari *et al.* (2019) who found the positive correlation between acidity and microbial load of the table spread.

Peroxide value (meq/kg). Peroxide value increased steadily during storage study may be contributed due to level of oil and extend of storage and formation of primary oxidation substances i.e. peroxides during initial stages of lipid oxidation in table spread. Similar findings was found by Honfo *et al.* (2011), in which peroxide value exhibited increasing trend while studying the storage study. It can be observed that peroxide value (expressed as meg/kg oil) was increasing gradually during storage significantly ($p<0.05$). The peroxide value content increased from 0.17±0.04 to 0.18±0.04 and 0.19±0.05 when stored at 5° and 37° respectively for 75days.

Table 2: Effect of carotenoid-enriched table spread on moisture content (%) at different days of storage.

Storage days	Storage Temp(°)	Treatments				
		T_0	T_1	T_2	T_3	T_4
0	5	36.03±0.014	36.04±0.028	36.05±0.014	36.15±0.014	36.22±0.014
	37	36.03±1.14	36.04±1.14	36.05±1.14	36.15±1.14	36.22±1.14
15	5	35.97±0.021	36.00±0.014	36.00±0.028	36.11±0.014	36.19±0.014
	37	35.88±1.14	35.9±1.14	36±1.14	36.01±1.14	36.1±1.14
30	5	35.93±0.021	35.96±0.014	35.93±0.014	36.07±0.021	36.15±0.021
	37	35.75±1.14	35.85±1.14	35.98±1.14	36±1.14	36.05±1.14
45	5	35.89±0.021	35.91±0.021	35.99±0.014	36.02±0.014	36.09±0.021
	37	35.6±1.14	35.8±1.14	35.7±1.14	35.9±1.14	36±1.14
60	5	34.12±0.021	34.55±0.01	34.89±0.021	35.11±0.021	35.45±0.014
	37	34.02±0.01	34.12±0.014	34.47±0.014	34.62±0.021	34.86±0.028
75	5	33.92±0.014	34.02±0.021	34.12±0.028	34.44±0.021	34.65±0.02
	37	33.06±0.021	33.12±0.014	33.45±0.021	33.62±0.021	33.68±0.014

Table 3: Effect of carotenoid-enriched table spread on titratable acidity (%) at different days of storage.

Storage days	Storage Temp(C°)	Treatments				
		T_0	T_1	T_2	T_3	T_4
0	5	0.27±0.028	0.28±0.028	0.29±0.028	0.30±0.028	0.31±0.028
	37	0.27±0.028	0.28±0.028	0.29±0.028	0.30±0.028	0.31±0.028
15	5	0.29±0.014	0.30±0.014	0.31±0.007	0.32±0.007	0.33±0.007
	37	0.29±0.014	0.30±0.014	0.31±0.014	0.32±0.014	0.33±0.028
30	5	0.30±0.007	0.32±0.007	0.32±0.007	0.33±0.007	0.34±0.007
	37	0.31±0.021	0.32±0.014	0.33±0.014	0.34±0.028	0.35±0.028
45	5	0.31±0.007	0.33±0.014	0.34±0.007	0.35±0.007	0.36±0.014
	37	0.33±0.028	0.34±0.028	0.35±0.028	0.36±0.014	0.37±0.028
60	5	0.32±0.007	0.33±0.014	0.35±0.007	0.37±0.028	0.38±0.014
	37	0.36±0.014	0.38±0.028	0.40±0.014	0.42±0.014	0.45±0.007
75	5	0.33±0.014	0.34±0.014	0.36±0.028	0.38±0.007	0.39±0.028
	37	0.40±0.028	0.43±0.028	0.45±0.014	0.48±0.028	0.49±0.014

Table 4: Effect of carotenoid-enriched table spread on peroxide value (meq/kg) at different days of storage.

Storage days	Storage Temp(°)	Treatments				
		T_0	T_1	T_2	T_3	T_4
0	5	0.17±0.04	0.16±0.05	0.15±0.07	0.14±0.04	0.13±0.04
	37	0.17±0.05	0.16±0.08	0.15±0.07	0.14±0.04	0.13±0.07
15	5	0.18±0.04	0.17±0.07	0.16±0.08	0.15±0.02	0.14±0.04
	37	0.18±0.04	0.17±0.07	0.17±0.07	0.16±0.05	0.15±0.07
30	5	0.19±0.014	0.19±0.07	0.19±0.07	0.18±0.03	0.16±0.07
	37	0.20±0.014	0.20±0.07	0.20±0.08	0.19±0.05	0.17±0.05
45	5	0.21±0.011	0.20±0.014	0.20±0.08	0.19±0.07	0.18±0.04
	37	0.23±0.011	0.22±0.01	0.21±0.04	0.20±0.07	0.19±0.05
60	5	0.25±0.01	0.24±0.04	0.23±0.01	0.22±0.08	0.20±0.01
	37	0.27±0.07	0.26±0.01	0.25±0.04	0.24±0.01	0.23±0.014
75	5	0.28±0.01	0.27±0.07	0.26±0.014	0.25±0.011	0.24±0.07
	37	0.30±0.04	0.29±0.014	0.28±0.08	0.27±0.01	0.26±0.01

Thiobarbituric acid value (OD). The data referring to thiobarbituric acid content of carotenoid enriched fat spread is presented in Table 5. It was observed that there was steady increase in TBA value after two weeks of storage. There was significant difference between TBA values ($p<0.05$). The TBA content was highest in T_0 which increased from 0.428±0.011 to 0.593±0.012 and 0.630±0.00 when stored at 5° and 37° respectively for 75 days. While studying the effect of storage on functional mixed fat me at spread, Kumar *et al.* (2015) observed arise in TBA value over the progression of storage. The findings of present study also accord with results of Patange *et al.* (2013) also observed the similar trends of increasing TBA value in their respective spreads samples during storage.

Free fatty acids (%). Rancidity in fat rich products is mainly contributed by FFA's. There was steady increase in FFA during storage days and significant difference ($p<0.05$) was observed and is tabulated in table 6. The FFA content was highest in T_0 and increased from 0.79±0.12 to1.79±0.04 and 2.10±0.05 when stored at 5° and 37° respectively for 75 days. The possible explanation for increase in FFA's values during storage is the lipolytic breakdown of fat during storage. Shakerardekani *et al.* (2015) observed the similar trend during storage studies of their respective spreads.

Table 5: Effect of carotenoid-enriched table spread on thiobarbituric acid value (OD) at different days of storage.

Storage days	Storage Temp(°)	Treatments				
		T_0	T_1	T_2	T_3	T_4
0	5	0.428±0.011	0.417±0.004	0.407±0.007	0.391±0.04	0.358±0.01
	37	0.428±0.011	0.417±0.004	0.407±0.007	0.391±0.04	0.358±0.01
15	5	0.448±0.008	0.420±0.006	0.420±0.025	0.401±0.01	0.362±0.004
	37	0.452±0.008	0.422±0.006	0.428±0.025	0.408±0.01	0.375±0.004
30	5	0.476±0.025	0.480±0.005	0.436±0.005	0.423±0.007	0.387±0.007
	37	0.497±0.025	0.454±0.005	0.446±0.005	0.435±0.007	0.393±0.007
45	5	0.502±0.004	0.498±0.007	0.486±0.005	0.468±0.008	0.402±0.009
	37	0.513±0.004	0.503±0.007	0.493±0.007	0.478±0.008	0.418±0.009
60	5	0.560±0.004	0.552±0.004	0.543±0.005	0.532±0.008	0.526±0.004
	37	0.592±0.008	0.584±0.007	0.571±0.007	0.568±0.004	0.562±0.008
75	5	0.593±0.012	0.587±0.012	0.573±0.004	0.562±0.007	0.558±0.007
	37	0.630±0.008	0.624±0.008	0.618±0.008	0.606±0.008	0.598±0.009

Table 6: Effect of carotenoid-enriched table spread on free fatty acids (%) at different days of storage.

Storage days	Storage Temp(°)	Treatments				
		T_0	T_1	T_2	T_3	T_4
0	5	0.91±0.04	0.89±0.08	0.86±0.02	0.83±0.02	0.79±0.12
	37	0.91±0.04	0.89±0.08	0.86±0.02	0.83±0.02	0.79±0.12
15	5	1.02±0.09	0.98±0.02	0.94±0.02	0.92±0.014	0.80±0.09
	37	1.09±0.09	1.02±0.02	0.98±0.02	0.96±0.014	0.83±0.09
30	5	1.16±0.08	1.12±0.08	1.08±0.12	1.02±0.13	0.89±0.02
	37	1.17±0.08	1.16±0.08	1.11±0.12	1.06±0.13	0.91±0.02
45	5	1.49±0.08	1.40±0.04	1.36±0.08	1.24±0.05	1.20±0.05
	37	1.60±0.05	1.52±0.04	1.44±0.08	1.37±0.03	1.28±0.02
60	5	2.02±0.02	1.88±0.09	1.65±0.02	1.41±0.02	1.35±0.04
	37	2.82±0.08	2.45±0.08	2.18±0.03	1.69±0.05	1.60±0.02
75	5	2.20±0.09	2.04±0.02	1.93±0.08	1.85±0.08	1.79±0.04
	37	3.19±0.05	2.62±0.05	2.42±0.05	2.27±0.09	2.10±0.05

CONCLUSIONS

The experiment was carried out with 4 treatments and 3 replications. Table spread was developed with different proportion of carotenoid rich emulsion. The prepared table spreads were stored for 75 days and storage study on qualitative and bio-functional properties were carried out in an interval of 15 days. Treatment T_2 with 6% carotenoid rich emulsion was the best among all other treatments after proper sensory analysis.

FUTURE SCOPE

In the present study carotenoid-enriched table spread has more health benefits compared with table spreads available in local market. Carotenoid enriched table spread has higher future scope in uplifting existing vitamin A defficiency in the country. The research work put forward added benefits and extended scope in various research field. Now there is possibility to fortify table spread with other essential nutrients with biofunctional properties which can have positive correlation to the human health.

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Conflict of Interest. None.

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