Effects of Rosemary extracts on oxidative stability of chikkis fortified with microalgae biomass

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Abstract The present study evaluates the oxidative stability in chikkis enriched with omega 3 fatty acids using natural antioxidant from *Rosmarinus officinalis*. The best condition for the extraction of phenolic compounds from *Rosmarinus officinalis* L. (rosemary) was established, and the antioxidant activity was demonstrated based on inhibition of DPPH free radical formation. *Nannochloropsis oculata* and *Isochrysis galbana* are rich sources of Eicosapentanoic acid (EPA) and Docosahexaenoic acid (DHA). Biomass of these microalgae were incorporated in chikkis as omega 3 fatty acid source. Effects of addition of natural and synthetic antioxidants (BHA) on oxidative stability of chikkis were analyzed for storage period of 2 months. Evaluation of peroxide value (PV) and fatty acid profile showed that the process of oxidation slowed down. Natural antioxidant was found to be more effective when compared to synthetic antioxidant (BHA). Omega-3 PUFA levels (EPA+DHA) of 75 and 240 mg/100 g chikkis were observed if enriched with 1 and 3 % *Nannochloropsis oculata* biomass respectively. Similarly, Omega-3 PUFA levels (EPA+DHA) of 102 and 320 mg/100 g chikkis were observed if enriched with 1 and 3 % *Isochrysis galbana* biomass respectively. The effects of microalgae and antioxidant incorporation on the chikkis showed that color values remained stable during storage period of 2 months with no significant change ($P < 0.05$) in texture. Sensory evaluation revealed that up to 3 % microalgal biomass incorporation was positively classified and accepted.

Keywords Marine microalgae · Food fortification · Lipid oxidation · Antioxidants · PUFA

Introduction

The conception of healthy food has been evolving with time all over the world. Currently, consumers are increasingly making better food choices that provide health benefits. Functional food generally contains one or more functional ingredients that provide an additional health benefit in addition to the energetic and nutritional aspects that every food must confer (Plaza et al. 2010). Functional food ingredients have biological benefits beyond normal nutrition and have the potential to improve human health. These ingredients are available as dietary supplements or may be incorporated into food products.

Chikki is one of the popular and traditional ready-to-eat indian sweet generally made from roasted peanuts (*Arachis hypogaea*) and jaggery. It is a very popular sweet item in both rural and urban South Asia (spanning India, Pakistan, Bangladesh, Nepal and Sri Lanka). They are also known popularly as Peanut brittle in western countries. Jaggery is a concentrated product of date, cane juice or palm sap without separation of molasses and crystals. It contains proteins, minerals and vitamins and a potent source of iron and copper (Manay and Swamy 2001). There are different varieties of chikkis in addition to the most common peanut chikki. Usually, ingredients such as puffed or roasted bengal gram, sesame, puffed rice, beaten rice and khobara (desiccated coconut) are used (Chetana and Sunkireddy 2011). If properly stored it has a relatively long shelf life. Because of their acceptability among all age groups, longer shelf life and better taste, they are considered as a good product for Omega 3 fatty acid fortification and nutritional improvement.

Long chain omega-3 fatty acids (LC PUFA) decrease the risk of cardiovascular diseases, certain types of cancer, and

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autoimmune disorders. Moreover, omega-3 LC PUFA has a positive impact on the proper development and function of the central nervous system as well as visual properties (Bermúdez-Aguirre and Barbosa-Cánovas 2011). The common omega-3 LC PUFAs are eicosapentaenoic and docosahexaenoic acids (EPA C20:5 and DHA C22:6). Approximately, 5–10 % of dietary α -linolenic acid (ALA) can be converted to EPA, and an additional 2–5 % may be further converted to DHA in healthy adults (Whelan and Rust 2006). For these reasons, daily diet should ensure adequate intake of ω -3 PUFAs.

But the problem of lipid oxidation could lead to off-flavor development in foods and reduces the nutritional and economic value of food products (Li et al. 2013). Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxy toluene (BHT) are extensively used as food additives, but their safety has been questioned (Mildner-Szkudlarz et al. 2009) stimulating the search and evaluation of natural compounds with antioxidant properties. Many naturally-occurring compounds from herbs and spices have been extensively studied for their antioxidant activity (Martínez et al. 2013). Among these, rosemary extracts (RE) have been widely used in food systems.

Currently oily fish (e.g. salmon, mackerel, sardines) and fish oils are the major dietary sources of EPA and DHA whereas canola, walnut, soybean, and flaxseed oils contain ALA (18:3 ω 3) (Kris-Etherton et al. 2003, Khandelwal et al. 2013). Declining fish stocks, fishy odor, seasonal variations in quality (Belarbi et al. 2000) as well as the presence of pollutants such as heavy metals and polychlorinated biphenyls (PCBs) in fish (Domingo and Bocio 2007) may compromise consumption of fish as the major source of EPA and DHA. Moreover fish oil does not meet the needs of a vegetarian diet. Therefore; other dietary sources of EPA and DHA are being sought. Food enrichment is probably the best long-term solution to boost intake of long chain ω -3 PUFA (Molendi-Coste et al. 2011). Microalgae are the primary producers of PUFAs in the trophic chain (Barclay et al. 1994). This favors production of EPA and DHA from microalgae.

Isochrysis galbana (Ig) belongs to the class Haptophyceae and recognized as a natural rich source of EPA and DHA. These fatty acids accumulate as oil droplets in prominent lipid bodies in the cell (Liu and Lin 2001). *Nannochloropsis oculata* is a marine eustigmatophyte currently cultivated in many aquaculture hatcheries as the basis of an artificial food chain due to its high EPA content (Huang et al. 2013).

The present research work aims at utilization of biomass from *Nannochloropsis oculata* and *Isochrysis galbana* as a natural source of Omega 3 fatty acids in chikkis. In addition, the effects of natural and synthetic antioxidants on oxidative stability of chikkis were analyzed.

Materials and methods

Peanut (*Arachis hypogaea*) and jaggery were used as raw materials. All ingredients were purchased from the Spencer's Daily super market, Chennai.

Microalgal cultivation

Nannochloropsis oculata and *Isochrysis galbana* used in this study were obtained from Central Institute of Brackishwater Aquaculture (CIBA), Muttukadu, Chennai. The microalgae were cultured in 20 L bubble column glass photo bioreactors with bubbling air and 2 % CO₂. Microalgae was cultured using Conway medium and kept illuminated with fluorescent lamps (Philips TLM 40 W) at an irradiance level of 175–190 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with photoperiod 12:12 h (L:D). The cultures were continuously stirred by filtered air and kept at 24 $\pm$ 1 °C until cell growth reached early stationary phase. The culture salinity was maintained at 25 g/L. The microalgae biomass recovered was concentrated by centrifugation (5,000 rpm, 10 mins) and freeze drying.

Growth was monitored by counting cells with the help of Neubaer Haemocytometer.

Chikki preparation

Peanuts were roasted at 120 °C until it turns golden brown in color. Roasted peanuts were dehusked, degermed and crushed into small pieces in a domestic mixer. Clear jaggery syrup (comprising sugar and water) was heated, immediately pre-weighed, roasted and added with dehusked peanuts. It was mixed thoroughly till the nuts get coated with jaggery syrup. It was then transferred to a wooden mould and rolled uniformly with help of a roller. Later it was placed in a steel plate for cooling and then cut into uniform slabs and packed.

Nannochloropsis oculata and *Isochrysis galbana* biomass were added to it at 1.0, 2.0 and 3.0 % concentration levels (w/w). Peanut composition was adjusted according to the level of microalgae added. Chikkis without biomass was prepared as control. After preparation, chikkis were stored in bottles at room temperature and protected from light for 2 months.

Preparation of extracts

Leaves of *Rosmarinus officinalis* were macerated and placed in a closed vessel. The extract was mixed with solvents (hexane, methanol, ethanol and water) in the ratio of 1:5 (w/v) individually and kept for shaking overnight. The extract from each solvent was concentrated by vacuum rotary evaporator at 50 °C. The obtained solvent free extract was stored in amber colored air-tight containers at 4 °C, until further use.

Addition of antioxidants in chikkis

Synthetic antioxidant BHA (200 ppm) and Rosemary extracts (RE) (1–5 %) were added to chikkis that contained 3 % microalgal biomass. After the addition of antioxidants, chikkis were stored at room temperature (27 ± 2 °C) for a period of 2 months and analyzed periodically (15 days) for oxidative stability.

Proximate analysis

The proximate chemical composition of microalgal biomass and chikki was performed according to the methods of AOCS (2006): dry weight (DW) by drying in an oven at 105 °C until constant weight; crude protein ($N \times 6.25$) by the Kjeldahl method after acid digestion; fat content by Soxhlet extraction with petroleum ether and moisture by oven dry method. All the analysis was done in triplicates.

Determination of antioxidant activity

Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical inactivation method. Ethanolic extracts (50 µL) were mixed with 6×10^{-5} M methanolic DPPH• solution (2.00 mL) in 1 cm path length disposable cuvette (Bandoniene et al. 2002). The decrease in absorbance at 515 nm was recorded after incubation period of 20 min at room temperature. The absorbance was read on UV spectrophotometer. The percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical was calculated according to the formula:

$$\text{DPPH}^\bullet (\% \text{ inhibition}) = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

where A_{blank} - is the absorbance of the blank solution and A_{sample} - is the absorbance sample and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical solution after 20 min.

Peroxide value

Oil from chikki was extracted with chloroform, solvent was removed under vacuum and peroxide value (PV) was determined as per AOCS (1995) procedure.

Color measurement

The chikki color was measured instrumentally using a Minolta CR-300 (Japan) tri-stimulus colorimeter (8 mm Ø contact area). The results were expressed in terms of L^* , lightness (from 0 (black) to 100 (white)), a^* , greenness/redness (from $-a^*$ (green) to $+a^*$ (red)) and b^* , blueness/yellowness (from $-b^*$ (blue) to $+b^*$ (yellow)), as per CIELab system. The measurements were conducted under constant lighting conditions,

at room temperature (27 °C) using a white control ($L^*98.76$, $a^*-0.04$, $b^*2.01$), replicated 5 times in duplicate.

Measurement of texture

Chikki texture was measured objectively by following triple beam snap method using a texturometer TA.XT-Plus (Stable Micro- Systems, UK). Sample was taken and 3-point bending/breaking test was performed using a load cell of 100 N with 6 replicates with a cross head speed of 50 mm/min. The peak force at break, representing breaking strength (N) was recorded and average values were reported.

Lipid extraction from microalgae

Lipid was extracted from lyophilized algal biomass using modified method of Bligh and Dyer. Freeze-dried cells (100 mg or more) were weighed accurately in a 15 mL centrifuge tube. For extraction, 3 mL chloroform: methanol (2:1) containing 0.5 mg/mL butylated hydroxytoluene (BHT) were used and the tube was shaken gently overnight at room temperature. After centrifugation at $1,500 \times g$ for 5 min, the supernatant containing the extracted lipid was stored at 4 °C. The extract was evaporated in a water bath (40 °C) to remove solvents. The final lipid concentration was determined gravimetrically.

Fatty acid profiling

The fatty acid composition of oil in chikki was determined by extracting oil from chikki with chloroform and converting into fatty acid methyl esters (FAME) using 14 % boron trifluoride/methanol as per AOCS (1995) method. The fatty acid methyl esters (FAMES) were extracted with hexane and analyzed by Agilent 7890 gas chromatography equipped with a flame ionization detector and a Supelco sp-2380 A capillary column (60 m $\times$ 250 µm $\times$ 0.2 µm) with helium as a carrier gas at a flow rate of 0.7 mL/min. One micro liter sample was injected in the split (20:1) injection mode. The inlet and detector temperatures were 260 °C, and the oven temperature was programmed at an initial temperature of 100 °C, then raised to 250 °C, in steps of 10 °C min⁻¹ and maintained at 250 °C for 3 min. Fatty acid methyl esters were identified by chromatographic comparison with authentic standards (Sigma Chemical Co., St. Louis, MO).

Sensory evaluation

A sensory evaluation of the fresh and stored chikkis (2 months) containing microalgae biomass along with natural antioxidant were observed in order to evaluate the impact of microalgal biomass incorporation on the sensorial performance. Sensory attributes like color, texture, odor, sweetness and overall acceptability for all samples were assessed using nine point

hedonic scales by a group of nine panelists. Fresh and stored samples were served in random order to the panelists, whom were asked to taste and rate the attributes. Hedonic scale was in the following sequence: like extremely - 9, like very much - 8, like moderately - 7, like slightly - 6, neither like nor dislike - 5, dislike slightly - 4, dislike moderately - 3, dislike very much - 2, dislike extremely - 1 (Stone and Sidel 1985).

Statistical analysis

All analyses were carried out in triplicate. Duncan's Multiple Range Test was applied to differentiate among the means of different samples ($p \leq 0.05$).

Results and discussion

Proximate analysis

Quality and stability of the product was determined by its moisture content. It was observed to be in the range of 2.5–3.5 % for chikki and microalgae. This was in agreement with the results reported by Chetana and Yella Reddy 2011. *Nannochloropsis oculata* and *Isochrysis galbana* freeze dried biomass have protein content of 36 ± 0.2 g/100 g DW, 39.2 ± 0.1 g/100 g DW and fat content of 22.1 ± 0.3 g/100 g DW, 23.4 ± 0.1 g/100 g DW respectively. Protein and fat content of chikki was 13 ± 0.3 g/100 g DW, 23.2 ± 0.2 g/100 g DW respectively.

Antioxidant activity in extracts

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al. 2002). The percentage inhibition of DPPH for BHA was found to be 88.3 ± 2.08 %.

The antioxidant activities of rosemary extracts varied significantly ($P < 0.05$) for solvents water, hexane, methanol and ethanol. It was found to be 41.4 ± 0.72 %, 62.55 ± 1.34 %, 77.45 ± 1.59 and 88.88 ± 1.48 % respectively. Higher antioxidant activity has been positively correlated with the concentration of phenolic compounds in extracts (Bryngelson et al. 2002; Sun et al. 2007). The greatest antioxidant activity was found in ethanol extract and the lowest in water. Polarity of the solvent plays a major role in extracting the compounds responsible for radical scavenging activity. The high antioxidant activities were due to high values of polarity. Strong antioxidant activity of rosemary was not surprising, because of various findings (Terpinc et al. 2009, Visentin et al. 2011) reported on its stabilizing effect and the presence of components such as carnosol, rosmanol, rosmariquinone, carnosic and ursolic acids etc. World-wide food regulations recognize rosemary extracts as GRAS ("generally recognized as safe") additives. There are no regulations that limit their use.

Peroxide value (PV)

PV is one of the important parameter in determining the quality of food product by the indication of degree of oxidation. The oxidative stability of Natural (Rosemary extract (1–5 %)) and Synthetic (BHA) antioxidant added in chikkis were analyzed for a storage period of 2 months. An initial PV value of 4.4 was observed for the control and the antioxidant incorporated chikkis. After 15 days of storage at room temperature (27 ± 2 °C) PV values started increasing for all the samples. After 30 and 45 days of storage PV values of control increased significantly ($P < 0.05$) when compared to antioxidant (both BHA and rosemary) incorporated chikkis. This demonstrates the effect of antioxidants in controlling the level of oxidation. After 60 days of storage, PV value of 23 was observed for control. PV values were found to be lower for chikkis incorporated with rosemary extracts when compared to chikkis

Fig. 1 PV values for 3 % *Nannochloropsis* incorporated chikkis

Fig. 2 PV values for 3 % *Isochrysis* incorporated chikkis

incorporated with BHA. It was observed that chikkis incorporated with 3 % rosemary extract provided better oxidative stability when compared to others (Figs. 1 and 2). Hence 3 % rosemary extract was chosen for further incorporation and analysis. In biscuits, addition of purified extracts of amla, drumstick leaves and raisins was reported to have an excellent antioxidant effect compared to the effect of BHA (Reddy et al. 2005). Similarly, biscuits enriched with 1 % Green Tea Extract provided better antioxidant activity when compared to BHA (Mildner-Szkudlarz et al. 2009). Combination of Rosemary extract, Ascorbyl palmitate and Tert-butylhydroquinone (TBHQ) showed best antioxidant performance on storage of walnut oil as observed by Martínez et al. 2013.

Color evaluation

With increasing range of microalgal concentration from 1 to 3 % the lightness values (L^*) decreased significantly ($P < 0.05$), which led to the formation of darker chikkis (Fig. 3). Similar results were reported in previous studies carried out with the incorporation of flaxseeds in chikkis

(Chetana and Yella Reddy 2011) and for microalgae biomass incorporated cookies (Gouveia et al. 2010 and 2008). Microalgae biomass addition exceeding 2 % increased the chikkis green color which results in negative a^* values (Fig. 4), while the yellow tonality (b^*) decreased (Fig. 5). Dominant yellow chromaticity (positive b^*) with lower a^* values was observed for control. Chikkis color parameters, L^* , a^* and b^* , remained very stable ($P < 0.05$) during the storage time period of 2 months. Incorporation of rosemary extracts did not have any significant influence on the surface color characteristics. Chikki was found to be acceptable in terms of color.

Texture analysis

Texture analysis was carried out for control, chikkis incorporated with microalgal biomass (1–3 %) and for chikkis incorporated with microalgal biomass (3 %) + antioxidant (3 %). The firmness value (breaking strength N) of the chikkis ranged from 45 to 57 N. Chikkis firmness decreased significantly ($P < 0.05$) after 45 days of storage, due to absorption of

Fig. 3 L^* values for Chikkis at different time intervals

Fig. 4 a^* value for Chikkis at different time intervals

moisture content (Chetana and Yella Reddy 2011). Addition of microalgal biomass and antioxidants did not affect the texture of chikkis. It was in accordance with the results obtained by Reddy et al. 2005 for incorporation of natural extracts in biscuits.

Fatty acid profile determination

Fatty acid profile was analyzed for control, microalgae and for chikkis incorporated with microalgal biomass (1–3 %) + antioxidant (rosemary extract 3 %). The fatty acid profile of *Nannochloropsis oculata* showed the presence of monounsaturated fatty acids as a dominant group, followed by saturated and polyunsaturated fatty acids (43, 33 and 24 %, respectively). Nineteen percent of EPA and 1 % of DHA were present in polyunsaturated fatty acids. The fatty acid profile of *Isochrysis galbana* showed the presence of polyunsaturated fatty acids as a dominant group, followed by saturated and monounsaturated fatty acids (40, 35 and 25 %, respectively). EPA content of 20 % and DHA content of 10 % were observed in

polyunsaturated fatty acids. The fatty acid profile of control showed the presence of monounsaturated fatty acids as a dominant group, followed by polyunsaturated and saturated fatty acids (45, 33 and 22 %, respectively (Table 1). This was in accordance with the fatty acid profile obtained for chikki by Pallavi et al. 2014.

International Society for the Study of Fatty Acids and Lipids recommends an intake of at least 500 mg per day of EPA and DHA (Cunnane et al. 2004). The World Health Organization and North Atlantic Treaty Organization have made formal, population-based recommendations: for EPA plus DHA, daily recommended amounts range from 300 to 500 mg/day and 800–1,100 mg/day for ALA (Kris-Etherton et al. 2003, Komprda 2012).

After 2 months of storage, fatty acid profile of chikkis incorporated with microalgal biomass (3 %) + antioxidant was analyzed. *Nannochloropsis oculata* incorporated chikkis presented PUFA- ω 3 levels (EPA + DHA) of 75 and 240 mg/100 g chikkis, for 1 and 3 % microalgal biomass incorporation, respectively (Table 1). *Isochrysis galbana* incorporated

Fig. 5 b^* values for Chikkis at different time intervals

Table 1 Fatty acid profile of Chikkis and *Nannochloropsis* incorporated chikkis (with 3 % rosemary extract). Expressed as average values (three replicates) of the percent of fatty acids

Fatty acids (%)	Control	<i>Nannochloropsis</i> biomass incorporated chikki (Fresh)			<i>Nannochloropsis</i> biomass incorporated chikki (After 2 months)		
	Chikkis	1.0 %	2.0 %	3.0 %	1.0 %	2.0 %	3.0 %
14:0	0.37 ^a	0.44 ^b	0.59 ^b	0.98 ^c	0.57 ^b	0.68 ^b	1.37 ^c
16:0	15.38 ^a	15.82 ^a	16.47 ^b	17.22 ^c	16.31 ^a	16.95 ^b	18.12 ^c
18:0	2.98	2.85	2.69	2.73	2.97	2.95	3.03
Other SFA	0.45	0.23	0.31	0.52	0.42	0.68	0.84
ΣSFA	18.73	19.34	20.06	21.45	20.27	21.26	23.36
16:1	0.11 ^a	1.79 ^b	2.65 ^c	2.28 ^c	1.64 ^b	2.36 ^c	1.88 ^{ab}
18:1	43.41 ^a	43.96 ^a	44.54 ^{ab}	44.37 ^{ab}	43.29 ^a	43.14 ^a	44.25 ^{ab}
Other MUFA	1.52	0.47	0.82	0.63	0.38	0.79	0.97
ΣMUFA	45.04	46.22	48.01	47.28	45.31	46.29	47.1
18:2	29.66 ^c	27.27 ^c	28.39 ^d	27.18 ^c	26.14 ^b	26.07 ^b	24.11 ^a
18:3	0.84 ^b	0.81 ^b	0.75 ^b	0.55 ^a	0.71 ^b	0.59 ^a	0.27 ^a
20:4	—	0.26 ^a	0.48 ^b	0.73 ^b	0.39 ^a	0.75 ^b	0.99 ^c
20:5	—	2.97 ^a	5.24 ^b	8.25 ^c	2.46 ^a	4.18 ^b	8.25 ^c
22:0	1.43 ^b	0.97 ^a	1.18 ^{ab}	0.89 ^a	1.15 ^{ab}	1.47 ^b	1.29 ^b
22:6	—	0.29 ^a	0.56 ^b	0.93 ^c	0.22 ^a	0.47 ^b	0.80 ^c
Other PUFA	1.28 ^d	0.10 ^a	0.42 ^b	0.87 ^c	0.08 ^a	0.67 ^b	0.83 ^c
ΣPUFA	35.59	32.67	37.02	39.4	31.15	34.2	36.54

chikkis presented PUFA- ω 3 levels (EPA + DHA) of 102 mg/100 g and 320 mg/100 g chikkis, for 1 and 3 % microalgal biomass incorporation, respectively (Table 2). These results

confirmed the importance of microalgae incorporation in traditional foods as a way to enrich the nutritional value by bioactive molecules. Gouveia et al. 2008 reported almost

Table 2 Fatty acid profile of Chikkis and *Isochrysis* incorporated chikkis (with 3 % rosemary extract). Expressed as average values (three replicates) of the percent of fatty acids

Fatty acids (%)	Control	<i>Isochrysis</i> biomass incorporated chikki (Fresh)			<i>Isochrysis</i> biomass incorporated chikki (After 2 months)		
	Chikkis	1.0 %	2.0 %	3.0 %	1.0 %	2.0 %	3.0 %
14:0	0.37 ^a	0.49 ^b	0.67 ^b	1.08 ^c	0.60 ^b	0.76 ^b	1.43 ^c
16:0	15.38 ^a	15.57 ^a	16.20 ^b	16.80 ^c	16.02 ^a	16.35 ^b	17.85 ^c
18:0	2.98	2.89	2.73	2.68	3.04	2.98	3.00
Other SFA	0.45	0.27	0.19	0.12	0.35	0.48	0.34
ΣSFA	18.73	19.22	19.79	20.68	20.01	20.57	22.62
16:1	0.11 ^a	0.92 ^b	1.77 ^c	2.12 ^c	0.82 ^b	1.43 ^c	1.69 ^{ab}
18:1	43.41 ^a	43.56 ^a	44.18 ^{ab}	43.96 ^{ab}	43.03 ^a	42.94 ^a	43.73 ^{ab}
Other MUFA	1.52	0.47	0.18	0.23	0.35	0.49	0.37
ΣMUFA	45.04	44.95	46.13	46.31	44.2	44.86	45.79
18:2	29.66 ^c	28.08 ^c	28.83 ^d	27.76 ^c	27.25 ^b	26.45 ^b	24.33 ^a
18:3	0.84 ^b	0.81 ^b	0.73 ^b	0.54 ^a	0.72 ^b	0.58 ^a	0.30 ^a
20:4	—	0.26 ^a	0.48 ^b	0.73 ^b	0.39 ^a	0.75 ^b	0.99 ^c
20:5	—	2.99 ^a	5.47 ^b	8.42 ^c	2.47 ^a	4.26 ^b	8.36 ^c
22:0	1.43 ^b	0.97 ^a	1.18 ^{ab}	0.85 ^a	1.14 ^{ab}	1.45 ^b	1.29 ^b
22:6	—	1.29 ^a	2.54 ^b	4.97 ^c	1.21 ^a	2.34 ^b	4.75 ^c
Other PUFA	1.28 ^d	0.10 ^a	0.37 ^b	0.27 ^c	0.08 ^a	0.37 ^b	0.53 ^c
ΣPUFA	35.59	34.5	39.6	43.4	33.25	36.2	40.54

Table 3 Sensory analysis of *Nannochloropsis* incorporated Chikkis

Sample	Color	Odor	Texture	Sweetness	Overall acceptability
Initial					
Control	8.2±0.20	8.0±0.19	7.9±0.23	7.8±0.16	7.9±0.28
1.0 %	7.1±0.26	7.0±0.33	7.1±0.23	6.9±0.28	6.9±0.19
2.0 %	5.9±0.21	6.0±0.15	6.1±0.14	5.5±0.31	5.8±0.23
3.0 %	4.3±0.16	4.8±0.12	5.0±0.26	4.6±0.33	4.7±0.28
3.0 % + RE(3 %)	4.4±0.17	5.0±0.21	4.9±0.19	4.5±0.23	4.5±0.23
After 2 months					
Control	8.0±0.21	7.8±0.33	7.7±0.24	7.6±0.32	7.8±0.27
1.0 %	7.0±0.16	6.6±0.35	6.5±0.27	6.1±0.36	6.4±0.19
2.0 %	5.5±0.25	5.2±0.29	5.4±0.20	5.0±0.25	5.2±0.28
3.0 %	4.0±0.17	4.9±0.14	4.4±0.26	4.3±0.23	4.5±0.32
3.0 % ± RE(3 %)	4.1±0.15	5.1±0.21	4.3±0.18	4.4±0.19	4.4±0.24

Data are reported as means±standard deviation of triplicates

similar level of Omega 3 fatty acids in biscuits enriched with *Isochrysis galbana* biomass. Successful incorporation of *Isochrysis galbana* and *Diacronema vlkianum* biomass as a source of Omega 3 fatty acids in pasta was reported by fradique et al. 2013.

Beneficial changes achieved by increasing polyunsaturated fatty acids in chikkis are susceptible to the oxidation. PUFAs, in particular, were easily attacked by free radicals that react with their double bonds and yield products such as short-chain aldehydes, forming objectionable off-flavor compounds (Frankel 1998). As a consequence, it is important to control the intensity of this process in the products using antioxidants. Only minor changes were observed in fatty acid levels of chikkis incorporated with natural antioxidant up to 2 months of storage (Tables 1 and 2).

Sensory analysis

It is necessary to maintain typical sensorial characteristics of a product in order to meet commercial success in the development of functional foods. Chikkis without microalgae was preferred than microalgae incorporated chikkis. However chikkis incorporated with 3 % microalgal biomass were positively classified and appreciated (Tables 3 and 4). The panel identified a fishy taste for the higher concentration of microalgae (>4 %) in chikkis which may lead to weak appreciation.

Mean score for sensory evaluation of chikkis revealed that there are significant differences ($p \leq 0.05$) between various composition of biomass for sensory attributes like taste, color, flavor and overall acceptability. No significant differences ($s \leq$

Table 4 Sensory analysis of *Isochrysis* incorporated chikkis

Sample	Color	Odor	Texture	Sweetness	Overall acceptability
Initial					
Control	8.4±0.22	8.2±0.22	8.1±0.21	7.9±0.23	8.2±0.25
1.0 %	7.3±0.23	7.3±0.26	7.2±0.23	6.8±0.27	7.1±0.21
2.0 %	5.9±0.19	5.9±0.17	6.4±0.18	5.7±0.29	5.9±0.22
3.0 %	4.5±0.18	5.0±0.15	5.3±0.23	4.8±0.25	4.9±0.25
3.0 % + RE(3 %)	4.6±0.21	5.3±0.19	5.4±0.24	4.6±0.27	4.9±0.21
After 2 months					
Control	8.2±0.20	7.9±0.21	7.9±0.25	7.8±0.23	7.9±0.24
1.0 %	7.2±0.17	6.8±0.23	6.7±0.26	6.2±0.25	6.7±0.23
2.0 %	5.7±0.22	5.3±0.22	5.6±0.21	5.2±0.23	5.4±0.24
3.0 %	4.2±0.19	5.0±0.17	4.6±0.22	4.5±0.21	4.5±0.20
3.0 % + RE(3 %)	4.3±0.14	5.2±0.20	4.5±0.18	4.3±0.22	4.5±0.18

Data are reported as means±standard deviation of triplicates

0.05) were observed between chikkis incorporated with and without antioxidants.

Incorporation of edible seaweed wakame (*Undaria pinnatifida*) up to 20 % had sensorial acceptance resulting in improved amino acid and fatty acid profiles, increase of antioxidant activity, and higher content of fucoxanthin and fucosterol in seaweed pasta (Prabhasankar et al. 2009). Sensory acceptance of up to 2 % microalgal incorporation was reported for pasta by Fradique et al. 2010.

Conclusion

Nannochloropsis oculata and *Isochrysis galbana* biomass were successfully incorporated in cheap, tasty, nutritionally rich chikkis for the enrichment of omega 3 fatty acids EPA and DHA. Oxidative stability studies showed that chikkis incorporated with 3 % rosemary extract had better antioxidant effect when compared to synthetic antioxidant (BHA). Only minor losses in omega 3 fatty acid profiles were observed for stored chikkis (2 months) added with natural antioxidant (Rosemary extract). Sensory evaluation revealed that up to 3 % microalgae biomass incorporation was positively classified and accepted. Chikkis added with 3 % rosemary extract were found to be acceptable in terms of color and texture. Moreover microalgae contain high amounts of protein. The presence of carotenoids in microalgae could be used as food colorants.

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