Fortified Ghee - A Step towards Quality Enhancement and Retinol

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Abstract:
Raw and fresh Ghee samples, both home-made (Gh)& packaged (Gp), were analysed for estimating the initial FFA and PV and these remained the initial values for the entire experiment, based on which the changes in FFA & PV due to β-carotene fortified products were compared, where 'h' & 'p' denote home-made & packaged ghee respectively. Home-made ghee was itself sufficiently potent to combat against the in situ FFA generation and resulted lowest value (1.46%) amongst all the samples after 5-week experimental period and fortification of β-carotene didn't impart a remarkable role towards combating the lipid-oxidation For home-made and packaged ghee respectively, 200 & 100ppm fortifications may furnish 333 & 166.50 IU Vit A/gm, thus one can replenish the Vitamin A deficiency and may partially meet up his daily need.
Keywords: *Home-made & packaged Ghee, β-carotene fortification, Free Fatty Acid Value, Peroxide Value, Vitamin A deficiency.*

Introduction

In our daily diet ghee is one of the most important sources of essential fatty acids (Tahir, Bokhari and Adnan, 2015) (Butyric acid, Capric and myristic acid,some long chain saturated fatty acids and MUFA (mainly oleic acid) (Mehta, 2013). Ghee is generally prepared from cow's milk, buffalo's milk or a combination of two types of milk. It is a good source of fat-soluble vitamins (A, D, E and K) and Cholesterol content is very high at about 0.2–0.4% which may serve a detrimental effect on heart if it consume in excess amount (Achaya, 1949; Mehta, Darji and Aparnathi, 2015).

Rancidification is one of the major problems during storage of ghee. There are so many methods are applicable for determining the deterioration like Free Fatty Acid value, Peroxide value, Saponification value etc. (Achaya, 1949; Mehta, Darji and Aparnathi, 2015).

In this study we focused on the need for addition of antidegradating agent for arresting the degradation of ghee. Beta-carotene is one such naturally occurring and potentially strong antioxidant (Rice-Evans et al., 1997) and also have a nutraceutical value that can act towards retarding or lessening the normal degradation of ghee because plant pigment not only act as an antioxidant but also it has a photo protective effect (Clevidence and Bieri, 1993; Burton and Ingold, 1993). All those information regarding the field of research motivate us to study the effects of most important plant pigment, beta-carotene to exert effects on the shelf life of ghee in commercial manufacturing level as well as in home scale preparation (Hornero-Méndez, 2000).

Review of Literature

Literatures study reveals that there are several analytical works had been done in the field of oxidative deterioration effects on ghee or other types of fats and oils as well as the fortification of beta carotene and its antioxidant effects (Handelman et al., 1991; Castenmiller and West, 1998).

Mehta and Aparnathi (2015) suggested that FOX (ferrous oxidation-xylenol orange) method was the most suitable method tested to determine peroxide value in oxidized ghee instead of other four methods (BIS, AOAC, AOCS, IDF) which are examined by them.

In the experimental study by Asha et al. (2015), the antioxidant activities of butylatedhydroxyanisole (BHA) and orange peel powder extract in ghee stored at different storage temperatures were evaluated during storage period of 21 days and showed that the ghee incorporated with orange peel extract (OPE) showed stronger activity in quenching DPPH radicals and least development of PV, TBA and FFA than ghee incorporated with BHA and control.

A further study found that by adding beta-carotene (50 to 300µg/g) to the corn, rapeseed and sunflower oils, no significant changes was observed in saturated fatty acids but have a significant effect on unsaturated fatty acids in the tested oils (Goulson and Warthesen. 1999; Habibullah et al., 2007). The study reveal that the concentration of stearic, arachidic and behenic acid did not changed with increasing the concentration of beta-carotene. It was also observed that the presence of different concentration of each individual fatty acid in the three oils showed similar non-significant effects on the stability of the oils (Hazra, Meheta and Aparnathi, 2014).

Aims and Objectives

1. To observe the differential stage of oxidative degradation of non-fortified &beta-carotene fortified (100, 200 and 300ppm) homemade ghee and packaged ghee to control the oxidative breakdown of saturated fat for a long time by free fatty acid (FFA) value and peroxide value (PV) estimation.
2. To compare the stability or shelf-life between the fortified & non-fortified ghee for one and half month at a gap of 7 days incubation.
3. To formulate a nutrient-rich product through fortification of ghee thereby aims to control Vit A deficiency partially.

Materials and Methods

Sample Preparation:

Raw and fresh carrots were purchased from local market, washed with clean water, air dried, cut into pieces, grinded with mixer grinder and extract the beta-carotene with acetone AR grade (Merck). Extraction were done several times using magnetic stirrer to swirl the mixture for optimum extractions until the color of grinded carrot becomes faint yellow. The combined extractions containing beta carotene was then clarified through petroleum ether (40-60˚c) wet alumina columnar and eluted with acetone after freeing the soluble starchy materials.

The purified beta-carotene was then dried on water bath at 50˚C and then added to ghee in different amounts to achieve the desired concentrations of 100, 200 and 300 ppm in the final product.

Determination of Acid Value- AOCS Cd 3d-63 method (Aricetti and Tubino, 2012):

Definition: The acid value is defined as the number of milligrams of sodium hydroxide required to neutralize the fatty acids present in one gram of fat to measure the percentage of rancidity as free fatty acids (lauric acid) during decomposition of glycerides of ghee.

Principle: The acid value is determined by titrimetric method against the standard sodium hydroxide in alcoholic medium.

Apparatus: 250 ml conical flasks.

Reagents:

a. Ethyl alcohol: 95% ethyl alcohol or rectified spirit neutral to phenolphthalein indicator.
b. Phenolphthalein indicator solution: dissolve one gram of Phenolphthalein in 100 ml ethyl alcohol.
c. 0.1 N Standard aqueous sodium hydroxide solution.

Procedure:

Accurate amount of sample was taken (about 1gm) in a 250 ml conical flasks and 25 ml of freshly prepared neutralized hot ethyl alcohol was then added to the sample and again heated in hot water bath for 5 min. Titration was done in hot condition against standardised NaOH shaking vigorously during titration using 1ml of phenolphthalein as an indicator

Calculation:

$$\text{Acid value} = \frac{20 \times \text{buret reading} \times \text{strength of NaOH}}{\text{Weight in gm of the sample}} \quad (\%)$$

Determination of Peroxide Value– AOCS method (Takeshita et al., 1994; Crowe and White, 2001):

The rancidity can be analyzed by the determination of peroxide value. This is an indication of the extent of oxidation suffered by the fats.

Reagents and Solution:

1. Acetic Acid - chloroform solution (1:3).
2. Saturated Potassium Iodide solution.
3. Sodium thiosulfate solution, 0.1N.
4. 1% Starch solution.
5. Distilled or deionized water.

Procedure:

1. (±0.01) g of weighted sample was taken in a 250 ml glass stoppered flask.
2. 30 ml of the acetic acid - chloroform solution was then added by graduated cylinder.
3. Flask was swirled properly until the sample is completely dissolved.
4. After that 0.5 ml of saturated potassium iodide solution was prepared
5. Stopper the flask and swirl the contents of the flask for exactly one minute.

6. 30 ml of either distilled or deionized water was immediately added by graduated cylinder, stopper the flask and shaken vigorously to liberate the iodine from the chloroform layer.
7. Then yellowish orange colour was appeared in the solution which in turn blue grey colour by adding 1% starch solution.
8. Titration was done against std 0.1N sodium thiosulfate until the blue grey colour disappears into the aqueous solution.

Calculation:

S = titration of sample

B= titration of blank

$$\text{Peroxide value} = \frac{(S - B) \times N \text{ thiosulfate} \times 1000}{\text{Weight of sample}} \text{ (mEq/kg.)}$$

Result and Discussion

SAMPLE	FFA (%) WEEK						PV (mEq/kg) WEEK					
	0	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**
Non-fortified Ghee (G_h)	2.41	2.34	1.99	1.86	2.63	1.46	1.52	9.24	9.13	22.96	32.86	40.28
***β*-carotene fortified Ghee (100ppm) (G_hCAR1C)**		1.79	2.42	2.06	2.61	2.46		7.93	13.97	22.57	53.89	44.93
***β*-carotene fortified Ghee (200ppm) (G_hCAR2C)**		2.39	2.31	2.64	2.91	2.20		6.25	10.64	22.43	54.84	48.24
***β*-carotene fortified Ghee (300ppm) (G_hCAR3C)**		1.71	2.62	2.13	2.99	2.26		11.27	36.71	47.82	60.20	76.05

Table 1: ***Free Fatty Acid (FFA) Value & Peroxide Value (PV) of Raw &β-carotene fortified Home-made Ghee***

Non-fortified means without β-carotene.

Week means PV and FFA values were estimated in a gap of seven days.

Highlighted Values (green): lowest FFA & PV Values after 5-week

Highlighted Values (yellow): lowest FFA & PV Values in the whole experiment

SAMPLE	FFA (%) WEEK						PV (mEq/kg) WEEK					
	0	1	2	3	4	5	0	1	2	3	4	5
Non-fortified Ghee (G_h)	1.07	0.94	0.76	0.95	1.03	**1.36**	5.05	6.57	6.07	1.60	1.85	71.38
β-carotene fortified Ghee (100ppm) (G_hCAR1C)		0.97	0.83	0.93	1.05	0.59		5.66	5.42	1.56	1.35	34.88
β-carotene fortified Ghee (200ppm) (G_hCAR2C)		0.66	0.73	0.89	1.16	1.78		3.66	4.75	1.17	1.43	36.66
β-carotene fortified Ghee (300ppm) (G_hCAR3C)		0.94	0.43	0.95	0.92	1.54		7.73	5.26	1.01	1.46	53.15

Table 2: ***Free Fatty Acid (FFA) value & Peroxide Value (PV) of Raw &β-carotene fortified* Packaged Ghee**

Non-fortified means without β-carotene.

Week means PV and FFA values were estimated in a gap of seven days.

Highlighted Values (green): lowest FFA & PV Values after 5-week

Highlighted Values (yellow): lowest FFA & PV Values in the whole experiment

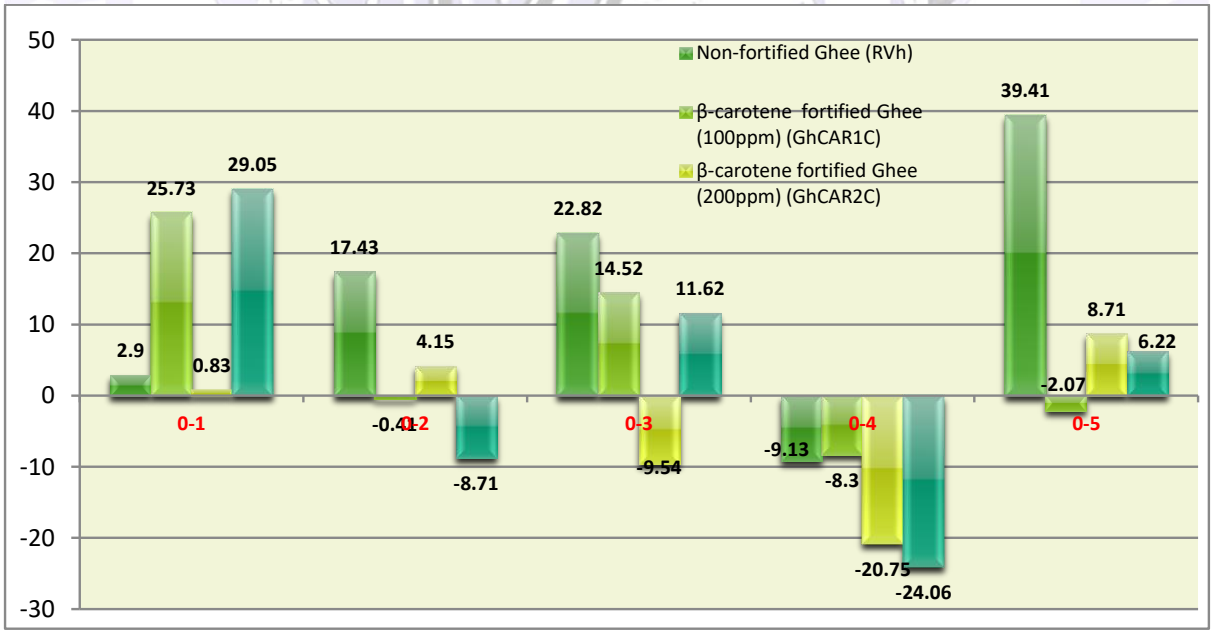

Figure 1: ***Percentage (%) change of FFA value of Raw &β-carotene Fortified Home-made Ghee***

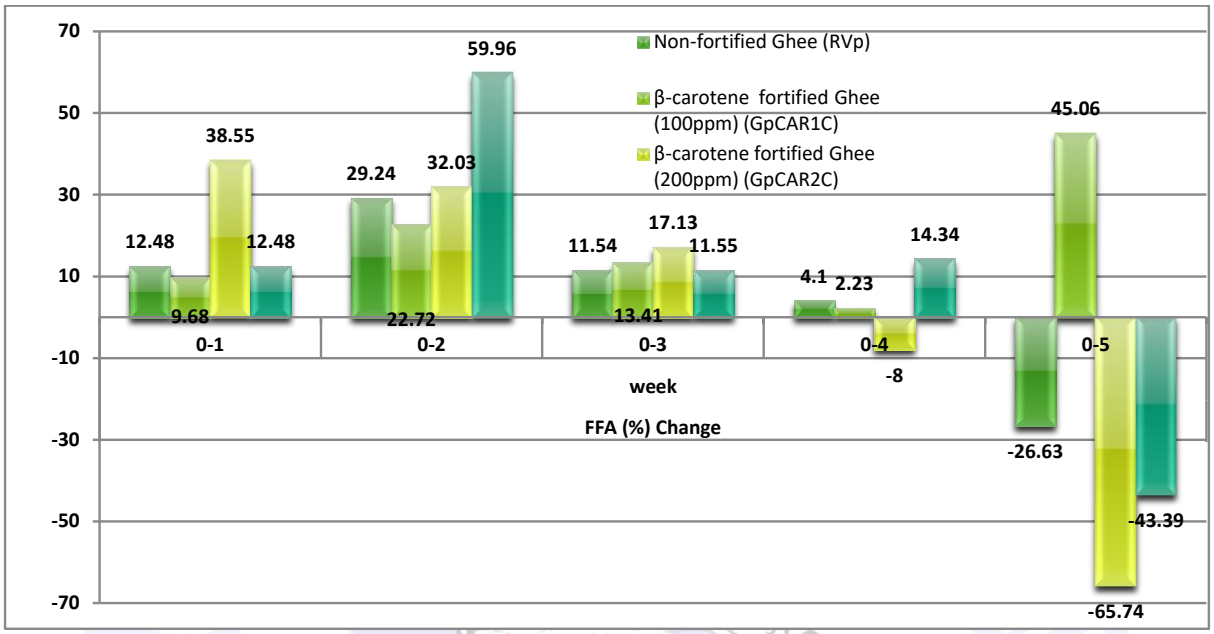

Figure 2: ***Percentage (%) change of FFA Value of Raw &β-carotene Fortified Packaged Ghee***

The samples, both home-made (Gh)& packaged (Gp), were analyzed for estimating the initial FFA (Gh: 2.41%; Gp: 1.07%) and PV (Gh: 1.52mEq/kg; Gp: 5.05mEq/kg)and these remained the initial values for the entire experiment, based on which the changes in FFA & PV due to beta-the samples was fortified with carotene in different concentration like 100ppm:GhCAR-1C, GpCAR-1C; 200ppm:GhCAR-2C, GpCAR-2C; 300ppm: GhCAR-3C, GpCAR-3C were compared. The experiment was carried out for a period of five (5) weeks at 32-34ºC temperature in 60-85% humid condition. All the samples were kept open for 1hour each day to have the normal home storage and usage effect. Data were taken after an interval of 7 days from the beginning.

A. Free Fatty Acid (FFA) Value:

The FFA of the non-fortified samples changed from the initial 2.41% to 1.46% for Gh and from 1.07% to 1.36% for Gp, during the 5-week experimental period. Three fortified products ended up at different degradation levels. While for home-made ghee the final FFA values ranged from the lowest 2.20% (GhCAR-2C) to the highest 2.46% (GhCAR-1C), for packaged ghee the values ranged between 0.59% (GpCAR-1C) and 1.78% (GpCAR-2C) (Table1 & Table2).

Home-made Ghee: It is clear from Table1, the prepared product from fresh cow milk was itself sufficiently potent to combat against the in situ free fatty acid generation and resulted lowest FFA value (1.46%) amongst all the samples after 5-week experimental period. Here, the 200ppm (GhCAR-2C) fortification proved to be the best among all fortifications done to control the FFA generation (2.20%) and GhCAR-1C proved to be the least effective and does not impart a remarkable role towards combating the oxidation of lipids.

Packaged Ghee: On contrary to from Table2 that 100ppm (GpCAR-1C) fortification depicted the highest controlling power against lipid oxidation and resulted in the lowest free fatty acid generation (0.59%) after 5-week experimental period where non-fortified and other fortified products shows higher FFA than the initial value, although GpCAR-3C resulted in lowest value during the experimental period (0.43%) after 2-week storage.

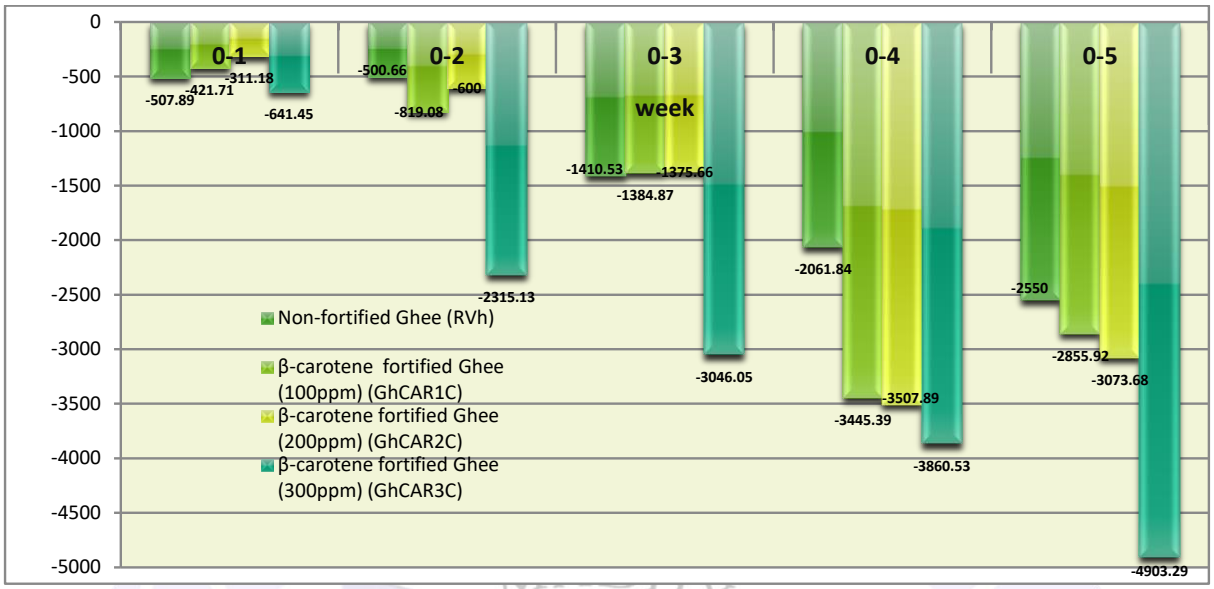

Figure 3: ***Percentage (%) change of Peroxide value (PV) of Raw &β-carotene Fortified Home-made Ghee***

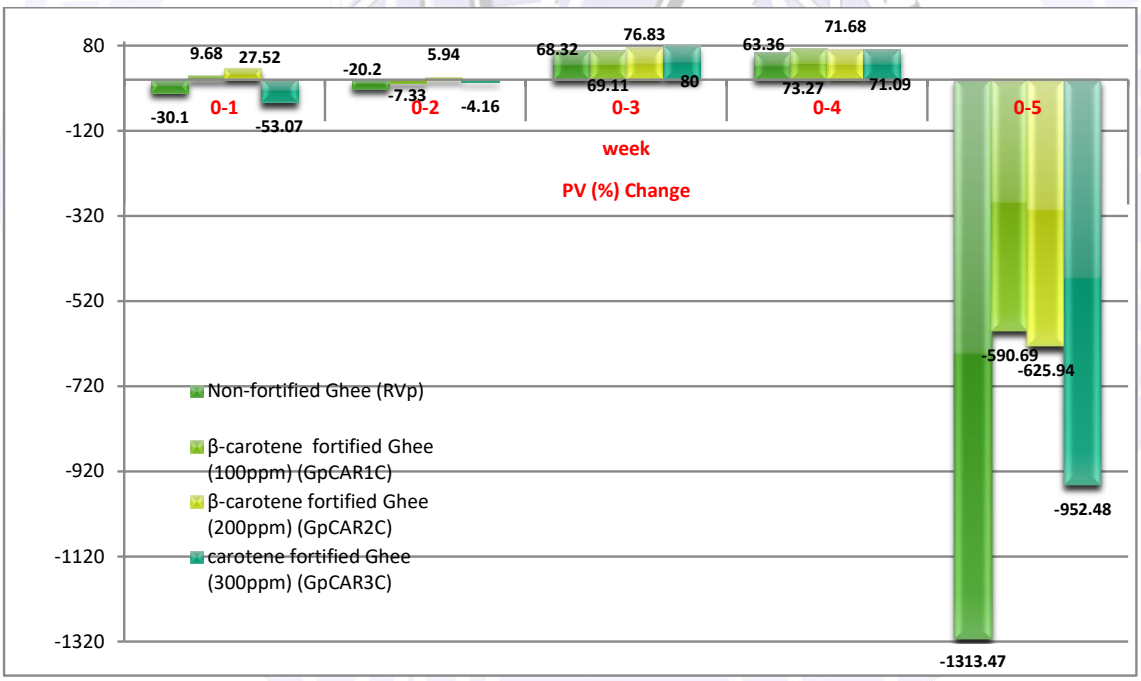

Figure 4: ***Percentage (%) change of Peroxide value (PV) of Raw &β-carotene Fortified Packaged Ghee***

B. Peroxide Value (PV)

The PV of the non-fortified samples changed from the initial 1.52 to 40.28mEq/kg for Gh and from 5.05 to 71.38mEq/kg for Gp, during the 5-week experimental period. Three fortified products ended up at different degradation levels. For home-made ghee the final PV ranged from the lowest 44.93mEq/kg (GhCAR-1C) to the highest 76.05mEq/kg (GhCAR-3C), for packaged ghee the values ranged between 34.88mEq/kg (GpCAR-1C) and 53.15mEq/kg (GpCAR-3C) (Table1 & Table2).

Home-made Ghee: Table1 depicts that home-made ghee have enough potential to quench the in situ peroxides generation and resulted lowest PV value (40.28mEq/kg) amongst all the samples after 5-week experimental period. Here, the 100ppm (GhCAR-1C) fortification proved to be the best among all fortifications and GhCAR-2C expressed comparable result with the former (48.24mEq/kg). Thus, non-fortified home-made ghee showing antioxidant property to control the peroxide formations for a period of at least 5-week and fortification ghee does not play an effective action towards combating the oxidation of lipids.

Packaged Ghee: From Table2, 100ppm (GpCAR-1C) fortification depicted the highest controlling power against peroxide formation and showed remarkable effect non-fortified product (71.38mEq/kg).

C. Retinol Availability

For a healthy population, the major factors that affect the bioavailability of food carotenoids and the bioconversion of food provitamin A carotenoids to vitamin A in humans are food matrices, food preparation, and the fat content of a meal (20). Because the body converts all dietary sources of vitamin A into retinol, 1 mcg of physiologically available retinol is equivalent to the amounts from dietary sources: 1 mcg of retinol, 12 mcg of beta-carotene. (21)

Conversion:

- 1 IU retinol = 0.3 mcg RAE
- 1 IU beta-carotene from dietary supplements = 0.15 mcg RAE
- 1 IU beta-carotene from food = 0.05 mcg RAE

As the 200ppm fortification for Gh and 100ppm fortification for Gp proved to be the best among the fortified products, retinol availability from such may be considered.

200ppm beta-carotene = 200mg per 1000gm = 200,000mcg per 1000 gm = 200mcg beta-carotene per gm.

and

100ppm beta-carotene = 100mg per 1000gm = 100,000mcg per 1000 gm = 100mcg beta-carotene per gm

Now, 0.6 mcg beta-carotene= 0.3 mcg retinol = I IU Vitamin A.

Thus, 200mcg beta-carotene = 100mcg retinol (RAE) = 100 x 3.33 = 333.00 IU Vitamin A and

100mcg beta-carotene = 50mcg retinol (RAE) = 50 x 3.33 = 166.50 IU Vitamin A

(Ref: Vitamin A: 1 IU is the biological equivalent of 0.3 mcg retinol, or of 0.6 mcg beta-carotene)

So, for the home-made ghee, a 200ppm fortification may furnish 333 IU Vitamin A per gm and for packaged ghee 166.50 IU Vitamin A per gm may be obtained from the corresponding 100ppm fortified product; thereby, one can replenish the Vitamin A deficiency and may partially meet up his daily need.

Conclusion

The experiment revealed that in home-made ghee, fortification was not fruitful and that the freshly prepared product from cow milk proved to be more efficient in combating the generation of either free fatty acid or peroxides. On the contrary, the packaged ghee from

market could not produce the same effect. It exhibited excellent quenching power as far as the FFA value & PV are concerned and in particular, the 100ppm fortified packaged ghee proved to be the best. The study revealed that due to some differences – either in processing or in ingredients, the antioxidant activity getting hindered. Therefore, further research is required to analyze the differences which counteract the natural capacity of this product.

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