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Comparative evaluation of irradiated and non-irradiated expeller-pressed virgin coconut oil for the design of novel functional antioxidant-rich non-carbonated ready-to-serve and dry beverages

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Abstract

Expeller-pressed virgin coconut oil was subjected to 0–10 kGy gamma irradiation for removal of its rancid-acid odour as judged by sensory evaluation and electronic nose systems. The optimized dose for the removal of the rancid-acid odour was 4.2 kGy. The oil was then blended with green tea extract (3:1 w/w) to produce a novel antioxidant-rich non-carbonated ready-to-serve (RTS) still beverage, which was evaluated against a control beverage prepared with non-irradiated oil. The beverages were stored at 4°C and 23°C and periodically assayed for microbial growth, sensory attributes and physiochemical and phytochemical properties. The beverage containing irradiated coconut oil had a shelf life of 13 days at 4°C. Microencapsulation of this RTS beverage produced a 'dry beverage' with appreciable phytochemical properties and a 29-times longer half-life compared to the RTS beverage. The reconstitution ratio of this beverage in water was 1:5 w/w. This is the first report of an oil-based beverage and describes a unique application for expeller-pressed virgin coconut oil. The antioxidant-rich RTS and dry beverages designed in this study could be promising novel health drinks for the beverage industry.

Introduction

India is a leading global producer of coconuts (*Cocos nucifera* L.), particularly the West Coast Tall variety [1]. Coconut oil is commonly obtained from dried kernel (copra) and is rich in antioxidants [2–5]. Our previous work established that expeller-pressed virgin coconut oil extracted from the copra of West Coast Tall coconuts is a rich source of antioxidants, chiefly phenols, alkaloids and glycosides [6]. However, coconut oil has a characteristic rancid-acid odour which is unacceptable to the general population.

The conventional method for deodorizing oil involves exposure of the oil to high temperature steam, which can, however, damage the phytochemicals [7]. Therefore, the present investigation presents an alternative approach for removing the rancid-acid odour from coconut oil by employing the non-thermal green technology of gamma (γ) irradiation. To the best of our knowledge, there are no previous reports on odour removal using γ-irradiation except for a single report on the application of γ-irradiation together with H_2O_2 oxidation for the decolourization and deodorization

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of sludge protein foaming solution [8]. It has been reported that γ-irradiation does not leave radioactive residues in food products and is safe for food applications [9]. We therefore envisage that irradiated coconut oil without a rancid-acid odour would have increased acceptability globally as an edible oil. This work aimed to formulate a beverage using γ-irradiated coconut oil.

Green tea (*Camellia sinensis* L.) is a rich source of phytochemicals such as phenols, flavonoids and polyphenols [10]. The polyphenols in green tea, such as the catechins, have antioxidant and anticancer activities [11]. To obtain a nutraceutically enriched health drink, an antioxidant-rich, non-carbonated ready-to-serve (RTS) still beverage was formulated using a blend of coconut oil (after γ-irradiation) and green tea extract. The shelf stability of the beverage was assessed microbiologically for bacterial and fungal growth and also sensorially, and its physicochemical and phytochemical properties assessed.

Since phytochemicals are sensitive to light, heat and oxygen, microencapsulation of the beverage would enhance its shelf stability and preserve its phytochemical properties. Microencapsulation using spray drying is the most popular technique since it is flexible, allows continuous operation, produces particles of good quality and is economical [12]. The microencapsulated form of this beverage could serve as a 'dry beverage'. This work describes for the first time the microencapsulation of an oil-based dry beverage.

The specific objectives of this investigation were: γ-irradiation of coconut oil for the removal of the rancid-acid odour; design of a novel health drink (a non-carbonated RTS beverage) using the irradiated oil and green tea extract; shelf life assessment of the beverage (bacterial and fungal load); assessment of sensory, physicochemical and phytochemical properties; microencapsulation of the best shelf stable beverage to obtain a 'dry beverage'; and assessment of the phytochemical properties of the dry beverage including its rehydration ability. To the best of our knowledge, this work is the first to describe an oil-based beverage, and therefore a unique application of expeller-pressed virgin coconut oil.

Materials and methods

Materials

A gift sample of a leading brand of expeller-pressed virgin coconut oil (from coconut copra of the West Coast Tall variety) with no added antioxidants or preservatives was used in this work; green tea powder (Chamong Tea Company, Kolkata) and sugar were procured from a local supermarket in Kolkata, India. Specialty chemicals and standards were procured from Sigma, USA. AR grade chemicals and reagents and media for microbiological culture were procured from Merck, India and HiMedia, India, respectively. HDPE/LDPE co-polymer screw-capped bottles were purchased from a local supermarket in Kolkata, India.

Methods

Gamma irradiation of coconut oil samples

HDPE/LDPE co-polymer screw-capped bottles were UV sterilized in a laminar flow cabinet and filled with coconut oil (500 ml). The oil samples were irradiated at doses ranging from 0 to 10 kGy in a GC 5000 unit (BRIT, India) with source ^{60}Co, at a dose rate of 5.201 kGy/h. The upper dose limit was 10 kGy since this is the maximum permissible dose of irradiation for food products at which no heat is produced and the nutritional quality of foods is unaffected [9]. The surface radiation of the GC 5000 unit was measured before the irradiation experiment with a Minirad survey meter (Pulsecho, India) and was within safe operational limits (<2 mrad). After irradiation, the oil samples were stored at 23±2°C in the dark until analysis.

Sensory evaluation of irradiated coconut oil samples

The effects of γ-irradiation on the odour profile of virgin coconut oil samples were assessed by a semi-trained panel of university faculty members and research scholars (15 men and 15 women) aged 20–45 years. The panelists were selected based on their interest and performance in a screening test. The panelists evaluated the oil samples on overall appearance, colour, odour, taste and aftertaste. All samples were coded using three-digit numbers and

served randomly to panelists. The 30 ml oil samples were given to panelists in 50 ml glass beakers. The panelists used a 9-point hedonic scale to evaluate the samples (9: like extremely and 1: dislike extremely), between 10 a.m. and 12 noon in a ventilated room under white light. Crackers were also served to the panelists as a carrier after analysis of each sample. This methodology was in accordance with reported methods where the acceptability of irradiated food products is judged by a semi-trained panel using an affective method-based acceptance test (hedonic score rating). The panel responses were represented graphically by radar plots [13, 14]. The panelists used flavour strips (Oror Flavors & Chemicals Pvt. Ltd., India) for odour analysis and were allowed a rest period of 5 min between consecutive samples. Each sample was served in triplicate in a session and rounded off mean scores were analyzed. Sensory evaluation of the oil samples revealed that the rancid-acid odour of coconut oil was removed when the oil was irradiated at 4.2 kGy (Fig. 1a). ENOVISION (C-DAC, India) and Heracles (Alpha M.O.S., France) e-nose systems further validated this finding previously published by our research group [15]. The odour profile of 4.2 kGy-irradiated coconut oil obtained by the Heracles e-nose has also been published by us [15]. This oil without a rancid-acid odour was used to formulate the RTS beverage.

Design of the RTS beverage

The RTS beverage was prepared according to the flow diagram shown in Fig. 2a. The beverages were broadly coded as B_1 (for beverage prepared with irradiated oil), B_2 (for beverage prepared with non-irradiated oil) and control oil samples as coconut oil irradiated at 4.2 kGy (C_1) and non-irradiated coconut oil (C_2) (Fig. 2b). The coconut oil:tea extract ratio, quantity of lecithin and sugar were optimized by sensory evaluation in preliminary trials at 3:1 w/w, 1% w/w and 10% w/w, respectively.

The beverages were homogenized in an Ultra-Turax homogenizer (Ika, Germany) for 10 min and immediately filled into UV-sterilized HDPE/LDPE bottles and flushed with nitrogen. They were labelled according to their storage condition: B_{1a}, B_{2a}, C_{1a} and C_{2a} for samples stored at 4±1°C and B_{1b}, B_{2b}, C_{1b} and C_{2b} for samples stored at 23±2°C (in the dark). All samples were prepared in triplicate.

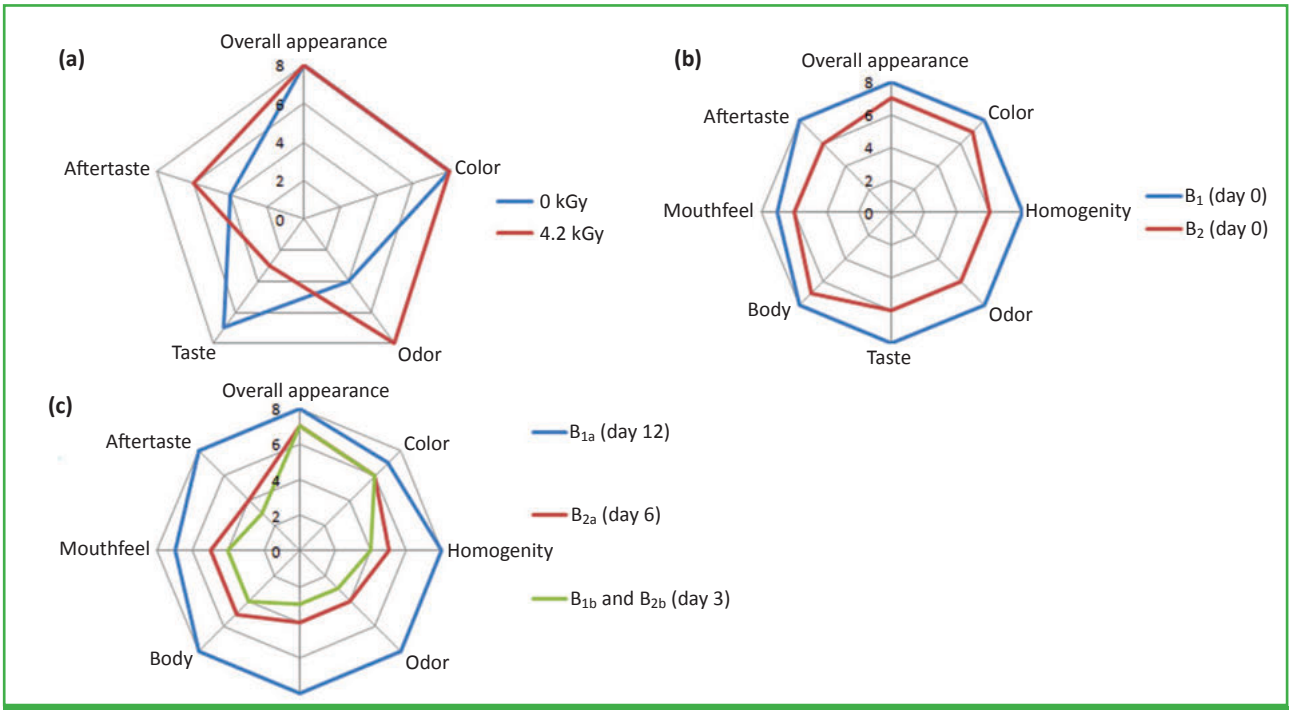

Figure 1 - Radar plots for sensory evaluation of **a** irradiated (C1) and non-irradiated oil (C2) samples, **b** beverages prepared with irradiated and non-irradiated oil evaluated on day 0, and **c** beverages on the last day of their recommended shelf life.
B_1: beverage prepared with coconut oil irradiated at 4.2 kGy, B_2: beverage made with non-irradiated coconut oil, B_{1a}: beverage made with coconut oil irradiated at 4.2 kGy, B_{2a}: beverage prepared with non-irradiated coconut oil (stored at 4±1°C), B_{1b} and B_{2b}: beverages prepared with irradiated (at 4.2 kGy) and non-irradiated oils, respectively (stored at 23±2°C)

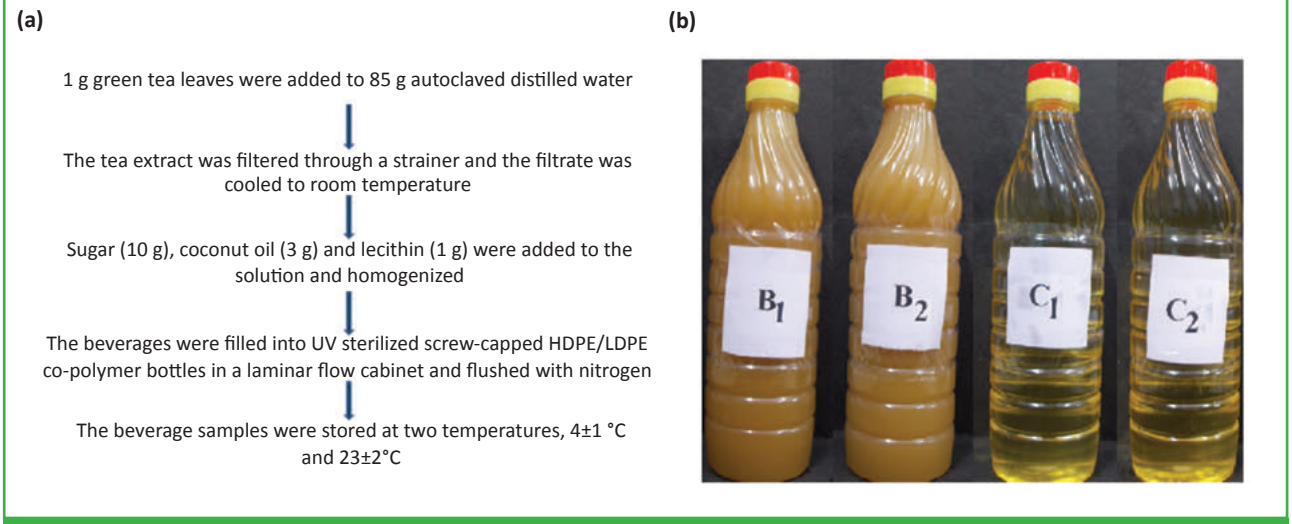

Figure 2 - Beverage preparation: **a** process flow sheet, and **b** photographs of the beverages (immediately after formulation) and control oil samples. B_1: beverage made with coconut oil irradiated at 4.2 kGy, B_2: beverage made with non-irradiated coconut oil, C_1: coconut oil irradiated at 4.2 kGy, and C_2: non-irradiated coconut oil

Proximate analysis of RTS beverages

The proximate analysis included estimation of energy [16] and used AOAC methods for the measurement of moisture (method 934.01), fat (method 920.39A), protein (method 984.13), total ash (method 942.05) and carbohydrates (by difference) [17].

Analysis of microbial growth in the beverages and oils

The total plate count of beverage samples was determined by the spread plate technique for both bacteria and fungi. The plates were incubated at 37±1°C for 24 h for bacteria and at 25±1°C for 72 h for fungi. The total plate count for bacteria and fungi was determined and represented as CFU/ml beverage. The beverages with microbial growth safe for human consumption, in accordance with USFDA guidelines [18], were subjected to sensory evaluation. Three replicate samples were analyzed during storage.

Sensory evaluation of beverages

A same semi-trained panel evaluated the beverages on the following attributes: overall appearance, colour, homogeneity, odour, taste, body, mouthfeel and aftertaste, using the same methodology as described above for oil samples. The 30 ml beverages (encoded and served at random) were served in 50 ml glass beakers and assessed by panelists on a 9-point hedonic scale, at intervals of 3 days from day 0 until day 15. This time interval and duration were chosen since in our preliminary trials we observed off-flavours from day 14 in all samples. The samples for which sensory evaluation was conducted were then subjected to physiochemical and phytochemical analyses. The mean scores of the three replicate samples were considered for sensory analyses.

Physicochemical analyses of beverage and oil samples

The physicochemical properties of beverages assayed were specific gravity, pH, total solids (gravimetrically), total soluble solids (°Brix, using a hand refractometer [Erma, Japan]), colour (Y+5R, using a Lovibond Tinctometer Model-F [The Tintometer Ltd., UK]) and viscosity (Pa·s, using a Brookfield Digital DV-E Synchro-Electric Viscometer (Brookfield Engineering Company, USA) with spindle LV-1 at 23±2°C at a speed of 3–60 rpm). Total sugar (after inversion) of beverages was assayed by the DNSA method [19]. The hydrophilic-lipophilic balance (HLB) values of the beverages and the dry beverage were calculated according to the reported method [20]:

$$HLB=20(1–SV/AV) \quad (1)$$

where SV is saponification value and AV is acid value.

The free fatty acid (FFA) value (expressed as %

oleic acid; AOAC method 940.28 [17]), peroxide value (PV) (method 965.33 [17]), *p*-ansidine value (*p*-AV) (IUPAC method 2.504 [21]) and TOTOX value (2 PV+*p*-AV) of the beverage samples were also analyzed. Specific gravity, colour, viscosity, FFA value, peroxide value, p-anisidine value and TOTOX value were assayed for C_1 and C_2.

Phytochemical analysis of the beverage and oil samples

The phytochemical parameters assayed for all three replicate beverage samples included antioxidant activity as IC_{50} values (mg/ml) by the DPPH assay [22], total phenols as mg gallic acid eq./ml beverage [23], tannins as mg tannic acid eq./ml beverage [24], caffeine as mg caffeine eq./ml beverage [25] and epigallocatechin gallate as µg epigallocatechin gallate eq./ml beverage analyzed spectrophotometrically according to the method reported by He *et al.* [26]. Antioxidant activity (DPPH assay, mg/ml) and total phenol content (mg gallic acid eq./g dry copra) were also determined for the oil samples. All quantifications were made from their respective standard curves.

Statistical analyses

One-way ANOVA was carried out to analyze the effects of different formulations on the physicochemical and phytochemical properties affecting the shelf lives of the three replicates of RTS beverages prepared in three independent experiments. A value of $p \leq 0.05$ was considered significant to establish differences in all tests. Duncan's multiple range test was used to determine significant differences between different treatments. All statistical tests were performed using STATISTICA Software version 8.0 (StatsSoft, Tulsa, OK).

Microencapsulation of the RTS beverage with the best shelf life

Microencapsulation of the best shelf stable RTS beverage was conducted using a spray dryer (B-290 spray dryer; BÜCHI, Switzerland) to obtain a 'dry beverage'. The optimized batch size, wall material composition and inlet air temperature were determined from several preliminary trials. The best combination of yield and phytochemical properties in the dry beverage was obtained with maltodextrin:gum arabic:beverage in the ratio 6.3:2.7:1. The inlet air temperature, spray gas flow rate, sample feed rate and system pressure were kept constant at 160°C, 357 l/h, 9 ml/min and –55 mbar, respectively, which were optimized from preliminary trials. After encapsulation, the dry beverage obtained was packed in aluminium foil, placed in a Ziploc pouch (Johnson, India), flushed with nitrogen and stored at 23±2°C in the dark until analysis.

Characterization of the dry beverage

The moisture content of the dry beverage was determined by AOAC method 934.01 [17]. The bulk density and tapped density of the dry beverage were also determined for evaluation of the Carr index and Hausner ratio, in accordance with the methods reported by Barman *et al.* [27]. This encapsulated beverage was further analyzed for % microencapsulation efficiency (ME) and % surface binding (SB) in terms of antioxidant activity, in accordance with the method described by Chatterjee *et al.* [28].

Study of release kinetics of antioxidants from the dry beverage

The release of antioxidant compounds from this encapsulate was studied in accordance with the method described by Chatterjee *et al.* [28] using 1 g dry beverage.

SEM analysis of the dry beverage

The outer structure of the dry beverage was studied using a scanning electron microscope (SEM). The powder was coated with gold using a vacuum evaporator (Emitech, The Netherlands) and analyzed using a FEG Quanta 250 field emission scanning electron microscope (FEI, The Netherlands) operated at 10 kV with a working distance of 8.0 mm. The scanned images were collected digitally using XT Microscope software.

Energy dispersive X-ray spectroscopy of the dry beverage

Energy dispersive X-ray (EDX) analysis of the dry beverage was conducted on a FEG Quanta 250 instrument (FEI, The Netherlands) for detection of toxic metals such as Pb, Cd, Hg, Cr, Cu and

As. The operational voltage for the instrument was 200 V–30 kV and an Everhart-Thornley (ET) detector was used for secondary electrons at high vacuum.

Study of the reconstitution of the dry beverage in water

Reconstitution of the dry beverage in water was carried out using the least quantity of distilled water required to obtain the original beverage. Preliminary trials showed that the best reconstitution was achieved when 1 g powder was mixed thoroughly in 5 ml distilled water at 60°C. The reconstituted beverage was assessed sensorially and also analyzed for specific gravity, pH, total soluble solids (°Brix), antioxidant activity (DPPH assay) and total phenol content. Each assay was conducted three times on each sample.

Study of storage of the dry beverage

The storage stability of the RTS beverage and dry beverage samples was estimated by determining their half-life values ($T_{1/2}$) when stored at 23±2°C, 80% RH. For estimation of half-lives, IC_{50} values were determined at intervals of 15 days for 60 days using the DPPH assay. $T_{1/2}$ values were calculated by determining the ratio of antioxidant activity on day 0 (TA_0) and on day (t) (TA_t). The natural logarithm of TA_0/TA_t was plotted against storage time and the slope of the line (k) was used to obtain the half-life ($T_{1/2}$=ln 2/k) [28].

Results and discussion

Proximate analysis of beverages

The results of proximate analyses of the beverages were identical. Proximate analysis showed that the beverages had a high energy content (40.63±3.00 kJ/100 ml) and high moisture content (90.94±2.50%), followed by crude fat (0.87±0.05%), protein (0.03±0.01%), total ash (0.1±0.01%) and carbohydrate (8.07±0.50%).

Microbiological studies of the beverages

The total plate count of the beverage samples showed significantly higher bacterial and fungal growth (p=0.000) in samples stored at 23±2°C compared to those stored at 4±1°C. Also, in samples stored at 4±1°C, microbial growth was in the order: $B_{1a}<B_{2a}$ and $C_{1a}<C_{2a}$ (Fig. 3a,b). Thus, irradiation caused a decrease in microbial growth in the oils as well as in the beverages. Beverage B_{1a} had the lowest total bacterial count of all beverages, demonstrating a bacterial count of 52 CFU/ml beverage and a fungal count of 36 CFU/ml beverage on day 15.

The microbial load in C_2 was significantly higher than in C_1 (p=0.00), although it was small in comparison to that in beverages. On day 15, bacterial and fungal counts were 4 CFU/ml and 3 CFU/ml, respectively, in C_{2a}, and 3 CFU/ml and 1 CFU/ml, respectively, in C_{1a} (4.2 kGy). After day 9, there was a significant increase in bacterial and fungal growth in all beverages (except B_{1a}), indicating spoilage. In particular for beverages containing non-irradiated oil, the bacterial counts were 40 CFU/ml beverage and 64 CFU/ml beverage for B_{2a} and B_{2b}, respectively, while fungal counts were 29 CFU/ml beverage and 59 CFU/ml beverage for B_{2a} and B_{2b}, respectively. This may be due to lower microbial growth in beverages stored at 4±1°C compared to those stored at 23±2°C.

Sensory evaluation of the beverages

The panel rated B_1 higher than B_2 on day 0 with respect to all beverage attributes including overall appearance, colour, homogeneity, odour, taste, body, mouthfeel and aftertaste (Fig. 1b), while B_2 was reported to have an oily taste with a characteristic coconut oil off- odour (rancid-acid odour). B_1 tasted sweeter than B_2. C_1 and C_2 were markedly different regarding odour, taste and aftertaste throughout the storage period under both storage conditions (4±1°C and 23±2°C).

Panelists rejected B_{2a} on day 9 and concluded that it should be consumed within 7 days of preparation (i.e., on or before day 6). B_{1a} became unacceptable on day 15 owing to a rancid-acid odour, non-homogeneity, disagreeable taste, thinner body and unacceptable aftertaste. Therefore, B_{1a} should be consumed within 13 days of preparation (i.e., on or before day 12). Thus, the beverage prepared with irradiated oil and stored at 4±1°C had a shelf life that was 6 days longer than that of its non-irradiated counterpart (Fig. 1c).

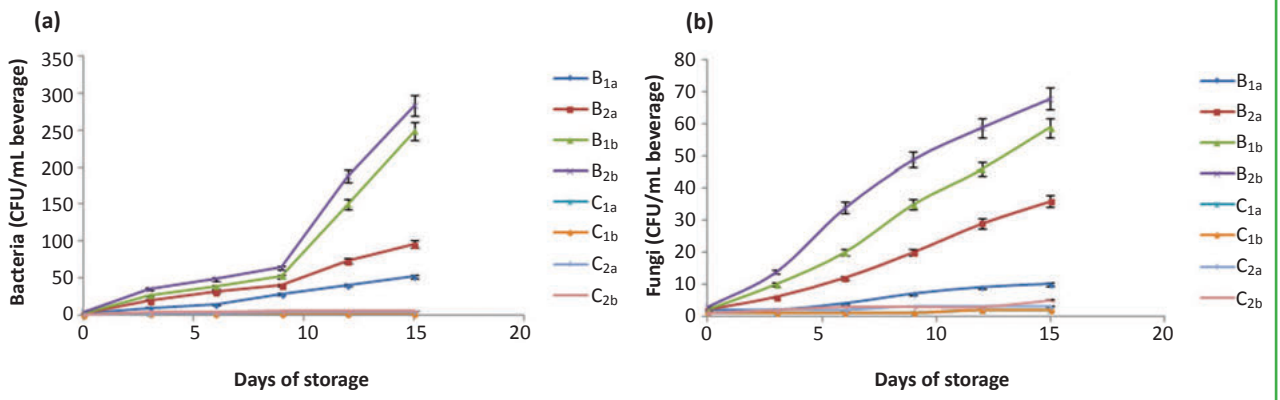

Figure 3 - Microbial growth in beverages: a bacterial growth and b fungal growth. B_{1a} and B_{2a}: beverages prepared with irradiated (at 4.2 kGy) and non-irradiated oils, respectively (stored at 4±1°C), B_{1b} and B_{2b}: beverages prepared with irradiated (at 4.2 kGy) and non-irradiated oils, respectively (stored at 23±2°C), C_{1a} and C_{2a}: irradiated (at 4.2 kGy) and non-irradiated coconut oils (stored at 4±1°C), respectively, C_{1b} and C_{2b}: irradiated and non-irradiated coconut oils, respectively (stored at 23±2°C)

For samples stored at 23±2°C, both B_{1b} and B_{2b} developed a rancid-acid odour and sour taste and had a thinner body on day 6. The panel found these samples unfit for consumption and rejected them on day 6. The panelists concluded that B_{1b} and B_{2b} should be consumed within 4 days of preparation. Beverages prepared with irradiated oil and stored at 4±1°C had a shelf life that was 9 days longer than that of beverages containing irradiated and non-irradiated oil and stored at 23±2°C. For all beverages throughout the study, the odour of beverages with irradiated oil was more acceptable than that of beverages prepared with non-irradiated oil.

Physicochemical analyses of beverage and oil samples

Changes with storage in specific gravity (from 1.01±0.01 to 1.02±0.01, p=0.280), total solids (from 90±9.12 to 95±9.50 mg/ml, p=0.651), total soluble solids (from 19.80±1.50 to 18.20±1.20°Brix, p=0.660), viscosity (Newtonian flow behaviour, from 0.054±0.02 to 0.059±0.02 Pa·s at 23±2°C, p=0.143), colour (from 21.00±1.80 to 19.50±1.50, p=0.331), HLB values (from 16.21±1.20 to 15.40±1.40, p=0.493) and p-anisidine values (from 1.93±0.10 to 1.92±0.10, p=0.280) were not significant. The HLB values of beverages showed that they were o/w type emulsions (hydrophilic).

With storage, the total solids of the beverages were in the order B_{1a} > B_{2a} and B_{1b} > B_{2b}, although these differences were insignificant. This observation was explained by the significantly higher decrease (p=0.000) in pH (from 5.98±0.10 to 3.41±0.10) and total sugar (from 14.36±1.20 to 4.78±0.34 mg/ml beverage) (p=0.000) in samples B_{2a} and B_{2b}, which may further be attributed to the higher microbial growth. These data were in agreement with sensory results where panelists reported that B_{2a} and B_{2b} had an unpleasant taste compared to their non-irradiated counterparts. The FFA, peroxide and TOTOX values in B_{2a} and B_{2b} were significantly higher than in B_{1a} and B_{1b} (Table 1). The trends in decreases in pH, total solids, total soluble solids and total sugar with storage were similar to those reported for a tamarind-based beverage [29].

Rancidity was detected in samples B_{1b} and B_{2b} earlier than in B_{1a} and B_{2a}, as was also demonstrated by sensory evaluation. Further, on day 9, physiochemical properties showed higher values indicating increased oxidation in B_{2b} compared to B_{2a}. For B_{1a}, the physicochemical properties showed significant changes on day 15, showing higher oxidation and the end of shelf life. The physicochemical properties of oil samples (C_{1a}, C_{2a}, C_{1b} and C_{2b}) such as specific gravity (0.92±0.01), viscosity (0.04±0.02 Pa·s at 23±2°C), flow behaviour (Newtonian), colour (5.17±1.28), FFA value (0.08±0.02% oleic acid), peroxide value (2.89±0.30 meq./kg oil), p-anisidine value (1.95±0.31) and TOTOX value (7.73±0.78) remained unchanged throughout the study.

Storage day	Sample	pH[z]	Total sugar (mg/ml beverage)[z]	FFA (% oleic acid)[z]	Peroxide value (meq./kg beverage)[z]	TOTOX value[y,z]	IC_{50} value of DPPH assay (mg/ml)[z]	Total phenol content (mg gallic acid eq./ml beverage)[z]	Total tannin content (mg tannic acid eq./ml beverage)[z]
Day 0	B_{1a}	5.98±0.10[a]	14.36±1.20[a]	0.59±0.02[a]	0.58±0.03[a]	3.09±0.25[a]	0.18±0.10[a]	0.30±0.01[a]	205±10.50[a]
	B_{2a}	5.96±0.20[a]	13.36±1.00[a]	0.59±0.03[a]	0.66±0.04[a]	3.25±0.30[a]	0.19±0.10[a]	0.30±0.01[a]	205±10.00[a]
	B_{1b}	5.98±0.20[a]	14.36±1.20[a]	0.59±0.01[a]	0.66±0.03[a]	3.25±0.30[a]	0.18±0.12[a]	0.28±0.02[a]	204±10.00[a]
	B_{2b}	5.99±0.20[a]	14.33±1.10[a]	0.59±0.02[a]	0.66±0.04[a]	3.25±0.30[a]	0.18±0.14[a]	0.28±0.01[a]	204±10.40[a]
Day 3	B_{1a}	5.98±0.10[a]	13.90±0.98[a]	0.59±0.02[a]	0.58±0.03[a]	3.09±0.25[a]	1.05±0.10[a]	0.30±0.02[a]	200±9.80[a]
	B_{2a}	5.97±0.20[b]	12.50±0.80[b]	0.84±0.04[a]	0.78±0.06[b]	3.49±0.32[b]	2.82±0.20[b]	0.22±0.02[b]	170±8.80[b]
	B_{1b}	4.92±0.30[c]	9.20±0.74[c]	1.17±0.10[c]	1.20±0.09[c]	4.33±0.36[c]	5.20±0.35[c]	0.18±0.01[c]	130±7.80[c]
	B_{2b}	4.52±0.30[d]	7.14±0.62[d]	2.90±0.20[d]	1.50±0.12[d]	4.93±0.40[d]	7.10±0.50[d]	0.12±0.01[d]	95±6.40[d]
Day 6	B_{1a}	5.51±0.30[a]	11.34±0.82[a]	0.84±0.07[a]	1.09±0.10[a]	4.11±0.32[a]	2.62±0.20[a]	0.24±0.02[a]	180±9.00[a]
	B_{2a}	5.10±0.30[b]	9.40±0.66[b]	2.46±0.10[b]	2.57±0.20[b]	7.07±0.60[b]	5.20±0.42[b]	0.12±0.01[b]	145±8.10[b]
	B_{1b}	3.91±0.21[c]	6.02±0.50[c]	4.02±0.30[c]	4.50±0.35[c]	10.93±0.92[c]	9.45±0.64[c]	0.08±0.01[c]	90±6.30[c]
	B_{2b}	3.82±0.20[d]	4.80±0.30[d]	6.90±0.40[d]	6.75±0.40[d]	15.43±1.10[d]	13.80±0.90[d]	0.04±0.01[d]	60±5.00[d]
Day 9	B_{1a}	4.61±0.30[a]	9.52±0.74[a]	1.17±0.10[a]	2.25±0.18[a]	6.43±0.50[a]	7.18±0.55[a]	0.12±0.01[a]	155±6.82[a]
	B_{2a}	4.02±0.20[b]	7.35±0.58[b]	2.90±0.20[b]	3.60±0.20[b]	9.13±0.80[b]	9.22±0.84[b]	0.08±0.01[b]	125±5.82[b]
	B_{1b}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	B_{2b}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Day 12	B_{1a}	3.99±0.10[c]	7.12±0.60[b]	2.90±0.25[b]	2.57±0.18[b]	7.07±0.60[b]	9.82±0.78[c]	0.10±0.05[a]	130±6.92[c]
	B_{2a}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	B_{1b}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	B_{2b}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Day 15	B_{1a}	3.41±0.10[c]	4.78±0.34[d]	6.90±0.40[d]	5.40±0.40[e]	12.73±1.10[e]	13.67±1.20[d]	0.05±0.02[e]	81±5.80[e]
	B_{2a}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	B_{1b}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	B_{2b}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

B_{1a} beverage made with irradiated coconut oil (4.2 kGy) stored at 4±1°C
B_{2a} beverage made with non-irradiated coconut oil stored at 4±1°C
B_{1b} beverage made with irradiated coconut oil (4.2 kGy) stored at 23±2°C
B_{2b} beverage made with non-irradiated coconut oil stored at 23±2°C
FFA free fatty acid, *n.a.* not analyzed (for samples which were sensorially rejected)
[x]The data showed significant difference with storage of beverages
[y]TOTOX value calculated using the *p*-anisidine value (1.93±0.1) for all beverages
[z]pH, total sugar, FFA, peroxide value, TOTOX value, IC_{50} of DPPH radical scavenging activity, total phenol content and total tannin content are the mean±SD of three replicate samples generated from three independent experiments
Different letters in a column for a particular assay and storage period indicate a significant difference at the $p \leq 0.05$ level

Table 1 - Physicochemical and phytochemical analyses of beverages during storage[x]

Phytochemical analyses of beverage and oil samples

The phytochemical analysis of beverage samples showed a significant decrease (p=0.000) in antioxidant potencies (by the DPPH assay) (from 0.18±0.10 to 13.67±1.20 mg/ml), total phenol content (from 0.30±0.01 to 0.05±0.02 mg gallic acid eq./ml beverage, p=0.000) and tannin content (from 205±10.50 to 81±5.80 mg tannic acid eq./ml beverage, p=0.000) with storage (Table 1). However, caffeine (from 0.06±0.01 to 0.05±0.01 mg caffeic acid eq./ml beverage, p=0.070) and epigallocatechin gallate (from 0.08±0.01 to 0.07±0.02 µg epigallocatechin gallate eq./ml beverage, p=0.073) did not change significantly in the beverage samples with storage. Our analysis showed that even when the beverages were sensorially rejected, they still had appreciable antioxidant properties. The decrease in antioxidants in beverage samples may be attributed to increased oxidation with storage. The residual antioxidant potencies of the beverages even when they were sensorially rejected, were due to retention of epigallocatechin gallate and caffeine, which reportedly have antioxidant activity [10, 30].

The phytochemical properties of beverages stored

Correlation coefficient (r)					Diffusion release exponent (n)	Release constant (K) (per minute)	Type of transport
Zero order	First order	Higuchi	Peppas	Hixson Crowell			
0.59	0.32	0.81	0.84	0.50	0.80	3.38	Anomalous

Table 2 - Release kinetics of antioxidants from dry beverage

at 23±2°C decreased more rapidly than those stored at 4±1°C, which may be due to the effect of storage temperature on rancidity. The phytochemical properties of irradiated and non-irradiated oils remained unchanged with storage (the antioxidant activity IC_{50} value of the DPPH assay was 0.04±0.01 mg/ml and the total phenol content was 0.96±0.04 mg gallic acid eq./g dry copra).

Microencapsulation of the best shelf stable beverage and characterization of the dry beverage

Beverage B_{1a} was microencapsulated by spray drying and yielded 62±0.90% powder (dry beverage). The moisture content of the dry beverage was 3.0±0.02% (dry basis). The bulk density of powder was calculated to be 0.42±0.01 g/ml, while its tapped density was 0.50±0.01 g/ml. Therefore for the dry beverage, the Hausner ratio was 1.19 (indicating low cohesiveness) and the Carr index was 16% (showing good flowability), in agreement with the findings of Jinapong *et al.* [31].

The total bioactive compounds were assessed using the IC_{50} values of the powder (3.24±0.10 mg/ml) and total phenol content (0.07±0.01 mg gallic acid eq./g dry beverage). %ME for the dry beverage was 72.15±1.50. %SB was low (10.00±0.50), indicating that the phytochemicals were located in the core of the encapsulate.

The release profile was found to best fit the Peppas model among all models with the highest regression coefficient r value (r=0.84). The release mechanism of antioxidants from the powder was determined from the Korsemeyer and Peppas equation [28]. The value of n in this study was determined to be 0.80, which showed that the mechanism of release was anomalous transport and followed the swelling-and diffusion-controlled-release phenomenon [28]. The value of the release constant K for the powder sample was 3.38/min (Table 2).

SEM images showed that the spray dried powder particles were mostly wrinkled with corrugated surfaces, while a few had rounded surfaces. The mean diameter was 8.0±0.92 µm (Fig. 4a and b).

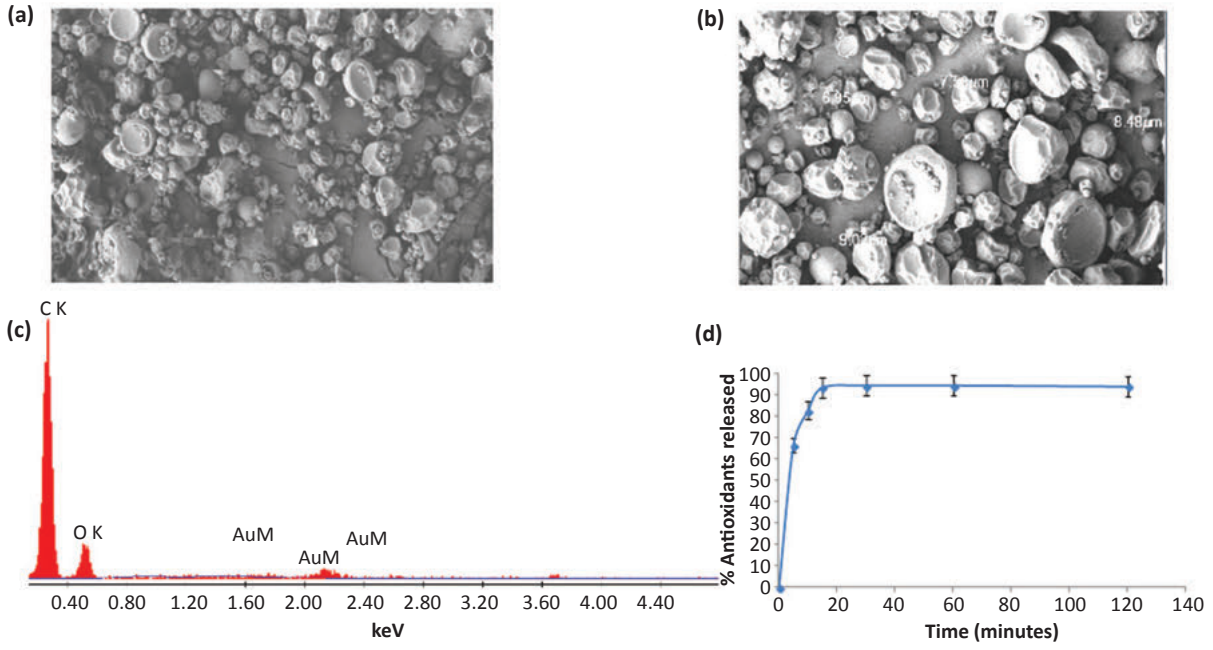

Figure 4 - Scanning electron micrograph of the dry beverage at a magnification of **a** 500× and **b** 1000×, **c** EDX analysis of the dry beverage, and **d** release profile of antioxidants from the dry beverage

Wrinkled particles were possibly formed when the micro drops were exposed to the high temperature of the drying unit, while the few that were exposed to a relatively lower temperature had rounded surfaces. Further, corrugations in spray-dried powder surfaces are known to assist dispensability [32]. EDX analysis showed that the spray-dried powder did not contain toxic metals such as Pb, Cd, Cu, Hg or As (Fig. 4c). The release kinetics study revealed that about 90% of antioxidants were released in the first 15 min (Fig. 4d). The HLB value of the dry beverage (15.80±1.00) showed it was hydrophilic.

The reconstituted beverage was similar to the original beverage in overall appearance and colour, but had a milder odour, a less sharp taste and a lighter body and mouthfeel, with no aftertaste. Overall, the reconstituted beverage was sensorially acceptable. The reconstituted beverage had a specific gravity of 1.03±0.01, pH of 6.80±0.03, total soluble solids content of 22±1.00°Brix, DPPH radical scavenging activity (IC_{50} value) of 3.52±0.20 mg/ml and total phenol content of 0.04±0.01 mg gallic acid eq./ml reconstituted beverage. The powder was also found to have a $T_{1/2}$ of 144 days (29-times increase) compared to a $T_{1/2}$ of 5 days for the non-encapsulated beverage.

Therefore, we suggest that the reconstituted beverage be served warm (50–60°C), while the original beverage (B_{1a}) could be consumed as a cold (4°C) RTS beverage. The specific gravity of the reconstituted beverage was slightly higher than that of the original beverage B_{1a}. The °Brix of the reconstituted beverage was also slightly higher than that of B_{1a} (19.80±1.50°Brix). Both these observations may be due to dissolution of capsulae into the reconstituted beverage.

Conclusion

A novel functional antioxidant-rich non-carbonated RTS still beverage was designed using γ-irradiated (4.2 kGy) expeller-pressed virgin coconut oil (devoid of rancid-acid odour) and green tea extract. Microbiological, sensory, physicochemical and phytochemical analyses showed that the best shelf stable beverage (B_{1a}, shelf life of 13 days) was obtained using irradiated oil with storage at 4±1°C. Microencapsulation of this beverage with maltodextrin: gum arabic (70:30) produced a novel dry beverage with 72.15% microencapsulation efficiency and considerable antioxidant properties. The $T_{1/2}$ of this dry beverage was 144 days, 29-times higher than that of the RTS beverage.

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Conflict of Interest

The authors declare no conflict of interest.

Human and Animal Rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

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