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Formulation of a novel mixed dried vegetables product for improved iron, zinc and vitamin A accessibility

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Abstract: Micronutrients are an important part of human nutrition that support survival and functioning of the body. Vegetables play a major role in the supply of micronutrients to human diet. In this study, different ratios of F1 (1:1:1), F2 (2:1:1), F3 (1:1:2) and F4 (1:2:1) by weight of solar-dried *Moringa oleifera* leaves (ML), *Ipomoea batatas* leaves (IBL) and *Daucus carota* (DC) were studied to determine the optimum mix for a novel product formulation. Atomic absorption spectrophotometer (AAS) was used for the evaluation of iron and zinc, while β-carotene was analysed by high-performance liquid chromatography (HPLC). Results for iron in the formulated products were 39.71, 58.54, 19.41 and 50.98 mg/100g for F1, F2, F3 and F4, respectively. On the other hand, zinc values were 1.75, 2.15, 1.40 and 1.80 mg/100g for F1, F2, F3 and F4, respectively. Beta-carotene values were 3.58, 4.16, 4.34 and 2.40 mg/100g for F1, F2, F3 and F4, respectively. A significant variation in zinc, iron and β-carotene among all formulations ($p < 0.05$) was observed. Formulation F2 was highly associated with zinc ($R^2 = 0.963$) and iron ($R^2 = 0.998$) and based on

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PUBLIC INTEREST STATEMENT

Vegetables are a good source of micronutrients in the diet. Micronutrients are an important part of human nutrition that supports functioning of the body. Lack of micronutrients in the diet results in several health consequences like anaemia, stunting and night blindness in particular, which are indicated as iron, zinc and vitamin A deficiencies, respectively. Solar-dried vegetables retain micronutrients that can supplement to the Recommended Dietary Allowances (RDA). This study formulated a novel product from solar-dried Moringa leaves, sweet potato leaves and carrots as an intervention to enhance iron, zinc and vitamin A accessibility to the community in need, for instance, women at reproductive age and children below age of five years who are at increased demand of micronutrients. The product was formulated in the powder form for convenient use. The product can be mixed with soup or stew and porridge to mention a few.

these parameters was selected as the best novel vegetable product formulation. Therefore, mixed solar-dried vegetables powder at a ratio of 2:1:1 for ML, IBL and DC, respectively, can be promoted to ensure the supply of iron, zinc and β-carotene in the diet throughout the year.

Subjects: Agriculture; Horticulture; Agriculture and Food; Food Additives & Ingredients; Food Chemistry; Food Engineering; FoodLaws & Regulations

Keywords: Dried vegetables; solar drier; iron; zinc; vitamin A; Moringa leaves; carrot; sweet potato leaves

1. Introduction

Vegetables represent the edible part of the plant such as stem and stalk (celery), tuber (potato), root (carrot), bulb (onion), leaves (amaranths, lettuce, Moringa), flower (globe artichoke) and fruit (tomato, cucumber, pumpkin) generally consumed raw or cooked with a main dish (Thompson, Willis, Thompson, & Yaroch, 2011). A combination of micronutrients, dietary fibre and phytochemicals makes the vegetables matrix beneficial to human health in controlling and preventing lifestyle non-communicable diseases, namely, type 2-diabetes, cardiovascular diseases, cancer, obesity and overweight (Slavin & Lloyd, 2012). Despite these benefits, vegetables are highly perishable and account for 30–50% postharvest loss in sub-Saharan Africa (Gustavsson, Cederberg, & Sonesson, 2011; Lipinski et al., 2013). Little effort to turn vegetables into valuable products makes them inaccessible throughout the year, which might be a contributing factor to micronutrient deficiencies or hidden hunger to most people globally (Gustavsson et al., 2011; Keding, Schneider, & Jordan, 2013).

Hidden hunger is the form of under-nutrition that occurs when intake or absorption of micronutrients such as iron, zinc, iodine and vitamin A is too low to maintain good health and development (Biesalski, 2013b). Contributing factors take account of poor maternal and child dietary patterns, limited access to nutritious food, increased micronutrients demand by body during pregnancy, lactation period as well as childhood stage below five years of age (Ojiewo et al., 2015; von Grebmer et al., 2014).

Globally, hidden hunger afflicts more than two billion people (Hoddinott, Rosegrant, & Torero, 2012). Iron, zinc, iodine and vitamin A are the big four micronutrients of public health concern. Zinc deficiency is indicated by a weak immune system and stunting, affecting 1.2 billion people, of which 165 million are stunted children (Bhutta et al., 2013; Saltzman et al., 2013, 2014). Iron deficiency disorder affects 1.6 billion people with nutritional anaemia, reduced physical activities, impaired cognitive development and risks of maternal death, low birth weight and premature birth (de Benoist, McLean, Egll, & Cogswell, 2008; Saltzman et al., 2014). Vitamin A deficiency is implicated by night blindness (xerophthalmia), irreversible blindness, poor epithelial health and weak immune system as well as risk of death in both pregnant women and preschool-aged children (von Grebmer et al., 2014). Global reports indicate that 19 million pregnant women and 190 million preschool children are vitamin A deficient (World Health Organization, 2009). Developing countries are at higher risks of hidden hunger as they are moving from traditional diets with minimally processed food to highly processed foods that are high in calories but poor in micronutrients. Moreover, these diets rely much on single staple foods that are energy dense, contributing to high levels of micronutrient deficiency in women at reproductive age and children below 5 years (Pauw & Thurlow, 2011). Linking micronutrients with vegetables, Fan, Olofinbiyi, and Gemessa (2013) and Pauw and Thurlow (2011) found that increased staple crops production has little impact on micronutrient deficiency in Tanzania since intake and inclusion of vegetables in diet are very low. Therefore, it is not feasible to underestimate the effect of vegetables on public health nutrition.

Increasing dietary diversification is one of the effective and sustainable interventions to prevent hidden hunger. At the same time, food fortifications with iron, iodine and vitamin A during processing have also proved to reduce micronutrient deficiencies (Zhou & Zhou, 2014). However, food fortification is affected by target micronutrient stability, bioavailability, not reaching the target population, overconsumption of micronutrients and monitoring of an individual taking fortified food (Dwyer et al., 2015). On the other hand, micronutrient supplementation to an individual is executed following diagnosis or is highly likely an individual or target population health status can be achieved by micronutrients supplementation (Zimmermann & Hurrell, 2007). Another approach is crop bio-fortification, which has met success in enhanced β-carotene in orange-fleshed sweet potatoes and cassava in Kenya, Uganda and Mozambique (Saltzman et al., 2013; Velu, Ortiz-Monasterio, Cakmak, Hao, & Singh, 2014). Likewise, processed vegetables have been reported to increase β-carotene bioavailability than meals containing raw vegetables (Biesalski, 2013a). Previous findings indicated increased blood levels of iron and vitamin A following consumption of solar-dried vegetables (Seidu et al., 2012). Moreover, it has been shown that dried *Moringa oleifera* leaves (ML) powder has been successfully used as supplement to supply micronutrients to children and women in West Africa and Uganda (Jilcott, Ickes, Ammerman, & Myhre, 2010; Kasolo, Bimenya, Ojok, Ochieng, & Ogwal-Okeng, 2010; Zongo, Zoungrana, Savadogo, & Traoré, 2013). On the other hand, mixed dried green vegetables have shown significant increase in serum retinol in women in India (Kushwaha, Chawla, & Kochhar, 2014). While other energy sources can be expensive and requires specialised equipment and trained personnel for effective vegetable drying, a cabinet solar drier uses solar energy, which is inexpensive, inexhaustible and non-polluting (Sobukola, Dairo, Sanni, Odunewu, & Fafiolu, 2007). The cabinet solar drier is simple to operate and proved to retain micronutrients and colour in dried vegetables (Kiremire, Musinguzi, Kikafunda, & Lukwago, 2010; Prakash & Kumar, 2013).

Therefore, this study aimed to formulate a novel solar-dried vegetable product using ML, *Ipomoea batatas* leaves (IBL) and *Daucus carota* (DC) for enhancing iron, zinc and β-carotene accessibility to populations affected with micronutrient deficiencies.

2. Materials and methods

2.1. Sample collection and preparation

ML was purchased from Nduruma farmers in Arusha, and IBL was obtained from AVRDC-The World Vegetable Centre farm plots Tengeru-Arusha, Tanzania. DC was purchased from Tengeru market, Arusha. ML and IBL were sorted and cleaned using tap water to remove dust, debris and any foreign matters. Carrots were sorted, cleaned, peeled and grated into thin slices of 2 mm thickness and 2–5 cm long using a grating (kitchen grater) machine. Prepared vegetables (ML and IBL) were blanched in boiling water at a temperature of 96.5 °C as described by Gamboa-Santos, Soria, Villamiel, and Montilla (2013) and Patras, Tiwari, and Brunton (2011). Grated carrots were blanched into hot water at 94 °C for 2–3 min. Blanching was performed aiming at inactivating enzymes to prevent enzymatic browning and micronutrients oxidation, sterilising vegetables, structural softening to facilitate moisture removal during drying and evaporating herb-like flavours (Chiewchan, Praphraiphetch, & Devahastin, 2010).

2.2. Drying of vegetables

Freshly prepared DC, ML and IBL, 400 g each, were blanched and loaded on the solar drier trays by spreading them to make a thin layer for effective drying during the morning hours at 0900 h. Drying was conducted from 0900 to 1700 h in the direct cabinet solar drier. Duration of drying for ML, IBL and DC was 1, 2 and 2–3 days, respectively. Constant moisture content was used to decide end of drying. Unloading of the dried vegetables from the drier was done in the afternoon when relative humidity was low to avoid moisture pickup. Dried vegetables were packed in polyethylene bags and stored at room temperature in a dark dry place for further analysis and novel product formulation.

2.3. Moisture content determination

Moisture content was determined using the oven drying method as described by Bradley (2010). Five grams of the dried formulated samples were placed in an oven at a temperature of 105 °C for 24 h. From the oven, the samples were cooled to room temperatures in a desiccator before weighing again for weight loss. Loss in weight was considered as moisture content. Moisture content was expressed in per cent.

$$\text{Moisture content (\% MC)} = \frac{(W1-W2)}{W1} \times 100$$

Here, W_1 = weight of vegetables before oven drying

W_2 = weight of dried vegetables after oven drying

2.4. Formulation of mixed dried vegetables

Dried vegetables were mixed in different formulation ratios of F1 (1:1:1), F2 (2:1:1), F3 (1:1:2) and F4 (1:2:1) ML, IBL and DC on dry weight. Then, they were ground into a fine powder using a Kenwood blender (Multi-Mill BL335). The powder was packed in polyethylene bags and stored in a dark dry place at room temperature for 7–14 days before further analysis.

3. Micronutrient determination

3.1. Mineral analysis

According to AOAC (1990) methods, dry ashing at 550 °C was performed for 6 h in a muffle furnace using 2 g of mixed dried vegetables powder on a clean porcelain crucible. The starting temperature was 450 °C, which was gradually increased to 550 °C at the rate of 50 °C/h. The obtained ash was digested in 10% HCl, filtered with an acid wash filter paper into a 100 mL flask and made up to volume using deionised water. Iron and zinc were determined using an atomic absorption spectrophotometer (AAS)-(GBC 906AA-USA). Iron absorption wavelength was set at 392 nm and zinc at 307 nm. Stock solution for zinc and iron was 1000 ppm of zinc standard and ferric nitrate for AAS (Fisher Scientific-UK), respectively. From stock solutions, standard solutions for Zn and Fe were prepared for standard curve calibration.

3.2. β-carotene extraction and HPLC quantification

Beta-carotene solvent extraction was performed using 95% n-hexane. Two grams of mixed dried vegetables powder were mixed with 10 mL of 95% n-hexane into a 50 mL polytetrafluoroethylene (PTFE) tube and shaken for 1 min. Four grams of $MgSO_4$, 1 g NaCl, 1 g $C_6H_8O_7$ and 0.5 g $Na_2C_6H_5O_7$ were added with distilled water to make 40 mL and mixed vigorously for 1 min, then centrifuged at 4000 rpm for 10 min in a Hettich ROTOFIX 32A-centrifuge. The hexane supernatant layer was transferred into a dispersive centrifuge tube containing 150 g of Primary Secondary Amine (PSA) and 900 mg of $MgSO_4$ and vortexed for 1 min, followed by centrifugation at 4000 rpm for 5 min in a Hettich ROTOFIX 32A-centrifuge. The clear supernatant was transferred into the high-performance liquid chromatography (HPLC) vial and injected into the HPLC (SHIMADZU L202347-Japan) with a column C18, 150 × 4.6, 5 µm (Thermo Scientific BDS hypersil) for β-carotene quantification. A Prominence Diode Array (SHIMADZU) detector at 436 nm wavelength was used. The mobile phase consisted of 88:10:2 (acetonitrile:methanol:ethyl acetate) at a flow rate of 1.5 mL/min (Szpylka & DeVries, 2005; Tee & Lim, 1992). All chemicals used were of analytical grade.

3.3. Data analysis

Microsoft Excel 2010 and XLSTAT software were used to organise data for descriptive statistics. R software version 3.2.1 (stats package) was used for statistical analysis. Data were subjected to one-factor analysis of variance (ANOVA) to test for significance variation (5%) of each micronutrient among dried vegetable formulations. Regression analysis was performed to find which formulation is associated more with micronutrients. Likewise, t-test was performed to compare for significance difference between micronutrients in the formulated novel product and fresh vegetables.

4. Results

4.1. Novel mixed dried vegetable products formulation

Four different formulations were prepared from a mixture of dried ML, IBL and DC in the ratio of F1 (1:1:1), F2 (2:1:1), F3 (1:2:1) and F4 (1:1:2), as shown in Table 1. Micronutrient values per formulation are as shown in Table 1. Formulation F2 indicated the highest iron (58.54 mg/100g) and zinc (2.15 mg/100g) values. On the contrary, insignificant difference in β-carotene was observed in F2 and F3 ($p > 0.05$) (Table 1). Formulation F2 showed significant differences ($p < 0.05$) in β-carotene, iron and zinc values as compared to fresh IBL and DC (Table 2). However, no significant differences in β-carotene and zinc between F2 and fresh ML were observed ($p > 0.05$) (Table 2). Furthermore, regression analysis of the individual micronutrients showed a positive association in all formulations (Table 3). Therefore, F2 was the best formulation with the highest values of iron and zinc, and hence may have contribution to Recommended Daily Allowances (RDA) as presented in Table 4.

Formulations F1 and F2 contributed 98.87% of micronutrients interaction effect. Formulation F2 was strongly positively correlated with all three micronutrients, which tended to have large values of β-carotene, iron and zinc.

5. Discussion

Four products in ratios of F1 (1:1:1), F2 (2:1:1), F3 (1:2:1) and F4 (1:1:2) were formulated from solar-dried ML, IBL and DC. Moisture content recorded was 8.43% for F1, 8.19% for F2, 8.36% for F3 and 10.98% for F4. The low moisture attained could prevent deterioration reactions, hence improving dried vegetable product storage quality for longer periods and making them available and accessible for dietary diversification throughout the year (Singh & Sagar, 2010; Vasantharuba, Banumathi, Premalatha, Sundaram, & Arumugam, 2012). All dried vegetables formulated products showed substantial amounts of β-carotene, iron and zinc to contribute to the Recommended Dietary Allowance (RDA). On performing a pairwise comparison for individual micronutrients between novel formulation F2 and each vegetable, significant differences were observed for iron, zinc and β-carotene in IBL and DC. However, there was no significant difference in zinc and β-

Table 1. Dried vegetable formulations micronutrients (mg/100 g)

Nutrients	F1	F2	F3	F4	*p*-values
β-carotene	3.58 ± 0.20	4.16 ± 0.37*	4.34 ± 0.33*	2.40 ± 0.29	< 0.05
Iron	39.71 ± 0.26	58.54 ± 0.36	19.41 ± 0.31	50.98 ± 0.40	< 0.05
Zinc	1.75 ± 0.02	2.15 ± 0.15	1.40 ± 0.01	1.80 ± 0.00	< 0.05

Key: All values represents mean ± standard error, β-carotene (n = 3), zinc and iron (n = 4). **F1** = ML:IBL:DC (1:1:1); **F2** = ML:IBL:DC (2:1:1); **F3** = ML:IBL:DC (1:2:1) and **F4** = ML:IBL:DC (1:1:2).

*No significant differences in β-carotene between vegetable formulation F2 and F3 ($p > 0.05$).

Table 2. Micronutrients values in formulation F2 and fresh vegetables (mg/100 g)

Nutrients	Formulation F2	Fresh ML	Fresh IBL	Fresh DC
β-carotene	4.16 ± 0.37	3.73 ± 0.18*	0.17 ± 0.01	6.72 ± 0.28
Iron	58.54 ± 0.36	28.24 ± 0.17	12.25 ± 0.17	3.91 ± 0.37
Zinc	2.15 ± 0.15	1.76 ± 0.17*	1.28 ± 0.19	0.41 ± 0.05

Key: All values are mean ± standard error, β-carotene (n = 3), zinc and iron (n = 4). Individual fresh vegetable β-carotene, iron and zinc values were compared with novel product F2 values ($p < 0.05$).

*No significant difference in micronutrients between novel product F2 and fresh vegetables ($p > 0.05$).

Table 3. Regression analysis of nutrients association in formulations

Nutrient	*F1	F2	F3	F4	R^2	*p*-value
β-carotene	3.58	0.58	0.77	−1.17	0.665	< 0.05
Iron	39.79	18.49	−20.44	11.09	0.998	< 0.05
Zinc	1.77	0.24	−0.35	0.04	0.962	< 0.05

Key: *Value for F1 as baseline coefficients. Values are presented as mg/100 g. A strong association of formulations and micronutrient was observed, with formulation F2 showing the highest micronutrients content.

Table 4. Iron, zinc and vitamin A Recommended Dietary Allowances (RDA)

		Nutrients		
Age groups (years)		Iron (mg/day)	Zinc (mg/day)	Vitamin A (μg RAEs/day)
Children	1–3	7	3	210
	4–8	10	5	275
Boys	9–13	8	8	600
	14–18	11	11	900
Girls	9–13	8	8	600
	14–18	15	9	700
Women	19–50	18	8	700
Pregnancy	14–18	27	12	750
	19–50	27	11	770
Lactation	14–18	10	13	1200
	19–50	9	12	1300

Source: Isselbacher & Estabrook (2001)

carotene values between F2 and fresh ML ($p > 0.05$) (Table 2). The highest mean value of iron was found in vegetables formulation F2 at 58.54 mg/100 g, followed by F4 at 50.98 mg/100 g (Table 1 and Figure 1). The lowest mean value of iron was 19.41 mg/100 g in formulation F3. Formulation F2 showed better association of iron micronutrient ($R^2 = 0.997$) compared to F1, F3 and F4 (Table 3 and Figure 2). The values of iron in all formulations were above RDA (Table 4) as reported from the USA-national academies press (nap); for instance, for women at reproductive age, RDA is 18 mg per day (Isselbacher & Estabrook, 2001). Hence, the formulated product can be a good source of iron when incorporated in the diet. The results were supported by a study conducted in Kenya by Nawiri, Nyambaka, and Murungi (2013), which reported a significant increase of haemoglobin by 4.6% among preschool children after eating dried vegetables, indicating that dried vegetable provides iron when incorporated in the diet. Moreover, anaemia prevalence in 4–9-year-old children was reduced from 37.3% to 33.3% after consuming dried leafy vegetables powder in their diet formulations in Ghana (Egbi, Gbogbo, Mensah, Glover-Amengor, & Steiner-Asiedu, 2018).

Formulation F2 indicated the highest zinc value of 2.15 mg/100 g compared to the other three formulations (Table 1 and Figure 1). Formulation F2 showed good association of zinc ($R^2 = 0.963$) (Table 3 and Figure 2) compared to F1, F3 and F4. Reported values of zinc in this study can have a good contribution to dietary intake of 2.5 mg/day for children below 5 years of age and 6.8–8 mg/day for women at reproductive age (Lukaski, 2004). Consumption of dried vegetable products has been reported to result in increased plasma and serum zinc values in different age groups (Gibson, Bailey, Gibbs, & Ferguson, 2010; Gregory et al., 2017; Osredkar & Sustar, 2011). For instance, Wijesinha-Bettoni, Kennedy, Dirorimwe, and Muehlhoff (2013) reported use of dried vegetable products to supply dietary micronutrients during the offseason period in most developing countries. Additionally, mixed dried vegetable products were reported to provide a balanced food

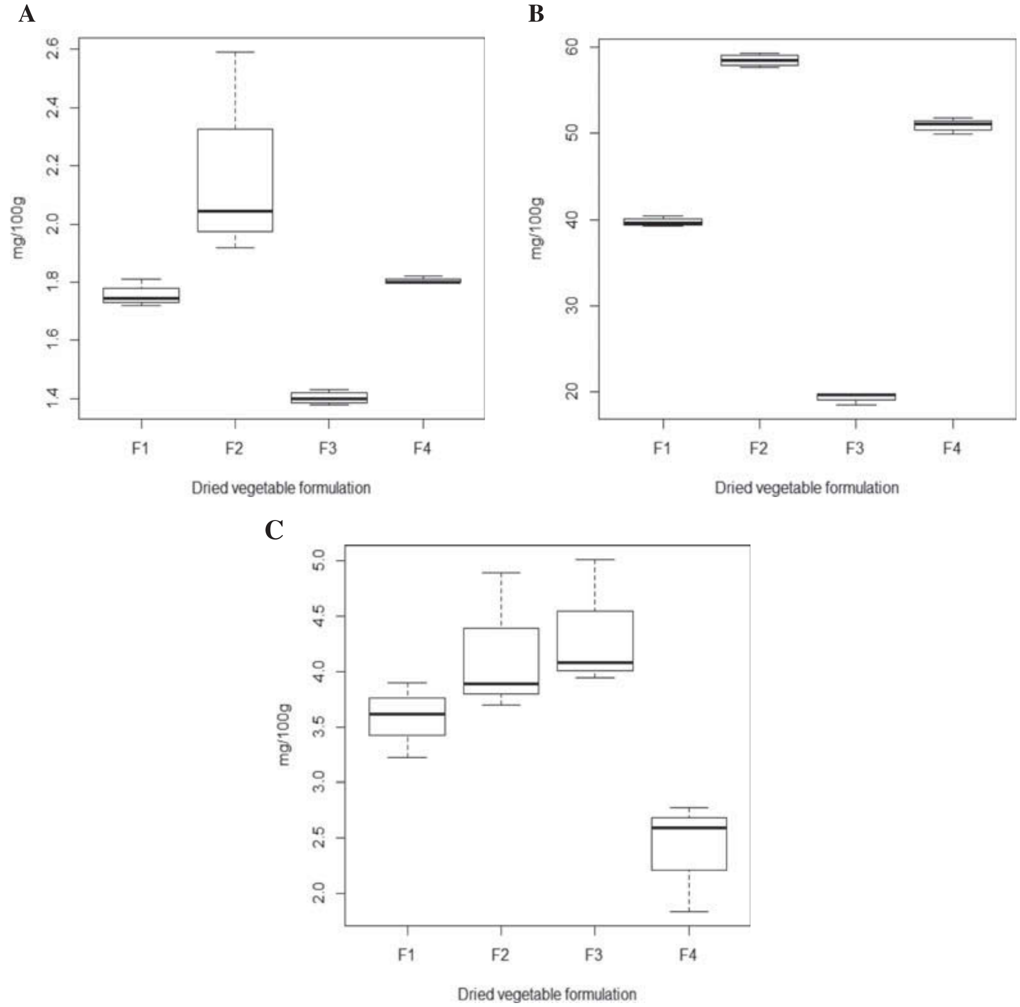

Figure 1. Box plot A: zinc; B: iron; C: β-carotene. Product F2 indicates high values of zinc and iron in box plots A and B, whereas product F3 indicates relatively high β-carotene in box plot C.

matrix that improved zinc bioavailability, and hence plasma zinc concentration (Agte, Jahagirdar, & Chiplonkar, 2006; Tidemann-Andersen, Acham, Maage, & Malde, 2011; Uusiku, Oelofse, Duodu, Bester, & Faber, 2010). Therefore, product F2 can be used in complementary foods to increase iron and zinc, as micronutrients of concern reported in complementary feeding programmes in developing countries (Dewey, 2013; Kulwa, Mamiro, Kimanya, Mziray, & Kolsteren, 2015).

Beta-carotene is both provitamin A and an antioxidant (Tang, 2010). From Table 3, it was observed that vegetable formulation F3 was highly associated with β-carotene values compared to other formulations at 4.34 mg/100 g (R^2 = 0.666). On the other hand, F4 indicated a value of β-carotene at 2.4 mg/100 g, which is 0.67-, 0.577- and 0.553-fold lower compared to F1, F2 and F3, respectively. At the same time, no significant differences were observed in β-carotene contents between products F2 and F3 ($p > 0.05$). Moreover, it was observed that all dried vegetable formulations contained low β-carotene value compared to fresh DC 6.72 mg/100 g as affected by solar drying (Tables 1 and 2). Similar findings of β-carotene losses during carrot processing have been reported due to oxidation, following exposure to solar energy at high water activity (Frias, Peñas, Ullate, & Vidal-Valverde, 2010; Sagar & Suresh Kumar, 2010). The values of β-carotene in the formulations can be converted to vitamin A in the body at an absorption rate of 7–65% and the conversion equivalence of 12 µg β-carotene to 1 µg retinol (Haskell, 2012). The observed β-carotene values can contribute to vitamin A, RDA, at 275 and 700 µg of retinol activity equivalent per day for children below five years and women at reproductive age, respectively (Isselbacher &

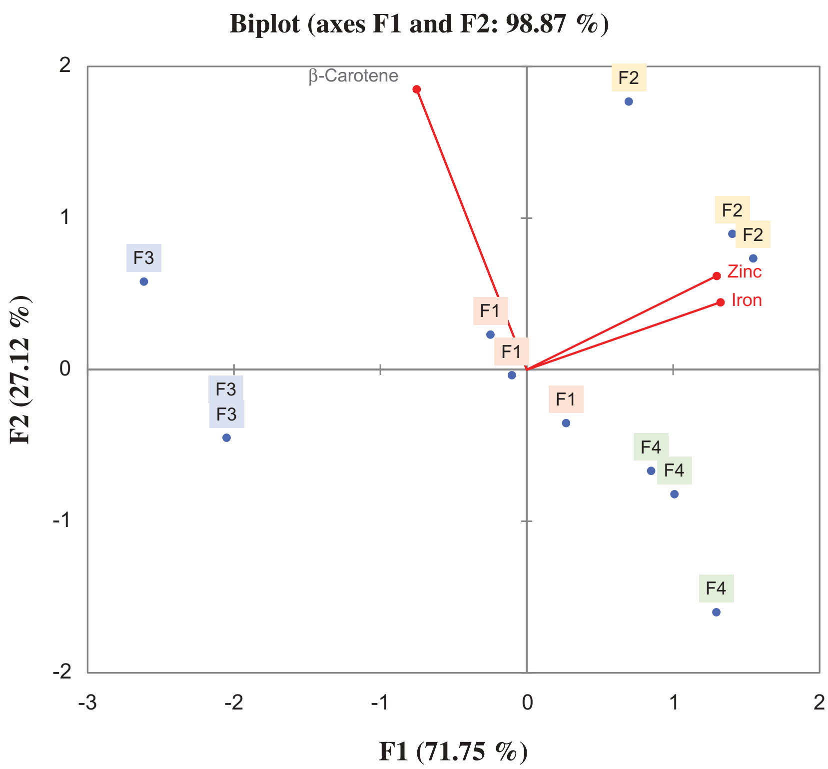

Figure 2. Vegetable product formulations micronutrient biplot.

Estabrook, 2001). Tang (2010) reported several findings that indicated increased total vitamin A body store in people with vitamin A deficiencies following consumption of vegetable products rich in β-carotene. For instance, feeding children with dried vegetables in a study conducted in Kenya resulted in increased serum β-carotene and retinol (Nawiri et al., 2013). Consumption of dried green leafy vegetables powder as an intervention to mitigate vitamin A deficiencies resulted in increased serum retinol from 26.96 ± 6.86µg/dl to 27.46 ± 7.28µg/dl in Ghanaian children 4–9 years (Egbi et al., 2018). Moreover, data from epidemiological studies from different populations have shown that diets rich in β-carotene containing foods like dried vegetables are associated with decreased risks of nutritional diseases such as nutritional anaemia and vitamin A deficiencies (Tang, 2010; Tanumihardjo, Palacios, & Pixley, 2010).

Therefore, as depicted from Figure 2, formulation F2 was considered as the good source of iron, zinc and β-carotene at 58.4, 2.15 and 4.16 mg/100 g, respectively, for enhancing their accessibility when incorporated in the diet. The formulated dried vegetables product can help address the challenges to meet nutritional need from highly cereal-based diet in the developing countries. Moreover, eating vegetable products enhances micronutrients in the diet, reduces the risks of nutritional related diseases and helps manage body weight when consumed in place of more energy-dense food (Freeland-Graves & Nitzke, 2013; Moore & Thompson, 2015). This is in agreement with various studies that had suggested dried vegetable products as good sources of iron, zinc, vitamin A and other nutrients in the diet (Arsenault et al., 2014; Kasolo et al., 2010).

6. Conclusion

Formulation F2 was shown and suggested to be superior in zinc and iron contents as well as good in β-carotene content. The novel formulated product F2 can be used to supplement micronutrients for increased micronutrients accessibility and dietary diversification as a food-based approach. The powder can be mixed with soup or incorporated in any food to suit recipes for micronutrients' accessibility. Therefore, vegetable products like mixed dried powder from ML, IBL and DC should be considered to complement other interventions for increased iron, zinc and vitamin A accessibility to the general population and communities at risk of micronutrient deficiencies.

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Competing Interest
The authors declare no competing interests.

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