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Orange-fleshed sweet potato: a potential source of antioxidants and provitamins "A" for the fortification of a local infant food flour.

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Abstract: Orange-fleshed sweet potato (OFSP) is a potential source of carotenoids (provitamins A), and its flour has been used to enrich the Misola, an infant food based flour. The new biofortified formulation presented nutritional qualities which could contribute in protein-energy recovery and improvement of the immune status of moderate malnourished children. By coupling chromatographic and spectral methods, β-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein and zeaxanthin have been identified in the extracts of Orange-fleshed sweet potato flour. The results indicated that enriching 30% of the Misola with OFSP flour causes optimal increases in carotenoid (3271%) and total antioxidant (109%) contents compared to the pure Misola. Total phenolic content was not significantly affected (only 11% relative increases) by the addition of OFSP flour. M_7J_3 represented the best formulation (802.56±5.14 kcal/ 200g) increasing the intake of energy values of about 5% and 10%, respectively, compared to Misola and OFSP pure flours.

Key words : OFSP, antioxidants, phenolics, carotenoids, FRAP, FCR

La patate douce à chair orange: une source potentielle d'antioxydants et provitamines "A" pour la fortification d'une farine infantile locale.

Résumé : La patate douce à chair orange (PDCO), une source potentielle de molécules provitamines A, a été utilisée pour enrichir une farine infantile locale appelée le Misola. La nouvelle formulation a présenté des qualités nutritionnelles qui contribuerait à la réhabilitation protéino-énergétique et à l'amélioration du statut immunitaire d'enfants malnutris modérés. En couplant des méthodes chromatographiques et spectrales, le beta-carotene, l'alpha-cryptoxanthine, la beta-cryptoxanthine, la lutéine et la zéaxanthine avaient été identifiées dans les extraits de la farine de PDCO. Les résultats ont montré qu'un enrichissement de la farine de Misola avec 30% de la farine de PDCO (M_7J_3) a entrainé des augmentations optimales des teneurs en caroténoïdes (3271%) et en antixydants (109%) totaux par rapport à la farine de Misola pure. La teneur en polyphenols totaux n'a pas été significativement affectée (seulement une augmentation de 11%) par l'apport de la farine de PDCO. M_7J_3 représente donc la meilleure formulation (802,56±5,14 kcal/200g) qui entraine une augmentation des valeurs énergétiques d'environ 5% et 10%, comparée respectivement aux farines pures de Misola et de PDCO.

Mots clés : PDCO, antioxydants, Phénols, caroténoïdes, FRAP, RFC

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1. Introduction

Vitamins are part of phytochemicals micronutrients vital for all human beings. Its deficiency limits the capacity of the body to ensure its own defense against diseases. In developing countries, vitamin A deficiency (VAD) remains a public health problem where children of 6-59 months, pregnant and lactating women are the most vulnerable groups of the population [1-3]. This deficiency is associated to children respiratory diseases, diarrhoea and immune deficiency, while for pregnant it is associated to blindness [stemming from xerophthalmia], and anemia. Sub-Saharan Africa presents the largest number of pre-school children suffering from night blindness and VAD[4]. In 1995 according to the Ministry of health and the WHO/MDIS, night blindness prevalence was 1.7% in children of 10 years old population in six provinces of the North of Burkina Faso while the night blindness prevalence was 1.2 to 7.9% in the Sanguié, Passoré and Bam provinces [5]. In 2002 in the department of Kaya, a study revealed that low serum retinol prevalence was 85% and 64%, respectively, for children from 12 to 36 months and for their mothers[1,6].

In Burkina Faso, recent statistical data in VAD that covering the whole country are not available. However, studies from different administrative districts confirmed the occurrence of VAD. To eradicate this nutritional problem, carotenoid-rich food consumption is one of the main approaches recommended in many countries [7]. OFSP has proved to be particularly promising regarding its high carotenoid content and its high bioavailability[1,8].

This work follows a previous study [9] on the identification of photosensitive molecules (carotenoids) but responsible of OFSP flour nutritional qualities. It concerned to take advantage of these microconstituants by strengthening the nutritional qualities of a local infant food flour (Misola). Then, it aims to formulate Misola-OFSP based infant food and to determine the physico-chemical properties and micronutrients in order to promote and popularize infant food that can be used in combating VAD in children under five population.

2. Materials and Methods

2.1. Materials

The OFSP flour was obtained from the storage roots of the OFSP variety called *Jewel* harvested in the centre-west region of Burkina Faso, at about 140 km from Ouagadougou. Jewel variety has been selected for the fortification of infant flour (Misola) due to its high total carotenoid content compared to other varieties grown in Burkina Faso. It is also a popular variety and presented good agronomic performances (30 t/ha). These storage roots were processed into flour using dried OFSP dried chips. The chips were dried away from sunlight to avoid the degradation of carotenoids which are photosensitive molecules.

The OFSP flour (Jewel) was added to that of Misola to improve its nutritional value including vitamin A. Misola is an infant food based flour (packaged in bags of 500 g) commercially available and widely used in nurseries in Burkina Faso.

Different proportions of this flour were mixed with the Misola for a total mass of 200 g. A total of nine (09) food flour compositions were made with the OFSP proportion varying from 10 to 90% (**Table I**).

The chosen variety of OFSP (Jewel) for the fortification of Misola flour has been previously studied to identify some of its major carotenoid molecules (photosensitive). Then, this previous study carried out in Koala et al. (2014) based on coupling TLC/MS using the system CAMAG TLC-MS interface.

Following results referred to this previous work in which carotenoid molecules were identified from the analysis of the LC-MS spectra. from the spectrum of Electrospray mass positive mode (ESI^+), five quasi-molecular ions (A, B, C, D and E) at m/z 535.4 $[M+H]^+$, m/z 537.4 $[M+H]^+$, m/z 551.4 [M+H] +, m/z 553.4 $[M+H]^+$ and m/z 569.4 $[M+H]^+$ were observed suggesting Atomic masses of 534 u, 536 u, 551 u, 552 u and 568 u respectively (figure 1). These atomic masses are characteristic of the molecular weight of some carotenoids previously reported. By analyzing mass spectra, it is clear that in addition to ordinary molecular ion peaks, there were very intense peaks so-called pseudo-molecular ions. Their high intensity reflected their greater stability. These pseudo-molecular peaks derived from fragmentation of molecular ions with appropriated structures [10,12]. The molecular ion $[M+H]^+$ m/z 537.4 of peak B primarily refer to β-carotene and lycopene, the α-carotene, γ-carotene, ε-carotene. The compound from peak A had been identified as α-cryptoxanthin while the D peak m/z 553 was β-cryptoxanthin, the xanthophyll provitamin A most commonly encountered [13]. The carotenoid from peak C m/z 551.4 was identified as lutein while the peak E m/z 569.4 was zeaxanthin.

Then, β-carotene, cryptoxanthin α, β-cryptoxanthin, lutein, and zeaxanthin and possibly beta-carotene isomers which are the α-carotene, ε-carotene, γ-carotene and lycopene have been identified in extracts of flour of the variety Jewel (**Figure 1 ; Table II**).

β-carotene, its isomers (α-carotene, γ-carotene, ε-carotene, lycopene) and β-cryptoxanthin are the most common precursors of vitamin A carotenoids considered in human diet [14 - 16].

On the other hand, lutein and Zeaxanthin are oxygenated-carotenoids from the subfamily of xanthophylls and have beneficial effects for consumers. Indeed, they have important antioxidant properties [17]. Lutein and zeaxanthin filter blue light, very energetic, which assaults the photoreceptors of the eye (indirect antioxidant effect); What would prevent the degeneration of the retina [18]. In addition, they contribute to the fight against degenerative diseases related to oxidative stress such as cancers [19]. Lutein and zeaxanthin also act as agents preventing the proliferation of cancer cells [20].

Finally, some controversy exists in the literature concerning whether or not α-cryptoxanthin is a provitamin A xanthophyll or not [21, 22].

The OFSP is therefore a potential source of molecules with important nutritional properties that can contribute significantly to the well-being of consumers. That explains the choice of the flour of Jewel variety to enhance the nutritional quality of this local infant food flour (Misola), which is much used in the recuperation of malnourished children.

Table I : Summary of the different infant food flour formulations

Codes	**$M_{10}J_0$**	**M_9J_1**	**M_8J_2**	**M_7J_3**	**M_6J_4**	**M_5J_5**	**M_4J_6**	**M_3J_7**	**M_2J_8**	**M_1J_9**	**M_0J_{10}**
Misola (%)	100	90	80	70	60	50	40	30	20	10	0
Jewel (%)	0	10	20	30	40	50	60	70	80	90	100

Table II: Structures of carotenoids identified

Molecular ion peaks	**m/z $[M+H]^+$**	**Formula**	**Pseudo molecular Ions**	**Structures of carotenoid suggested**
A	553.4	$C_{40}H_{56}O$	535.4 $[M\text{-}H\text{-}18]^{+*}$	α-cryptoxanthin
B	537.4	$C_{40}H_{56}$	-	β-carotene, α-carotene, γ-carotene, ε-carotene, lycopene
C	569.4	$C_{40}H_{56}O_2$	551.4 $[M\text{-}H\text{-}18]^{+*}$	lutein
D	553.4	$C_{40}H_{56}O$	-	β-cryptoxanthin
E	569.4	$C_{40}H_{56}O_2$	-	Zeaxanthin

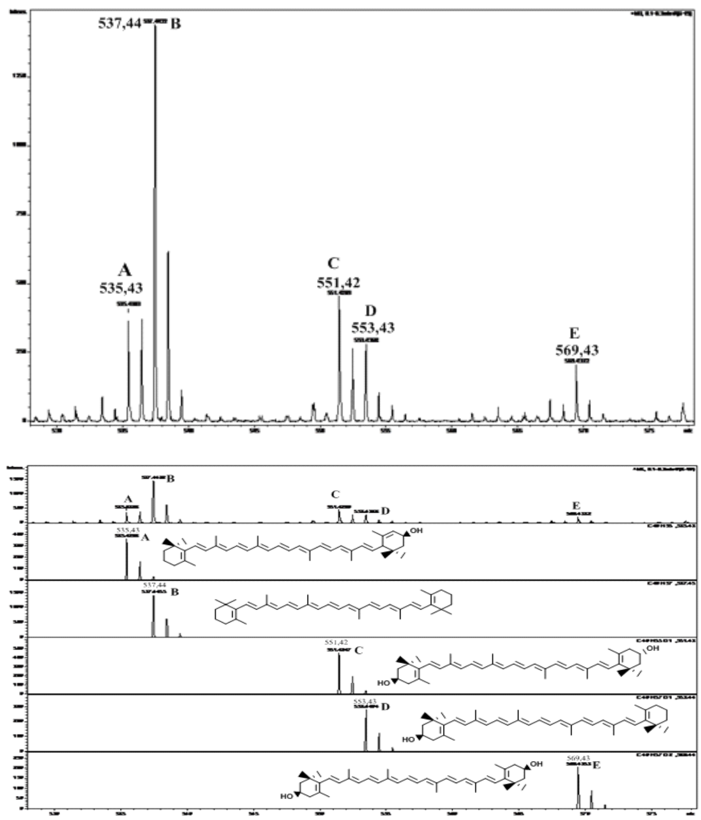

Figure 1: Liquid Chromatography – Mass Spectrometry and MS/MS spectra of OFSP flour extract

2.2. Methods

2.2.1. *Determination of physicochemical parameters*

Physicochemical parameters of this flour were determined using French standard V03-050, September 1970 for proteins, International standard ISO-659, 1998 for fats and a differential approach used in IRSAT-DTA for total carbohydrate contents.

2.2.2. *Extraction of total antioxidants, phenolic and carotenoids compounds*

Extractions of antioxidant and phenolic compounds were performed by maceration of storage root flour at 4 ° C with each of the following solvent systems: acetone-water-acetic Acid (70: 29.5: 0.5 v/v) [23] for assays of total antioxidant (TAC) and phenolic (TPC) contents and acetone-hexane (50: 50 v/v) [24] for the determination of total carotenoids contents (TCC).

2.2.3. *Determination of total antioxidant, phenolic and carotenoid contents:*

These contents were determined spectrophotometrically using a microplate in quartz 96 wells (MP96, SAFAS spectrophotometer) and choosing standards.

Thus, total carotenoids were assessed according to the method described by McMurry [25,26] slightly modified. β-carotene which is absorbed at 450 nm was taken as standard. Total carotenoids contents were obtained by reporting absorbance of extracts on a standard curve ($y = 25.56x + 0.016$; $R^2 = 0.999$) established using β-carotene. Total carotenoid contents are expressed in mg of β-carotene Equivalents (BCE) per gram of sample. All measurements were carried out in triplicate.

Using Ferric Reducing Antioxidant Power (FRAP)[27,28] method, total antioxidant contents which reacted with FRAP reagent to give intense blue color (λ=593 nm) were determined. Results obtained from the calibration curve equation ($y = 28.67x + 0.066$; $R^2 = 0.999$), are expressed in mg of Trolox Equivalent (TE) per gram of sample.

Total phenolic contents were determined using the Folin-Ciocalteu reagent [29]. Approximately 60 µL of appropriately diluted extract was mixed to 60 µL of diluted 10-fold Folin-Ciocalteu reagent. The mixture was left at room temperature for eight minutes to allow the complete reaction of the Folin-Ciocalteu reagent on oxidizable substances or phenoxide. Then 120 µL of Na_2CO_3 (7.5% in water) was added to neutralize the residual reagents. The absorbance of the extracts were measured at 765 nm with a microtiter plate in quartz 96 wells (MP96, SAFAS spectrophotometer) after incubation at 37 ° C for 30 minutes.

Using the Gallic Acid as standard, a calibration curve was established. Results, determined from the calibration curve equation ($y = 46.41x + 0.063$, $R^2 = 0.998$), are expressed in mg of Gallic Acid equivalents (GAE) per gram of sample. All measurements were carried out in triplicate.

2.2.4. *Statistical analysis*

The Experiments were conducted in three replications and the results are expressed as mean ± SD at the significance level of 5%. Analysis of variance (ANOVA) to appreciate the differences between formulations for their total antioxidant, phenolic and carotenoid contents was performed using the statistical software Genstat, edition 14.

3. Results And Discussion

3.1. Physicochemical parameters

The formulations energy values were calculated on the basis that one gram of protein, fats and total carbohydrate provides 4 kcal, 9 kcal and 4 kcal, respectively.

Compared to pure Misola ($M_{10}J_0$) and and OFSP (M_0J_{10}) flours which energy values were 764.62± 5.14 and 731.84 ± 5.14 kcal/200 g respectively, the energy value reached an optimum of 802.526 ± 5.14 kcal/200 g for the formulation M_7J_3 enriched with 30% of OFSP (**Figure 2d**).

3.2. Phytochemical contents

From different Misola-OFSP formulations obtained, there was a significant increase ($P< 0.005$) of total carotenoid contents in relation with the proportion of OFSP added. There was a good correlation of more than 98% between the percentage of OFSP added and the total carotenoids content (**Figure 2a**). Results in **Table III** show that pure Misola ($M_{10}J_0$) had total carotenoids content much less than that of the pure OFSP (M_0J_{10}); either a large relative variation of more than 99%. There was therefore a positive slope (**Figure 2a**) which showed that total carotenoid content increased with the proportion of OFSP added in the formulation. This increase in total carotenoids content is not surprising because several scientific studies revealed that OFSP has good nutritional qualities. This justifies its use in the proceeds of strengthening of local food (formulations) with bioactive micronutrients [30,31]. Amagoh and coworkers [32] arrived to the conclusion that OFSP complementary food is a good source of beta-carotene and would therefore contribute to the vitamin A requirements of infants. Also, Meenakshi and colleagues [33] found that if OFSP was incorporated into diets in Sub-Saharan Africa, the prevalence of vitamin A deficiency could be significantly reduced.

Initial Misola (0.2242 mg/g) and OFSP (0.364 mg/g) flours had TAC values significantly different ($P<0.05$); with a relative variation of 38.4 % (**Table III ; Figure 2b**). The TACs did not increase on a regular basis as in the case of total carotenoid contents (**Figure 2a-b**). Then, the same trend is not confirmed for TAC and TPC. However, some of the formulations presented particularly high TAC values, compared to the initial two extreme flours. Indeed, a significant increase ($p<0.005$) in TAC values when the percentage of OFSP varies from 10 to 30 % ; it reached a maximum TAC of 0.469 mg of Trolox equivalents/g of formulated flour ; either a variation of 109% (compared with Misola pure flour). Beyond this proportion, TAC values decreased globally up to 0.2481 mg of Trolox equivalents when the percentage of OFSP reaches 90%. The M_7J_3 formulation has therefore the optimal total antioxidant content. Also, M_8J_2 (0.4353 mg/g) and M_5J_5 (0.4447 mg/g) TAC values were not significantly different ($P > 0.05$) from M_7J_3 (0.4690 mg/g) (**Table III; Figure 2b**). These formulations (M_8J_2 and M_5J_5) containing optimal TACs were therefore good candidates for strengthening antioxidant and immune status of moderately malnourished consumers [34,35].

In addition, for bioethical reasons, it was preferable to choose a formulation containing optimal proportion in Misola (which is a flour used in the recovery of malnourished children)[36]. In this study, Jacques and his collaborators showed that a formulation Spirulina-Misola containing about 9% of spirulina was very effective for the rehabilitation of malnourished children. Spirulina and OFSP flour are characterized by a high proportion in glycides (57% and about 74%, respectively), and contain carotenoid molecules[37]. These characteristics confirm the suitability of supplementing Misola with 9% of Spirulina (this association gave an energy intake of 767 ± 5 kcal/day) or with 30% of OFSP flour (802.56 ± 5.14 kcal/200g).

Regarding the required bioethical elements and in the desire to improve the immune status of consumers, M_7J_3 could be chosen as the best formulation. In many processes of popularization (where OFSP was added to enrich staple food), it appeared that approximately 30% of OFSP was necessary.

For total phenolics typically responsible for more than 90% of the antioxidant activities of phytochemical extracts[38], it was noted that Misola (1.8218 mg/g) and OFSP (2.277 mg/g) pure flours were significantly different (**Table III; Figure 2c**). Total phenolics contents of the formulations did not vary significantly; with a low relative variation of about 20%. Then, TPCs did not influence qualitatively the choice of M_7J_3 as the convenient formulation. From Misola pure flour (1.8218 mg of GAE/g of formulated flour) to this chosen formulation (2.0309 mg of GAE/g of formulated flour), TPC has increased of about 11%

With regard to these results, M_7J_3 having the highest energy value (802.526 ± 5.14 kcal/200 g), optimal concentrations of total antioxidant (0.469 mg of Trolox equivalents/g of formulated flour), phenolic (2.0309 mg of GAE/g of formulated flour) and containing 0.8968 mg of BCE per gram of formulated flour of total carotenoids, was the best formulation to choose for infant porridges. In relation with Misola pure flour, TCC value of M_7J_3 has considerably increased of about 3271% (from 0.0266 to 0.8968 mg of BCE/g).

A study whose results have not yet been published was designed to evaluate the effect of the consumption of on the nutritional and immune status of moderate malnourished. It was made in a Nutrition Education and Rehabilitation Centre (CREN) at the Centre Medical St Camille (CMSC) of Ouagadougou through an appraisal prior-after intervention with a control group. Indeed, two homogenous groups of children (36 children per group) of moderate malnourished had to consume two types of porridge during one month. The first group consumed a porridge made with the formulated M_7J_3 flour. The second which is the control group received only Misola porridge.

At the end of the experiment, a significant improvement in the average rate of retinol serum with a gain of 0.33 µmol/L for children in the intervention group (first group) versus 0.01 µmol/L in the control group. It also contributed to correct anaemia of about 42% of children in the intervention group. In addition, using this formulation porridge, about 27.7% of children have left the form of underweight malnutrition. The same finding was observed for emaciation with a reduction of 36.1% of the number of children suffering from this form of malnutrition in relation with food consumed. Then, from reported results of this study, Misola and OFSP are good supplements in the evolution of immune status indicators.

Table III: Total antioxidants, phenolics and carotenoids contents

Formulations	TCC (mg/g)	TAC (mg/g)	TPC (mg/g)
$M_{10}J_0$	0.0266 ± 0.0010^{a}	0.2242 ± 0.0124^{A}	$1.8218\pm0.0764^{a'}$
M_9J_1	0.2740 ± 0.0032^{b}	0.3925 ± 0.0134^{CD}	$2.0861\pm0.0112^{b'c'}$
M_8J_2	0.5446 ± 0.0041^{c}	0.4353 ± 0.0043^{DEF}	$2.1842\pm0.0143^{b'c'd'}$
M_7J_3	0.8968 ± 0.0039^{d}	0.4690 ± 0.0233^{F}	$2.0309\pm0.0331^{b'c'}$
M_6J_4	1.1114 ± 0.0115^{e}	0.3967 ± 0.0170^{CDE}	$2.2156\pm0.0306^{c'd'}$
M_5J_5	1.4686 ± 0.0127^{f}	0.4447 ± 0.0103^{EF}	$2.3535\pm0.0429^{d'e'}$
M_4J_6	1.6192 ± 0.0127^{g}	0.3912 ± 0.0168^{BCD}	$2.4646\pm0.0669^{e'}$
M_3J_7	1.7175 ± 0.0226^{h}	0.3815 ± 0.0051^{BC}	$2.3669\pm0.0799^{d'e'}$
M_2J_8	1.7779 ± 0.0353^{h}	0.3396 ± 0.0136^{B}	$1.9989\pm0.0028^{a'b'}$
M_1J_9	2.0079 ± 0.0431^{i}	0.2481 ± 0.0136^{A}	$2.2933\pm0.0549^{d'e'}$
M_0J_{10}	2.367 ± 0.0290^{j}	0.364 ± 0.0103^{BC}	$2.277\pm0.0393^{d'e'}$

Data are expressed as means ±SE of triplicate experiments. Means in a column not having a common letter are different (P<0.05).

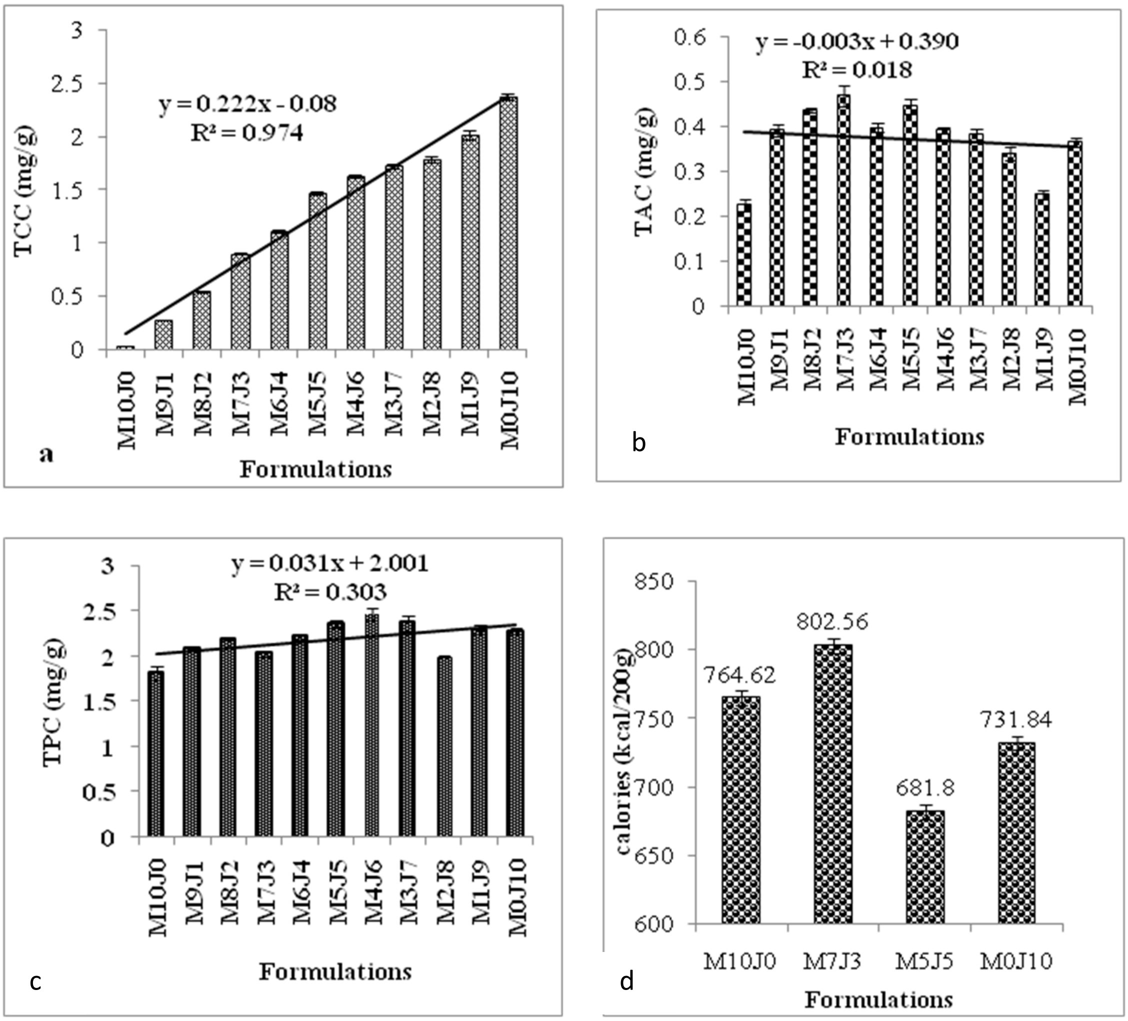

Figure 2: Changes in phytochemical contents [TCC (a), TAC (b), TPC (c)] and physicochemical parameters (d) of Misola-OFSP formulations

4. Conclusion

This study has revealed increase in five molecules of carotenoids when the existent infant food flour is enriched with OFSP flour.

Flour formulation containing 30% of OFSP lead to an optimum of total antioxidant content (0.469 mg TE / g), and total carotenoid contents (0.8968 mg BCE/g). This formulation with the best energy value (802.526 ± 5.14 kcal / 200g), can be recommended in combating vitamin A deficiency and protein-energy malnutrition in Burkina Faso.

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