



Formulation of a Yogurt Co-fermented with Carrot Juice (*Daucus carota*) and Evaluation of Its Nutritional Potential in the Fight Against Avitaminosis A

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Abstract: The WHO recommends the formulation and use of functional foods in response to persistent public health problems. There is a renewed interest in developing new products based on milk and fruit and vegetables to tackle certain public health problems. Avitaminosis A is a disease that affects thousands of people worldwide, particularly in sub-Saharan Africa. In order to contribute to the fight against Avitaminosis A in Cameroon, this study aims to formulate a yoghurt rich in provitamin A and to evaluate its nutritional potential. It is a yoghurt co-fermented with carrot juice which has the characteristics of a functional food. Five samples of co-fermented yoghurt were formulated and coded respectively YN, YC1, YC2, YC3, YC4 for the plain yoghurt made from 100% milk, then with increasing percentages of carrot juice substitution (70, 80, 90, 100% (V/V)) to reconstitute the milk. Physicochemical (pH and titratable acidity), nutritional (water content, total ash, crude protein, total lipids, total carbohydrates, crude fibers, vitamin C, β -carotene), sensory (color, taste, consistency, smell and general acceptability), microbiological (total coliforms, yeasts, molds and salmonella) analyses were carried out on the different formulated samples. From the results obtained, it appears that carrot juice significantly affects ($p < 0.05$) the pH, titratable acidity and dry matter of the different yogurts. The incorporation of this juice leads to an increase in protein, total sugar, crude fibers, ash, vitamin C and β -carotene content, but a decrease in lipid content ($p < 0.05$). Refrigerated storage at 4°C did not significantly ($p < 0.05$) change the pH and acidity of the yoghurts. Microbiological analysis revealed that all co-fermented yoghurts were fit for consumption. The results of the general acceptability showed that YN and YC3 yoghurts were the most appreciated by the panelists. Principal Component Analysis (PCA) showed that the yoghurt with 90% carrot juice (YC3) was the most correlated with β -carotene, vitamin C and crude fibers. Also, this sample has contents of 3.91% protein, 5.03% crude fibers, 3.20 mg β -carotene, 4.26 mg vitamin C. From the point of view of sensory analysis and PCA, this yoghurt was the best and could be recommended in the diet to contribute to the fight against vitamin A deficiency.

Keywords: Avitaminosis A, Carrot Juice, Co-fermented Yoghurt, β -carotene, Vitamin C

1. Introduction

Micronutrient deficiencies such as iodine and vitamin A remain the most common and important nutritional problems in many countries in Africa, Asia, Latin America and the

Near East [1]. Avitaminosis A is defined as a major health problem caused by a diet low in vitamin A, and its manifestations are mainly observed in infants and children, mostly resulting in eye damage [2]. It affects an estimated 19 million pregnant women and 190 millions pre-school children, [2] with more than 135,000 deaths of children under

5 in Cameroon. These alarming figures have prompted several governmental actions as well as those carried out by numerous researchers and public health actors to reduce the prevalence of avitaminosis A as much as possible [1]. Most of these actions aim at using locally available and nutritionally interesting food resources (protein and micronutrient sources such as milk and dairy products, fruits and vegetables) [2].

Milk processing allows the production of many by-products such as yoghurt, which is consumed by all segments of the population [3]. It is highly valued for its taste and texture and is considered a good carrier for other foods and appears to be a highly digestible food with high nutritional value [4]. It is rich in protein, fat, calcium but low in carotenes, iron, ascorbic acid, dietary fibers [5].

Carrot (*Daucus carota*) is a root, generally orange in color, belonging to the Apiaceae family and consumed as a vegetable. After the potato, it is the main root vegetable cultivated in the world [6]. It is widely cultivated in Cameroon and has several therapeutic virtues thanks to its high content of β -carotenes. It is rich in ascorbic acid, dietary fibers and tocopherol [7] but low in protein and lipids.

The formulation of a functional food in the form of yoghurt, based on milk and the carrot could help prevent the occurrence of avitaminosis A in the Cameroonian population. In this context, several studies have been conducted on the fortification of yoghurt with various ingredients. [8] in Algeria conducted work on yoghurt fortified with carrot juice in proportions of 5 to 20% whose β -carotene content was much lower than the recommended daily intake in pregnant women and preschool children. A study on the fortification of yoghurt with fruit cluster juices (blueberry, cherry, strawberry) as functional ingredients was carried out. This fortification increased the antioxidant activity of yoghurt by about 30 – 49%. Similar results were obtained by Tizghadam *et al.* [9] after adding dill extracts to yoghurt [10]. Further work by Baba *et al.*, [11] showed that supplementation of yoghurt with hazelnuts resulted in a significant decrease in microbial activity in yoghurt.

In Cameroon, the availability of animal (such as milk) and plant (carrots) resources, in time and space, offers an opportunity of choice that leads to the development of new products with high nutritional value. This action aims at the formulation of functional foods whose main role is to act efficiently in the body to prevent the occurrence of a pathology and, in turn, the valorization of local products. In this context, the general objective of the present study was the formulation of some co-fermented yoghurts based on carrot juice. More specifically, the carrot juice as well as the formulated yoghurts were characterized on the physicochemical (pH, titratable acidity), nutritional (water content, proteins, lipids, total carbohydrates, crude fibers, β -carotenes, vitamin C, ashes), microbiological (total coliforms, yeasts, molds and salmonella) and sensory levels.

2. Materials and Methods

2.1. Processing of Carrot Juice

Carrots roots collected from a farmer in the village of Babadjou in West Cameroon were taken to the Food and Nutrition Research Center where they were washed under running water, crushed and pressed using a *Moulinex* electric extractor. The carrot juice obtained was used to formulate the various yoghurts.

2.2. Processing of the Carrot Juice Co-fermented Yoghurts

The equipment used was previously sterilized in an autoclave after thorough cleaning with soap and water. Five samples of yoghurt YN, YC1, YC2, YC3 and YC4 were formulated using 0, 70, 80, 90, 100% (v/v) carrot juice to reconstitute the milk and powdered sugar mixture respectively, followed by homogenization to avoid the formation of lumps. The five reconstituted milk samples were heat treated at 95°C for 5 min and then cooled to 45°C. After cooling, the samples were inoculated with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and then placed in jars for fermentation in a ventilated oven at 45°C for 4 h and then cooled. The co-fermented yoghurts obtained were freeze-dried for analysis.

2.3. Physicochemical Analysis of Formulated Yoghurts

The physicochemical analyses of the five yoghurt samples were performed in triplicate on day 0. The pH and titratable acidity (TA) were determined by the methods described by AOAC [12]. These two parameters were assessed every 7 days for a total of 28 days. Water and dry matter contents were determined by steaming at 105°C [13], total lipids were determined by differential solubility in hexane [14], crude fibers by successive digestion with strong acids and strong bases using the methods described by Wolff [15]. The crude proteins content was obtained after mineralization of the samples according to the Kjeldahl method [16], followed by determination by the colorimetric technique of Devani [17]. The total sugar content was obtained by difference. Total ash was determined by incineration at a temperature of 550°C according to AFNOR [14] and vitamin C by the method described by Amra *et al.*, [18]. The β -carotene content was obtained by the method of Bandyopadhyay *et al.*, [19].

2.4. Microbiological Analyses of Yoghurts

The preparation of the samples was done according to the method described by AFNOR [20]. Total coliforms were determined using Neutral Red Crystal Violet Milky Agar (NRMLA) by the pour plate technique [20]. Yeasts and molds were counted by incubating 1 ml of 1/10 dilution of yoghurt by the acidified potato casting plate method in Agar Dextrose Medium (APDA) at 25°C for 5 days. Salmonella levels were determined using SS Agar (Salmonella Shigella Agar).

2.5. Sensory Analysis of the Yoghurts

The sensory analysis of the different types of yoghurt was carried out according to the method described by Richardson [21] according to the following descriptors: taste, color, smell and consistency. A five-level hedonic test (1=poor; 2=fair; 3=good; 4=very good and 5=excellent) was used by 30 panelists of both sexes, aged 16 to 50 years and of various social activities. The inclusion criteria for the panelists included being regular yoghurt consumers and being in good health. The general acceptability of yoghurt was subsequently assessed.

2.6. Statistical Analyses of the Results

Statistical analyses were carried out using the Statistical Package for Social Science (SPSS) version 20.0 for Windows. Graphs were plotted using Microsoft Excel 2016 for Windows. The results were represented as mean±standard deviation. The significance level was set at 5% based on an Analysis of Variance (ANOVA) coupled with a Post Hoc test (Turkey). The XL STAT trial software for Windows was used to perform the Principal Component Analysis (PCA).

3. Results and Discussion

3.1. Proximal Composition of Carrot Juice

Table 1 shows the proximal composition of the carrot juice obtained.

The results of the proximal analysis of the carrot juice showed that the juice has a pH close to neutral (6.23). This

would prevent the development of acidophilic and basophilic microorganisms. Moreover, its low dry matter content (1.55%) shows that carrots contain more than 98% water. This is of major interest for the hydration of the organism. However, this high moisture content could have the disadvantage of being a rapid growth medium for microorganisms. Carrot juice has relatively low levels of macronutrients (protein, fat and carbohydrates) but is a potential source of crude fibers and total ash. Also, the non-negligible content of β-carotenes (3.40 mg/ 100 g MF) reflects the important role that carrot juice has on the neutralization of free radicals as shown by acting as antioxidant [22, 23]. These results corroborate those obtained by Silva Dias [24] on the collection of nutritional data on several carrot varieties.

Table 1. Proximal composition of carrot juice.

Parameters	Values
pH	6,23±0,01
Titrate acidity (°D)	27,68±0,20
Dry matter (%)	1,55±0,12
Crude protein (g/100g MS)	0,81±0,05
Total fat (g/100g MS)	0,30±0,01
Total sugars (g/100g MS)	8,13±0,01
Crude fibers (g/100g MS)	5,57±0,37
β-carotene (mg)	3,40±0,06
Vitamine C (mg)	4,37±0,38
Total ash	3,04±0,05

3.2. Nutritional Composition of Yoghurts

The nutritional composition of yoghurt co-fermented with carrot juice is shown in Table 2.

Table 2. Nutritional composition of co-fermented yoghurts.

Parameters	YN	YC1	YC2	YC3	YC4
Dry matter (%)	24,11±0,57 ^a	24,71±0,51 ^a	24,68±0,17 ^a	24,38±0,35 ^a	24,14±0,15 ^a
Crude protein (g/100g MS)	3,52±0,19 ^a	3,67±0,05 ^{ab}	3,75±0,03 ^{bc}	3,91±0,11 ^{cd}	4,32±0,14 ^d
Total fat (g/100g MS)	7,87±0,70 ^b	6,97±0,57 ^{ab}	6,30±0,02 ^a	6,18±0,02 ^a	6,14±0,05 ^a
Total sugars (g/100g MS)	4,73±1,01 ^a	6,10±0,35 ^{ab}	7,42±0,15 ^{ab}	7,80±2,06 ^b	8,06±0,57 ^b
Crude fibers (g/100g MS)	0,24±0,05 ^a	3,12±0,20 ^b	4,15±0,17 ^c	5,03±0,15 ^d	5,75±0,28 ^d
β-Carotene (mg/100g FM)	0,08±0,00 ^a	1,88±0,07 ^b	2,86±0,05 ^c	3,15±0,04 ^d	3,27±0,07 ^d
Vitamine C (mg/100g FM)	0,002±0,002 ^a	3,80±0,26 ^b	3,81±0,16 ^b	4,26±0,31 ^b	4,35±0,41 ^b
Total ash (%)	2,34±0,29 ^a	2,75±0,27 ^a	2,86±0,22 ^b	3,02±0,05 ^b	3,03±0,08 ^b

YN (yogurt without carrot juice); YC1 (yogurt co-fermented with 70% carrot juice); YC2 (yogurt co-fermented with 80% carrot juice); YC3 (yogurt co-fermented with 90% carrot juice); YC4 (yogurt co-fermented with 100% carrot juice). The values are expressed as a mean±standard deviation. The values assigned to different letters on the same column are significantly different (p<0.05). MF=Fresh matter

The contents of dry matter, crude protein, total sugars, total lipids, crude fibers and total ash of the yoghurt samples range from 24.11 - 24.71%; 3.52 - 4.32%; 4.73 - 8.06%; 6.14 - 7.87%; 0.24 - 5.75% and 2.34 - 3.03% respectively (Table 2). Fortification of yoghurt with fruit or vegetable juice increases the nutritional value of yoghurt to some extent [8]. However, an increase in carrot juice produces a dilution effect of the nutrients in the yoghurt and significantly (p<0.05) decreases their contents. Yoghurt without carrot juice (YN) is very low in crude fibers (0.24±0.05 g/100 g DM) but very high in fat (7.87±0.70 g/100 DM). The 100% carrot juice (YC4) had the highest contents of crude protein (4.32±0.14 g/100g DM), crude fibers (8.06±0.57 g/100g DM)

and total ash (5.75±0.28 g/100g DM). These significant differences could be explained by the addition of carrot juice at different proportions [10]. For crude protein, it could be due to the fact that during lactic fermentation an amount of protein is degraded by *Streptococcus thermophilus* in order to develop the sensory qualities of yoghurt [25]. The decrease in lipid content could be explained by the fact that these nutrients are used to obtain the creamy and smooth texture of yoghurt [25]. The results are also in line with the IDF (International Dairy Federation) standard which recommends a minimum of 2.7% (m/m) protein in the finished product [26]. A protein-rich diet increases the activity of the enzyme 15-15'dioxygenase which is involved in the bioconversion of

provitamin A carotenoids to vitamin A and also the production of retinol binding protein [27]. While one of the effects of dietary fibers in the main point of lipid metabolism is the fecal extraction of bile acids, thus decreasing the intestinal reabsorption of lipids and fat-soluble substances including carotenoids [28]. Furthermore, it appears from Table 2 that the vitamin C values of the co-fermented yoghurts YC1 to YC4 ranged from 3.80 ± 0.26 to 4.35 ± 0.41 mg/100g MF respectively. They were significantly different at $p < 0.05$ with YN = 0.00 ± 0.00 mg/100g MF (yoghurt without carrot juice did not contain vitamin C). And vitamin C protects carotenes from oxidation during their absorption, storage and bioconversion [29]. The β -carotene contents of YC1, YC2, YC3, YC4 ranged from 1.88 ± 0.01 to 3.28 ± 0.07 mg/100g MF. These levels were significantly different at the 5% level with YN = 0.08 ± 0.002 mg/100g MF. According to

[30], the β -carotene values are higher than the recommended daily requirement which is on average 2.7 mg β -carotene/day (for children aged 0-5 years); 4.2 mg β -carotene/day (for pregnant women from the 4th month). In addition, vitamin A, which is derived from the bioconversion of β -carotene in vivo, is very important in the diet of children aged 0-59 months and pregnant women [31]. The human body converts beta carotene into vitamin A (retinol). Beta carotene is a precursor of vitamin A which is needed not only for good eye health and vision, but also for healthy skin, mucus membranes and immune system [32].

3.3. pH and Titratable Acidity

Figures 1 and 2 show the influence of storage time on pH and titratable acidity in different yoghurts.

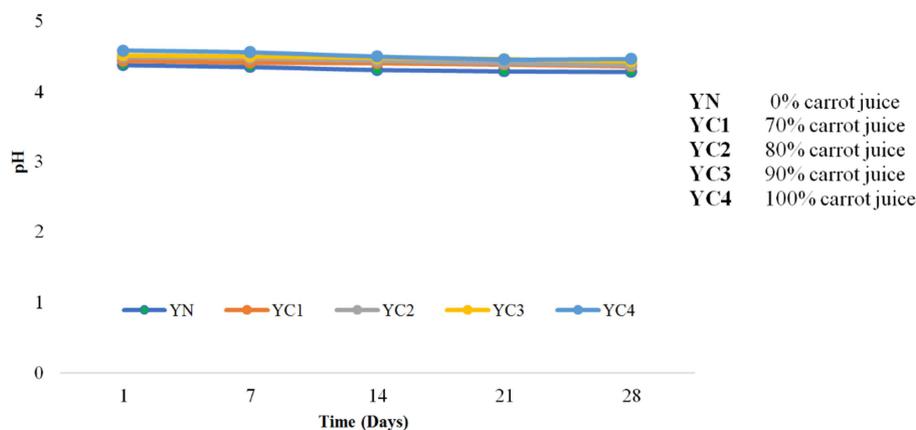


Figure 1. Influence of storage time on pH.

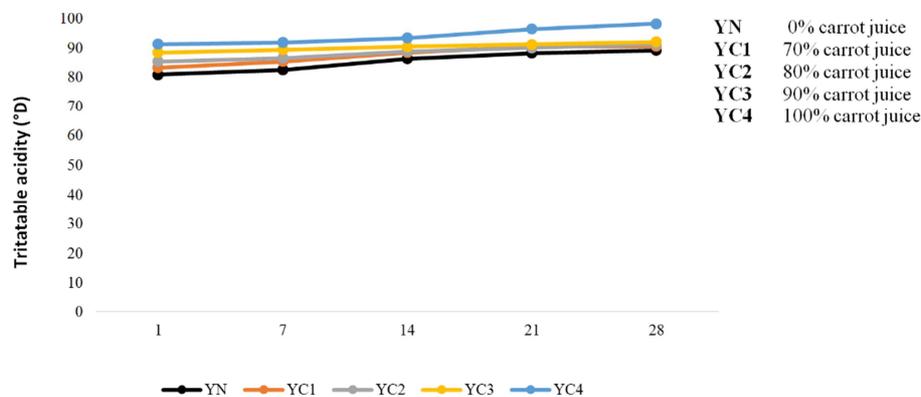


Figure 2. Influence of storage time on titratable acidity.

The pH of yoghurts obtained after fermentation are in accordance with those recommended by the IDF (International Dairy Federation) where the pH must be lower than 4.6. Moreover, it showed a significant difference at the 5% threshold and ranged from 4.36 ± 0.01 (YN) to 4.58 ± 0.02 (YC4) and increased with the addition of carrot juice. The pH values were comparable to those cited by Senarathne Wickramasinghe [33] for yoghurt enriched with carrot pulp and orange juice but higher than those cited by Kourdache and Ouchicha [34] for yoghurt spiced with ginger. The titratable acidity values of yoghurts co-fermented with carrot juice YC1,

YC2, YC3, YC4 were respectively 83.10 ± 0.94 ; 85.29 ± 0.31 ; 88.37 ± 0.28 ; 91.14 ± 0.16 °D. There was a significant difference at the 5% level with YN (80.80 ± 0.41 °D). These values are in line with the IDF standard which recommends values between 70°D and 120°D and higher than those cited by Njoya [35] for ginger spiced yoghurt. These differences would be due to the presence of lactic acid from lactic fermentation which increases the titratable acidity by decreasing the pH. In order to see the stability of the yoghurts, the evaluation of the pH and titratable acidity was carried out during 28 days. To this end, the results presented in figure 1 show that the carrot juice does

not significantly ($p < 0.05$) affect the pH during the 28 days of storage, but the evolution is characterized by a slight decrease of 2% on all samples. On the other hand, they remain in conformity with the IDF standard. They are lower than those of Kourdache and Ouchicha [34] who obtained a reduction of 3% over 28 days and those cited by Njoya [35] who obtained a reduction of 7% for spiced yoghurt with ginger over 30 days of storage.

For titratable acidity, an increase of 9% (YN), 8% (YC1), 6% (YC2), 4% (YC3), 7% (YC4) was recorded at a significant difference threshold of 5%. These values are lower than those of [35] who obtained an average increase of 15% over 28 days.

Table 3. Microbiological characterization of formulated co-fermented yoghurts.

	YN	YC1	YC2	YC3	YC4	CODEX STAN 243-2003 STANDARD
Total coliforms	Absent	Absent	Absent	Absent	Absent	$\leq 10^6$ ufc/ml
Yeast	10ufc/ml	Absent	Absent	Absent	Absent	$\leq 10^6$ ufc/ml
Moulds	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella	Absent	Absent	Absent	Absent	Absent	Absent

YN (yogurt without carrot juice); YC1 (yogurt co-fermented with 70% carrot juice); YC2 (yogurt co-fermented with 80% carrot juice); YC3 (yogurt co-fermented with 90% carrot juice); YC4 (yogurt co-fermented with 100% carrot juice).

From Table 3, it can be seen that total coliforms, molds and salmonella are completely absent. However, the presence of yeast in the yoghurt without carrot juice was observed at a value of 10 cfu/ml (10 colony forming units per milliliter). The results show that the co-fermented yoghurt complied with the CODEX STAN 243-2003 standard, which means that the co-fermented yoghurt is fit for consumption. Because coliforms present risks of infection for the consumer and have negative technological consequences: fermentation of sugars with production of gases, acids and other viscous substances with often unpleasant taste. Molds, on the other hand, contaminate foodstuffs and degrade their quality, some of them are toxigenic and represent a danger for the consumer. Yeasts do not pose a problem of food

poisoning, but they degrade acidic and sweet products by modifying the organoleptic quality of the product. And finally, salmonella are bacteria with a high pathogenic power. The presence of a single Salmonella in a product leads to its unsanitary condition, they can cause a food infection: Salmonellosis, one of the most widespread food poisoning diseases in the world [37].

3.4. Microbiological Characterisation

The results of the microbiological analyses of the co-fermented yoghurts formulated are given in Table 3.

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3.5. Sensory Analysis

Table 4 presents the scores of the different parameters of appreciation of the different yoghurts formulated.

Table 4. Scores of the different sensorial parameters.

	Color	Smell	Consistency	Taste
YN	4,00±0,43 ^b	4,07±0,53 ^c	4,07±0,40 ^c	3,86±0,61 ^c
YC1	2,86±0,63 ^a	2,50±1,00 ^{ab}	2,64±0,93 ^{ab}	2,00±0,71 ^a
YC2	3,00±0,71 ^{ab}	2,14±0,63 ^{ab}	2,86±0,73 ^b	2,57±1,02 ^{ab}
YC3	3,36±0,73 ^{ab}	3,50±0,57 ^{bc}	3,21±0,56 ^{bc}	3,29±1,20 ^{bc}
YC4	2,71±0,84 ^a	3,07±0,81 ^{ac}	1,86±0,61 ^a	2,86±0,78 ^{abc}

YN (yogurt without carrot juice); YC1 (yogurt co-fermented with 70% carrot juice); YC2 (yogurt co-fermented with 80% carrot juice); YC3 (yogurt co-fermented with 90% carrot juice); YC4 (yogurt co-fermented with 100% carrot juice). The values are expressed as a mean±standard deviation. The values assigned to different letters on the same column are significantly different ($p < 0.05$)

The values presented in Table 3 shows that there is a significant difference at the 5% level between the different co-fermented yoghurts YC1, YC2, YC3 YC4 and YN in terms of color, smell, consistency and taste. As for the taste of the formulated yoghurts, it was YC3 (yoghurts co-fermented with 90% carrot juice) with indices of 3.29±1.20 respectively. Figure 3 shows that in general, yoghurt without carrot juice (YN) is the most appreciated, but in terms of yoghurts co-fermented with carrot juice, YC3 is the most appreciated and YC4 the least appreciated because it is just made from 100% carrot juice and without any milk. This results in the following final ranking: YN - YC3 - YC2 - YC1 - YC4.

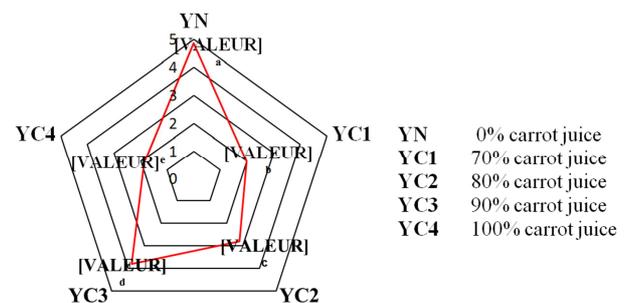


Figure 3. General acceptability of co-fermented yoghurts.

3.6. Principal Component Analysis (PCA)

Figure 4 shows the correlation circle between the different variables (the different results obtained on the analysis of

crude protein, total lipids, total sugars, crude fibers, vitamin C, β -carotene and total ash).

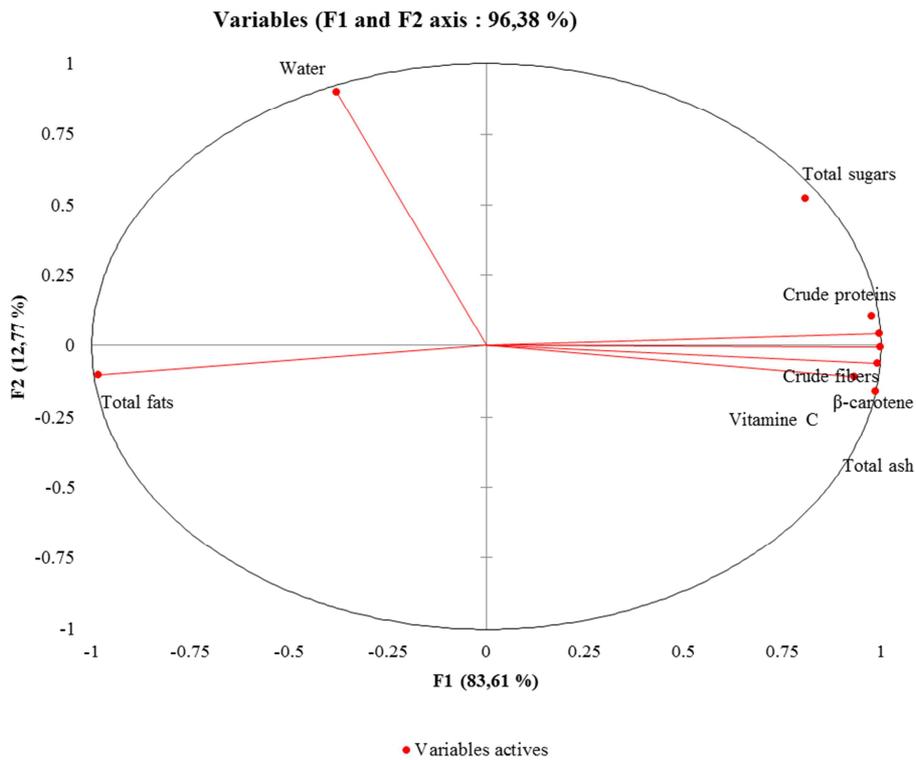


Figure 4. Correlation circle between variables.

The figure above shows that these variables contribute to the formation of the axis system (F1 x F2) at 96.38%. The F1 axis alone explains 83.61% of the observed variables and the second axis F2 explains 12.77%. The associated Principal Component Analysis (PCA) correlation circle between the variables is presented in Figure 5.

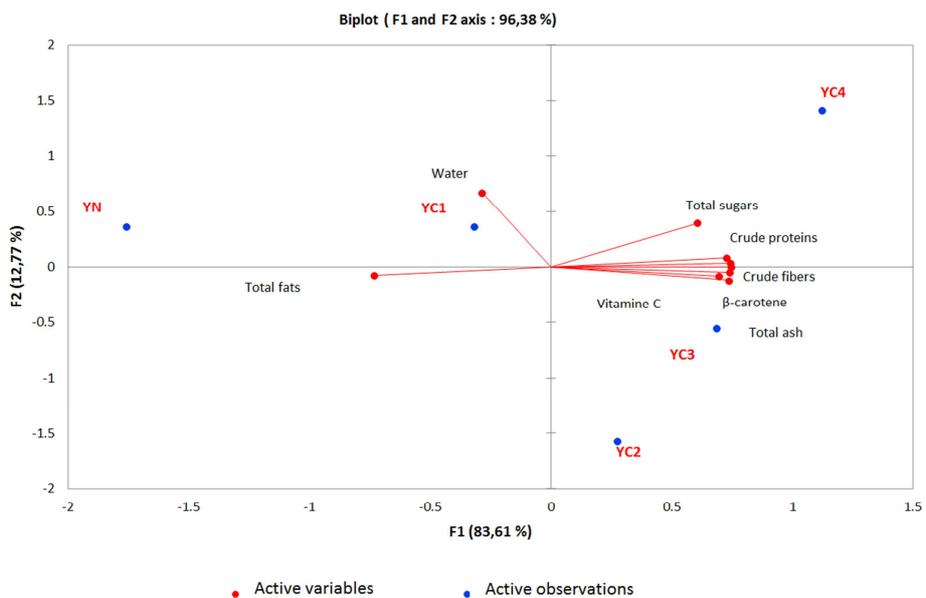


Figure 5. Principal Component Analysis (PCA) of formulated yoghurts.

YN (yogurt without carrot juice); YC1 (yogurt co-fermented with 70% carrot juice); YC2 (yogurt co-fermented with 80% carrot juice); YC3 (yogurt co-fermented with 90% carrot juice); YC4 (yogurt co-fermented with 100% carrot juice)

From Figure 5, it can be seen that the co-fermented yoghurts YC2 and YC3 are correlated in vitamin C, β -carotene, crude fibers and total ash. This gives them a high vitamin and mineral potential as well as good digestibility. Moreover, these correlations are stronger in Y3 than in Y2. Combining these correlations with the results obtained from the sensory analysis, it can be noted that the addition of 90% carrot juice to a yoghurt formulation results in a food that could not only contribute to the prevention of avitaminosis A, but also vitamin C, which promote the absorption of iron in the body. The Principal Component Analysis (PCA) of the co-fermented yoghurts formulated revealed that YC3 would be a good source of β -carotene, vitamin C, protein, crude fibers along the F2 axis. This could be advised in the diet to contribute to the fight against avitaminosis A subject to good bioavailability of β -carotene.

4. Conclusion

At the end of this study, it is noted that carrot juice is an excellent vector for the prevention of avitaminosis A in Cameroon. Yoghurts formulated and co-fermented with milk and carrot juice provide food with high nutritional potential, suitable for consumption and which could be stored in the refrigerator at 4°C for a relatively long time. Panelists judged that among the different co-fermented yoghurts formulated, the one made with 90% carrot juice is the most appreciated on the basis of several sensory descriptors. Further studies on the bioavailability of provitamin A and protein digestibility would help to confirm the use of this yoghurt in the fight against vitamin A deficiency

Declaration

Translated with www.DeepL.com/Translator (free version).

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