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Optimal conditions of steam blanching of spinach (*Spinacia oleracea*), a leafy vegetable consumed in Cameroon

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Abstract

Iron deficiency which is a public health problem in many developing countries is not only due to an insufficient supply, but also to a problem of bioavailability of iron in foods. The aim of this study was to determine the optimal conditions (time and temperature) of steam blanching of spinach leaves, and the effect of blanching on its chemical composition and functional properties. To achieve this, spinach was collected in the Ngaoundere main market, fresh and undamaged leaves separated and cleaned. The optimal conditions were determined using the plan of Doehler, where time and temperature of steam blanching varied from 2.5 to 12.5 minutes and 80 to 100°C respectively. Sample obtained was dried at 45°C ± 2°C for 24 hours and crushed to obtain a flour with a diameter of particles ≤ 500 µm. Responses sought were the maximum iron content and the minimum phytate/iron ratio in flour obtained. Chemical composition and functional properties of flour obtained with and without blanching was determined to evaluate the effect of steam blanching on spinach leaves, and data were analyzed using T-test at $P<0.05$. Results show that the optimal conditions of steam blanching of spinach leaves are 5 min at 95 °C. under these conditions, there was a significant increase ($p<0.05$) of iron content and a reduction of phytate/Iron ratio. Steam blanching of spinach leaves do not have any effect on carotenoids, magnesium and macronutrients content, but significantly reduces phosphorus content and increases calcium content. There is also a significant ($p<0.05$) decrease of anti-nutrients content (more than 50%). Water absorption capacity, water solubility index and bulk density are better in flour produced with blanched leaves. These results suggest that flour obtained with spinach leaves after steam blanching at 95°C for 5 min is a good source of bioavailable iron, so it can be used for infant food formulation and fortification.

Keywords: Iron Deficiency; Iron Bioavailability; Spinach leaves; Steam blanching.

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Introduction

Iron Deficiency is a problem of public health concern in many sub-Saharan countries in general and in Cameroon in particular, affecting mainly children under 5 years old and women in child bearing age [1]. The major consequences are anemia, birth defects and increased risk of maternal and child mortality [2]. In Cameroon, the situation remains worrying with 57% of children under 5 years suffering from Iron Deficiency [3]. To alleviate the situation, clinical and food strategies were set up by Cameroonian government. The principal food measures, were the fortification of some foodstuffs with iron [4]. The fact that the level of Iron Deficiency remains alarming shows that those measures were not sufficient [5]. This study leads to a sustainable complementary food approaches which integrate availability and accessibility to local resources of iron, an approach increasingly recommended [6].

In this light, spinach (*Spinacia oleracea*), a leafy vegetable grown and consumed in Cameroon, is one of the best plant sources of iron, with about 83.3 mg/100g DM [7]. Its high ash content (30.6%) indicates that it may contain other essential minerals [8]. It is also a good source of carotenoids [9]. Nevertheless, spinach contains high levels of anti-nutrients such as phytates, oxalates and tannins, which reduces the bioavailability of iron and others minerals [10]. Several authors having shown that steam blanching reduces these anti-nutrients in others leafy vegetables while preserving their nutritional quality [11, 12, 13]. Thus, the aim of this study was to determine the optimal conditions (time and temperature) of steam blanching of spinach leaves, and the effect of these treatment on its nutritional potential and some functional properties.

Materials and methods

Production of spinach leaves flour

Spinach leaves were collected in august 2017, early in the morning in the Ngaoundere main market, and transported to the Food Biophysics and Nutritional Biochemistry Laboratory of the National School of Agro Industrial Sciences of the University of Ngaoundere. The fresh and undamaged leaves were separated, washed with tap water and rinsed with distilled water. The sample of spinach leaves was then divided into two sub-samples, one blanched under optimal conditions of time and temperature, and the other used without steam blanching. This was to evaluate the effect of steam blanching on chemical composition and functional properties of spinach flour. The blanched and unblanched leaves were dried at 45°C ± 2°C for 24 hours and crushed with a micro hammer mill (Culatti type DCFH 48) to obtain a flour with a diameter of particles ≤ 500 µm.

Determination of optimal conditions of steam blanching

Doehlert plan at 2 factors was used to study the effect of time (x1) and temperature (x2) of steam blanching of spinach leaves on iron and molar ratio phytates/iron (Phy/Fe) by surface response method. The independent variables x1 and x2 varied respectively from 2 to 12 minutes (with 5 variations) and from 80 to 100°C (with 3 variations), as in other works [12, 14]. The expected response were the highest level of iron and the lowest phytates/iron ratio in spinach flour. For this model to be valid, at least two of the following three conditions are to be satisfied: coefficient of determination R2 ≥ 80% [15], absolute mean deviation analysis (AADM) close to zero [16], bias factor (Bf) between 0.75 and 1 [17].

Determination of proximal composition of flour

To determine the effect of steam blanching on the nutritional quality of spinach leaves, macronutrients composition of flours was determined. Dry matter was determined by drying the samples in a ventilated oven at 105°C during 24h until constant mass [18]. To determine oil content, the extraction of lipids in Soxhlet based on their differential solubility in hexane was done for approximately 12h [19]. Total protein content was obtained after mineralization of the samples [20], and quantification by spectrophotometric method at 412 nm [21]. The conventional conversion factor 6.25 of nitrogen into protein was used. Total sugars were extracted with sulphuric acid 1.5N, then quantified by spectrophotometric reading of absorbance at 420nm [22]. Crude fiber content was determined by boiling the sample in sulphuric acid, and then, in sodium hydroxide. The residue obtained was dried, calcined and weighed [23]. The determination of total ash content was done by complete incineration of the samples in a furnace adjusted at 550°C, to obtain white ashes [24].

Quantification of some micronutrients

Ash obtained was washed with concentrated hydrochloric acid [25], and the filtrates used for the quantification of iron, phosphorus, calcium and magnesium. Iron and phosphorus were determined by colorimetry, with orthophenanthrolin and ammonium molybdate in an acid medium respectively [26]. The determination of calcium and magnesium was done by titration with a solution of ethylene diamine tetra-acetic acid (EDTA) disodium salt [27]. Total carotenoids were extracted with a hexane-acetone mixture: 30/70 (v/v), dissolved in hexane, and the optical density was read with the spectrophotometer between 430 and 450 nm to determine the maximum absorbance [28]. The maximum optical density was used to determine the level of total carotenoids in the sample.

Quantification of some anti-nutrients

Phytates were extracted with hydrochloric acid and sodium chloride, then quantified using Wade's reagent [29]. Extraction of phenolic compounds and tannins was done with acetone (70%), and quantification with Folin Ciocalteu and polyvinylpyrrolidone (PVPP) [30]. The oxalates were measured by hot titration with potassium permanganate solution [31].

Functional properties of spinach flour

Water Absorption Capacity (apparent and real) was determined as the quantity of water (in grams) absorbed by 100g of powder after saturation and centrifugation (Centrifuge DL-6000B) by a method previously described [32]. The dried solids mass in supernatant was used to evaluate the Water Solubility Index [33]. Bulk Density (g/mL) was measured in a graduated cylinder by gently adding 2 g of spinach flour into an empty 10 mL graduated cylinder and vortexed (Vortex RS Lab_6Pro) for 1 min. The volume was read, recorded and the result was expressed as describe by the method [34].

Statistical analysis

The averages of three repetitions and standard deviations were calculated using Excel 2016, the Student T test was done using XL Stat 2016 to compare nutrient content with and without steam blanching. The plot of the curves was done using Sigmaplot 12.5 software.

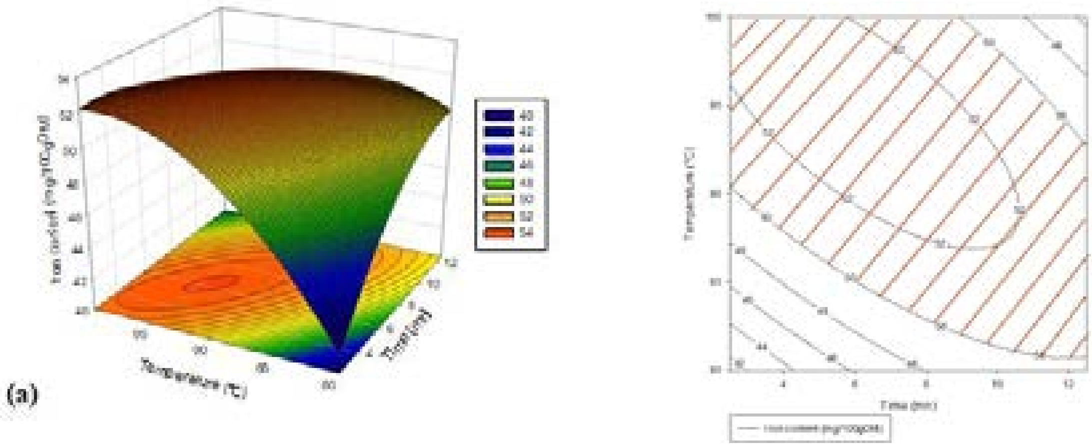
Results & Discussion

Optimal conditions of steam blanching of spinach leaves

The closer the molar ratio Phy/Fe is to 1, the more the iron contained in food is available, and when this ratio is ≥ 14, this iron becomes completely unavailable [35]. Thus, steam blanching to improve the bioavailability of iron consists in bringing the molar ratio Phy/Fe nearest to 1, and implying an increase in the iron content while the phytates content is reducing.

Response of iron content

Fig 1(a) shows the effect of time and temperature of steam blanching on iron content of spinach leaves. Iron content increases with time until 10 min, after what it begins to decrease. It is the same observation with temperature, an increase of iron content until 95°C, and a decrease after this temperature. From Eq (1), it appears that time and temperature of steam blanching have a positive linear effect on iron content while their interaction and quadratics effects have a negative effect. Knowing that the aim of blanching was to optimize iron content in spinach leaves, hatched area on fig 1 (b) represents the zone of experimental domain where iron content is highest. The increase of iron content in spinach leaves by a steam blanching at 95°C during 10 minutes is indeed a concentration, which could be due to the release of iron from its chelating agents on the effect of temperature [35]. After 10 minutes, the decrease observed is the fact of the scrubbing of water-soluble components like iron, because after condensation of the steam, the water droplets fall down and stream on the leaves [36].



(b)

Fig 1. Iron content response surface (a) and contour plot (b)

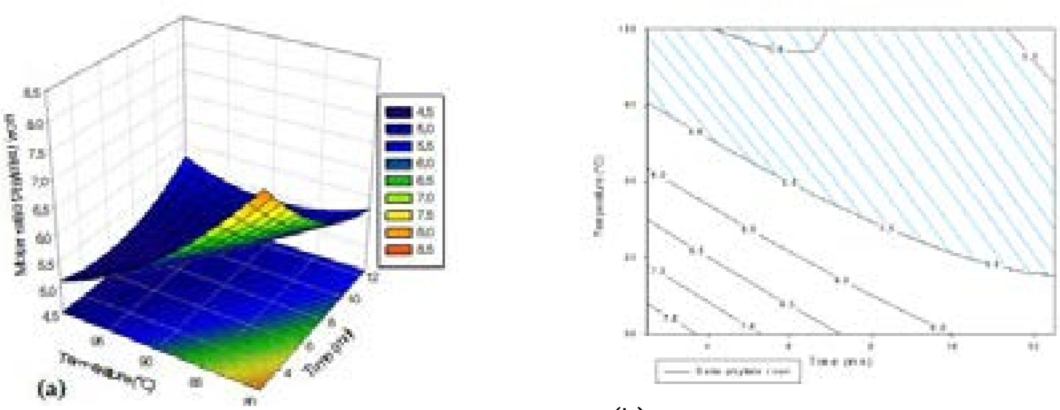
Eq1
$$\text{Iron content} = 52.535 + 0.496X_1 + 2.017X_2 - 2.252X_1^2 - 4.402X_1X_2 - 4.248X_2^2$$

(Written with the coded values); R2 = 88.69%; R2 ajusted = 85.99%

Response of Phy/Fe ratio

As shown in fig 2 (a) Phy/Fe ratio decreases with the time and temperature of steam blanching. The shape of the curve indicates that the fall of the Phy/Fe ratio is fast during the first 6 minutes, and it slows down thereafter. Time and temperature have a negative linear effect on this ratio.

while their interaction and quadratic effects positively affect it. This indicates that the lower the Phy/Fe ratio, the better is the bioavailability of iron. The hatchings on contour plot of fig 2 (b) indicate the zone where Phy/Fe ratio is lowest. The decrease of ratio phytate/iron during steam blanching is allotted to the destruction of phytates during heat treatment [37].



(b)

Fig 2. Ratio phy/Fe response surface (a) and contour plot (b)

Eq2
$$\text{Molar ratio}\ \frac{\text{phytate}}{\text{iron}} = 5.339 - 0.388X_1 - 0.822X_2 + 0.393X_1^2 + 0.815X_1X_2 + 0.537X_2^2$$

(Written with the coded values); R2 = 91.18%; R2 ajusted = 89.08%

Validation of models and compromise size

Table 1 shows that the values of coefficient of determination, Absolute Mean Deviation Analysis and Bias Factor respect the standards, from which the various models are validated.

Table 1: Validation of model

Elements of validation	Abbreviation	Iron	Phy/Fe	Standard values
Coefficient of determination	R^2	88.69 %	91.18 %	≥ 80 %
Absolute Mean Deviation Analysis	AADM	0.011	0.123	0
Bias factor	Bf	1	1	$0.75 \leq Bf \leq 1$

For the determination of the optimal zone, contour plots were superposed (fig 3). A point in this optimal zone was chosen, analyzed, and the results noted in table 2. From fig 3, the optimal zone is between 4 and 10 min for time and from 95 to 100°C for temperature. The compromise corresponding to the optimal conditions of production of steam blanching spinach leaves flour having the best iron bioavailability is 5 minutes at 95 °C.

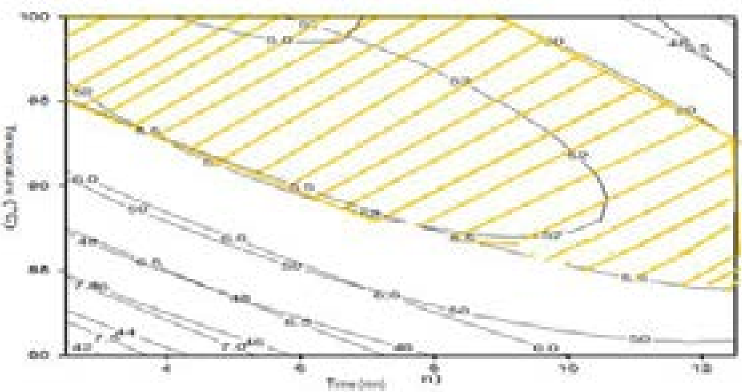


Fig 3. Compromise between iron content and molar ratio Phy/Fe ratio

Table 2: Optimal values of the responses

Responses	Experimental	Multi-response optimisation
Iron (mg/g DM)	52.22 ± 0.67^a	53.13 ± 0.81^a
Phy/Fe ratio	5.01 ± 0.02^b	4.99 ± 0.04^b

The averages on the same line carrying the same letters are not significantly different at $p > 0.05$

Determination of chemical composition of spinach leaves flour

Effect of steam blanching on proximal composition of spinach leaves

Proximal composition of spinach leaves flour produced with and without steam blanching is presented in table 3. In general, the macronutrients are not significantly affected by steam blanching ($p>0.05$). Steam blanching carried out on the spinach leaves under optimal conditions (5min / 95°C) does not affect macronutrients content, and several explanations can be given to this observation. The fact that the leaves are not in direct contact with water reduces considerably the possible reactions of hydrolysis and limits the cellular lysis due to the effect of heat. This absence of water contact also prevents the diffusion of the elements of the spinach leaves towards the cooking water. All this tends to preserve the macromolecular composition of the leaves. Similar results were obtained for Moringa oleifera leaves [14]. In the same light time and temperature of blanching are factors to be taken into consideration. The duration of the treatment (5 min) is short, and the temperature (95°C) low to initiate modifications which may lead to loss or a concentration in nutrients. Another study proved that prolonged steam cooking at higher temperature (15min / 100°C) increased proteins content in Solanum macrocarpum leaves, but reduced lipids contents in Ocimum gratissimum leaves [36].

Table 3: Macronutrients content (g/100g DM) of spinach flour

Flours	Water	Carbohydrates	Proteins	Fats	Fibers	Total Ash
Unblanched	5.29 ± 0.25	26.04 ± 2.06	16.81 ± 2.21	3.75 ± 0.66	28.98 ± 2.85	24.19 ± 1.05
Blanched	5.56 ± 0.96	25.89 ± 1.15	15.11 ± 0.25	3.80 ± 0.12	27.88 ± 2.56	25.47 ± 2.18
P-value	0.658	0.798	0.266	0.932	0.76	0.519

P-value- < 0.05 indicates a significant difference in a column

Effect of steam blanching on some micronutrients content

The effect of steam blanching on carotenoids and four bivalents cations contents of spinach leaves is presented in table 4. Results show that carotenoids and magnesium content is not significantly ($p>0.05$) influenced by steam blanching but significantly reduces ($p<0.05$) phosphorus content while significantly increasing ($p<0.05$) iron and calcium content. The level of reduction of phosphorus is 5.3%, while the level increase of iron and calcium is 35.7% and 18.9% respectively. Steam blanching of spinach leaves do not affect their carotenoids content. The same report was done by several authors in studies conducted on the effect of blanching and cooking on carotenoids content of spinach flours [38], pumpkin leaves [39] and yellow maize [40]. This could be due to the fact that, carotenoids are localized inside the internal membranes of chloroplasts surrounded by a double membrane [41], which protect them from heat treatment in certain conditions. Under the optimal conditions of steam blanching, iron content of spinach leaves have been significantly enhanced (35.7%). The same report was observed in a study on steam blanching of spinach leaves in others conditions (100°C/15min), an increase of 6.74% in iron content [42]. The low increase of iron content compared to this present study can be allotted to the experimental conditions. In general, iron is one of the most stable mineral during heat treatments [39, 42]. It is recommended that 100g of infant flour should contain a minimum of 7 to 18.6 mg of iron, according to the bioavailability of iron in the food matrix used [43]. With an iron content of 52.10 mg/100 g DM, flour obtained with blanched spinach leaves in optimal conditions can be used in infant food formulations and fortification.

Table 4: Carotenoids (µg/100g DM) and minerals (mg/100g DM) content in spinach flour

Flours	Carotenoids	Iron	Calcium	Phosphorus	Magnesium
Unblanched	30.76 ± 1.85	38.40 ± 1.01	101.32 ± 0.56	406.81 ± 1.21	24.19 ± 1.05
Blanched	26.98 ± 1.06	52.10 ± 2.33	120.45 ± 1.09	385.24 ± 1.51	19.67 ± 2.14
P-value	0.055	0.019	0.001	0.001	0.052

P-value- < 0.05 indicates a significant difference in a column.

Effect of steam blanching on some anti-nutrients and Phy/Fe ratio

Table 5 presents some anti-nutrients content and the Phy/Fe ratio of spinach leaves with and without blanching. Anti-nutrients content is considerably reduced in the steam blanched leaves. This reduction is about 47%, 74.1%, 68.8% and 71.3% of phytates, total tannins, phenolic compounds and oxalates respectively. There is also a significant reduction ($p<0.05$) of about 60.7% of the Phy/Fe ratio. The reduction of anti-nutrients content in blanched leaves of spinach could be allotted to the effect of steam cooking, knowing that anti-nutrients are destroyed under the effect of heat treatments [37, 13]. Blanching has been shown to be an efficient method to reduce level of phytates, total tannins, phenolic compounds and oxalates in other leafy vegetables like pumpkin leaves, thereby enhancing the level of nutrient bioavailability [39].

Steam blanching enhances iron content and reduces phytates in spinach leaves, with as consequence the significant reduction of the Phy/Fe ratio. Knowing that the bioavailability of iron is better when the Phy/Fe is closer to 1, steam blanching in the optimal condition defined in this study has improved the bioavailability of iron in spinach leaves. The same report has been done on Moringa oleifera leaves [13].

Table 5: Anti-nutrients content (g/100g DM) of spinach leaves flours

Flours	Phytates	Tannins	Phenolic compounds	Oxalates	Phy/Fe
Unblanched	0.45 ± 0.06	0.81 ± 0.01	1.25 ± 0.06	1.29 ± 0.05	11.72 ± 0.05
Blanched	0.24 ± 0.02	0.21 ± 0.01	0.39 ± 0.02	0.37 ± 0.08	4.61 ± 0.31
P-value	0.004	0.000	0.002	0.001	0.000

P-value- < 0.05 indicates a significant difference in a column.

Effects of steam blanching of functional properties of spinach leaves flour

Table 6 below presents the effect of steam blanching of spinach leaves on the rehydration properties of its flour. Water absorption capacity is significantly ($p<0.05$) reduced (about 5%) by steam blanching, while water solubility index and bulk density are significantly increased ($p<0.05$), with percentages of 15.7 and 4.7 % respectively. In food materials, water solubility index is complementary to water absorption capacity. The first provides indication of which portion of material can get solubilized in water upon soaking, while the second indicates the capacity of material to absorb water. The observation that blanched spinach leaves flour is able to hold water up to three times its mass, is very appreciable since solid-water interactions constitute a limiting factor in the utilization of food powders [44]. The decrease of water absorption capacity after blanching could be justified by a slightly decrease of carbohydrates and proteins content, which are the major component absorbing water in foods [45].

The bulk density provides an indication of the packing and arrangement of the particles, as well as the compaction profile of a material [46]. It is the capacity of the flour to be concentrated in a small volume. The increase in the bulk density by blanching indicates an improvement in nutrients density also. All these properties enhance the quality of blanched spinach leaves flour in the development of various food formulations such as the infant foods flours.

Conclusion

Steam blanching of spinach leaves at 95°C during 5 minutes produces flour with high content in iron and low content in phytates and others anti-nutrients. This flour treated under these conditions improves the bioavailability of iron in spinach leaves. The low water absorption capacity and high bulk density, associated to the high iron and low anti-nutrient content suggest that flour produced with spinach leaves blanched in optimal conditions (90°C/5min) can be used for formulation of infant foods.

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