Effects of Cooking and Defrost Methods on Retinol Concentration of Chicken Liver

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Abstract This study aimed to analyze the retinol concentration of chicken livers in various brands, batches and types of both organic and free range by HPLC. In addition, the effects of different cooking and defrosting methods on the retinol levels of chicken livers were also determined. Chicken livers samples were roasted for 35 minutes at 200°C, cooked for 40 minutes at (26 to 100)°C, fried for 5 minutes at 180°C. Other samples were frozen for 72 hours and divided to be defrosted by fridge for 8 hours at 3°C, at ambient temperature for 2 hours at 23°C, submersed in water for 35 minutes at 25°C, and microwaved on defrost function for 1 minute, where all methods were compared to its natural state (3°C). The mean retinol concentrations in brands A, B, C and organic and free-ranged were 9152, 4673, 5943, 3401, 30094 µg/100 g (P < 0.001). Significant losses of 39.9 % (P < 0.05), and 26.2 % (P < 0.01) were found when compared to natural state (raw) to roasting and microwave, respectively. This study showed a significant difference between brands and types of chicken livers, and a significant loss of retinol in chicken liver, in food processes commonly used. Otherwise, vitamin A values in chicken liver suggest that consumption could be recommended to prevent vitamin A deficiency.

Keywords: chicken liver, retinol, cooking, defrost, HPLC

1. Introduction

After World War II, due to food shortages, an alternative to beef was required. Consequently, the birds were raised on pastureland and in large warehouses with the objective of producing meat and eggs [1].

The liver is one of the principal parts of chicken giblets, and is considered an important economic and nutritional resource, rich in proteins, iron, and vitamin A [2,3,4].

Food products from animals are highly perishable, its low stability results from activity of diverse agents, where the microorganism, the enzyme activity, the wrong food processing, and the lack of information are the largest agents of food inadequacy for human consumption [5-9].

The use of high and low temperatures is an old method for food preservation due to the partial or total inhibition of the enzymatic and microbial activities. Defrosting stage conditions plays an important role for frozen meat to conserve the flavour of the products derived from meat and the texture of the final product. Thus, defrost performed in a wrong way causes microbial growth and chemical reactions of protein insolubility, and lipid oxidation, resulting in loss of nutritional quality of food [10,11].

Vitamins and minerals are the fundamental components of human metabolism, being involved in almost all known reactions and biochemical pathways involved in growth, reproduction and maintenance of life [12]. The food processing affects, in large scale, the vitamin level in foods, which may be a critical factor in the final quality of the food. This fact is dependent on nutritional value analysis, how this nutrient will contribute to the diet, and to whom it applies [13,14,15,16].

There is a need for knowledge on the vitamin A level evaluation, in different food preparation methods, from storage to preparation, being this knowledge of huge nutritional importance. The current study aimed to evaluate the concentration of retinol in chicken liver of different brands, batches and types (organic and free-range chicken), and the effects of different cooking and defrosting methodologies on retinol concentrations of chicken liver.

2. Materials and Methods

2.1. Biological Material

For this work, samples from three batches and three brands (named as Brand A, Brand B, Brand C) of chicken liver, chilled, within the expiry date, and traded and purchased in supermarkets in the greater area of Natal, Brazil, were used. One batch from one brand of organic chicken liver and one batch of free-range chicken liver were used too.

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2.2. Preparation of Samples

2.2.1. Homogenate Preparation

The preparation of the homogenates was performed in the Food Laboratory of the Department of Nutrition (DNUT)/Federal University of Rio Grande do Norte (UFRN), Brazil.

1 kg of chicken liver was weighed, these were then placed separately in a blender, Arno model, together with 1 L of a 0.9 % saline solution (NaCl), resulting in approximately 2 L of homogenate (50 %) and repeated for each brand, type and cooking and defrost methods.

2.2.2. Different Brands and Types

For retinol analysis of the different chicken brands livers (A, B, and C) and types (organic and free-range chicken).

In each of three 15 mL Falcon tubes, it was introduced 1 g of homogenate from brand A, and repeated the process for the B, C brands and organic and free-range chicken. The tubes were chilled and kept in a refrigerator at 3°C until the time of analysis. The sample preparation procedure was repeated on two additional batches of each brand.

The homogenates were transported in a refrigerated cooler to the Laboratory of Biochemistry of Food and Nutrition at the Center for Biosciences of the Federal University of Rio Grande do Norte, where there were subjected to retinol extraction.

2.2.3. Different Cooking Methods: Roasted

In order to make roasted liver, 1 kg of raw liver was put in a roasting dish and placed in an oven, preheated in 200°C, for 35 minutes. Following the cooking stage the liver was put apart to cool down.

2.2.4. Different Cooking Methods: Cooked

In order to make a cooked liver, 1 kg of raw liver was put in a large pan, approximately 1 L of water was added in order to cover the liver, and it was cooked in medium heat for 40 minutes, in a temperature starting (26 to 100) °C. After the procedure, the water was removed and the liver was put apart to cool down.

2.2.5. Different Cooking Methods: Fried

In order to make a fried liver, 6 mL of soybean oil (dessertspoon) was put in a pan, "Teflon" type, to heat. A small portion of raw liver was put at a time, frying for 5 minutes each side, reaching the temperature of 180°C and golden color. The same procedure was repeated with the rest of the liver. After the process, the liver was put on absorbent paper and cooled down.

Another kilo of raw liver was put in the fridge at 3°C.

The livers were submitted to homogenate preparation. Three Falcon tubes, with 15 ml capacity, were utilized to weighed 1 g of raw liver homogenate and for each other method: roasted, cooked, and fried.

2.2.6. Different Defrost Methods

One kilo of raw chicken liver was acquired and transported in a cooler to the Laboratory of Biochemistry of Food and Nutrition, of Biosciences Center, of Federal University of Rio Grande do Norte and it was submitted to homogenate preparation.

Fifteen Falcon tubes were utilized to weighed 1 g of fresh homogenized liver, where 12 tubes were put in a freezer at -18°C for 72 hours. After 72 hours, the 12 tubes were separated into four groups, such methodology has been adapted according to a study performed in Ireland with head chef and managers, on their practices with defrost, and a study on consumption and domestic preparation of meat in Turkey [17,18]: a group of three tubes was left in the fridge for defrosting for 8 hours, at 3°C, other group of three tubes was left in the table at ambient temperature (23°C) for 2 hours, another group of three tubes was submerged in water at 25°C for 35 minutes, and then, other group of three tubes was put in a microwave, Brastemp model, in defrost function for one minute. The other three tubes containing fresh liver, as well as the other 12 tubes, were taken for analysis as following described.

2.3. Retinol Analysis

2.3.1. Extraction of Retinol from the Liver

The extraction and measurement of retinol in the foods was performed in triplicate and according to [19]. The methods were adapted according to laboratory conditions.

Into each Falcon tube containing 1 g of homogenate, it was added 1 mL of 95 % ethanol to precipitate the proteins present in the sample.

Before the lipid extraction, saponification was performed by adding 1 mL of potassium hydroxide (KOH) 50 % to a bain-marie at 60°C for 1 hour, while stirring, with the objective of hydrolyzing the retinyl esters present in the samples.

For the lipid extraction, the samples were washed with 2 mL of hexane and stirred for 1 minute, allowed to stand for 5 minutes and then the supernatant was placed in a new tube. This process was then repeated two more times, resulting in approximately 6 mL of hexane. A 250-μL aliquot of hexane was removed, placed in a new 5-mL polypropylene tube and evaporated to dryness in a bain-marie at 37°C before storing the samples at -18°C, under a nitrogen atmosphere and protected from light.

Before the application of HPLC (*High-performance liquid chromatography*), the samples were redissolved in 1 mL of absolute ethanol and 20 μL of the samples were analyzed by HPLC. The samples were administered in duplicate and a mean between the two areas was obtained.

2.3.2. High Performance Liquid Chromatography (HPLC)

The concentration of retinol in the chicken liver was determined by a chromatograph consisting of a LC-20 AT pump coupled to an SPD-20A UV-VIS detector, a reversed phase Phenomenex® Luna® 5 μm C18(2) 100

Å, 250 mm x 4.6 mm column and a computer with the *LC solution software* for data processing.

The mobile phase used for retinol analysis of the samples was 100 % methanol in an isocratic flow system of 1 mL/min. The wavelength (λ) adopted for the monitoring of retinol absorbency was 325 nm.

The identification and measurement of the retinol in the samples was established by comparing the area obtained in the chromatographic profile with the areas of standard all-*trans* retinol (Figure 1).
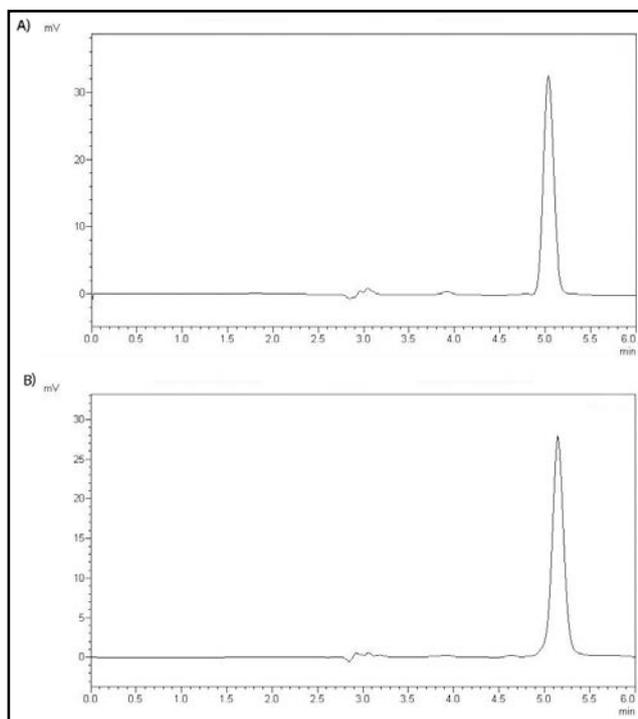

Figure 1. Retinol elution profile in HPLC. A) Chromatogram of all-trans retinol of 24.35 ng/20 µL; B) Chromatogram of chicken liver sample

2.3.3. Linearity, Precision and Accuracy of the Method

The linearity of the method is the instrumental response of the linear relationship of calibration (obtained area) with the standard concentration, it is satisfactory when the straight line correlation coefficient nears 1 [19].

Six different dilutions of standard retinol (3.37 ng/20 µL, 6.75 ng/20 µL, 13.49 ng/20 µL, 26.98 ng/20 µL, 53.96 ng/20 µL, and 107.92 ng/20 µL) were applied in the HPLC. A graph was then constructed from the straight line equation using their respective areas (26 426, 55 687, 119 135, 238 471, 498 955, 989 117). The straight line equation was obtained by linear regression (peak area *vs.* standard concentration), with R = 0.9999 (Figure 2).
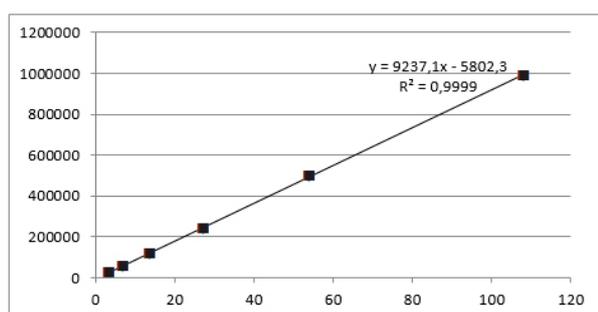
Figure 2. Gauging curve and straight-line equation obtained through application in the HPLC, at different concentrations of retinol standard

The precision of the method was analyzed by a repeatability test, using extractions from the same sample of each brand, on five alternate days. The coefficient of variation (CV) was calculated by the standard deviation about the mean and expressed as a percentage. The results were <15%, which in accordance with [21], is considered admissible.

The accuracy of the method was verified by recovery testing. Additional known quantities of standard retinol were added to the samples from the three brands. These samples passed through the same method of retinol extraction, described in section 2.3.1, and the method accuracy showed recoveries above 93 %.

2.3.4. Relationship between the Recommended Single Serving of Liver and Vitamin A Requirement

According to the Food Guide for the Brazilian Population, the recommended intake of giblets is one serving per week. Regarding its cooking measure, this portion corresponds to 2 units of chicken liver or 88 g [22]. The amount of retinol contained in a chicken liver portion was thus calculated following this principle and the ratio of the daily nutritional requirement was verified according to the recommendations from the Institute of Medicine [21]. Next, the chicken liver was classified as a source of vitamin A. A food is considered a certain nutrient source when it contains more than 5 % of the Dietary Reference Intakes (DRI) in one portion. A food is a good source if it contains between 10 % and 20 %, and an excellent source if it contains more than 20 % of the DRI in a single serving [22].

2.3.5. Statistical Analysis

The results were tabulated and submitted to descriptive statistics using Microsoft Excel. Retinol levels were expressed as means and standard deviations. Analysis of variance (ANOVA) was performed to verify statistical differences in retinol composition between batches of each brand and between brands. Afterwards, we used the post-hoc Tukey test, at a significance level of 0.01 ($P < 0.01$), to evaluate differences between means. The analyzes were performed using Statistica 7.0 software [23].

3. Results and Discussion

3.1. Retinol in Different Brands and Different Batches

The average concentration of retinol in the three batches of the three different brands is shown in Table 1.

This study found statistically different values among the means of the three lots of the three brands (Table 1).

Table 1. Mean Concentration of Retinol ± SD. Means Followed By Different Letters in the Same Column Differ from Each Other by the Tukey Test with P < 0.001. SD: Standard Deviation

Group	Mean Retinol Concentration in µg/100 g ± SD
Brand A	9152.9 ± 719[a]
Brand B	4673.1 ± 389[b]
Brand C	5943.6 ± 614[c]
Organic	3401,33 ± 597,12[b]
Poultry	30094,79 ± 4628,75[d]

Reference [26] assessed the concentration of vitamin A in two different strains of chicken liver, Cobb and Ross, subjected to the same breeding process, and they presented values of 6678 µg/100 g and 8324.1 µg/100 g, respectively. The results of the Cobb breed were close to the mean of brand C, and the Ross breed, to the mean of brand A.

Some breeds of chicken have higher requirements for certain nutrients due to differences in the absorption and conversion of pro-vitamin A compounds to retinol, thereby influencing the quantity stored in the liver [24].

Other studies analyzing the retinol concentration in chicken liver are shown in Table 2.

Table 2. Different Studies Showing Respective Retinol Concentrations Obtained, in µg/100 g, in Chicken Liver

Reference	Location	Vitamin A Concentration (µg/100 g)
[25]	Austria	5600
[26]	United Kingdom	9700
[27]	Brazil	10 455
[28]	Germany	13 220
[29]	Poland	10 389 to 16 218
[30]	Canada	18 760

Table 3 shows the quantity of vitamin A in chicken liver in the main tables of food composition. The present study revealed results superior to all the tables, except for the Tucunduva Food Composition Table [31].

Table 3. Retinol (µg/100 g) of Chicken Liver in the Food Composition Tables

Food Composition Tables	Retinol (µg/100 g) of Chicken Liver
[32]	3863
[31]	11 325
[33]	3296
[34]	3978
[35]	4000

Reference [28] mention that the incorrect description of foods, inadequate sampling, the use of counter analytical methods, variability of genetic and environmental factors, and the form of preparation and processing are key biases that may result in differences between the data in the various tables of food composition. They also cite the breed, as well as the management and age of the animals, as key influencing factors in the results of the composition of nutrients in food. In this regard, in order to diminish biases, a methodological standardization in nutrient analysis is recommended [36].

Reference [30] analyzed the concentration of vitamin A in the livers of 56 days old chickens, with vitamin supplementation of 10 IU/g to their diet, and found values of 25 820 µg of retinol/100 g, which represents about 5 times more than that found in brand C during the present study. This same study made a comparison with 42 days old chickens, and obtained results of 12 240 µg of retinol/100 g of liver. These different concentrations show a positive relationship between a higher intake of vitamin A and a greater amount of vitamin A in the liver of these animals, suggesting that chicken producers are not concerned with vitamin A supplementation in the diet, or, are using diets that lack pre-formed vitamin A.

Furthermore, the environment in which the animal lives, and factors such as temperature and stress, can influence food intake, causing problems in their development and performance [37].

The group represented by free-range chicken showed significantly higher values in relation to the farm and organic. This difference comes from, for the most part, the food. Hens are created predominantly in semi-intensive systems, characterized by the availability of an aviary, where are the feeders, drinkers and nests, however, also there is the option of having access to a free area of grazing and recreation [38].

In this kind of creation, characteristic of family farming, animals are fed corn-based diets, supplemented with specific concentrates, depending on the purpose of creation. Due to hens have some freedom, they consume what are available, such as grasses, seeds, insects, worms and crops. Some farmers in an attempt to improve the quality of chickens, even offer food waste such as rice and vegetable rich in beta-carotene [38].

3.2. Vitamin A in Different Cooking Methods

Changes in vitamin A values after diverse cooking methods are shown in Table 4.

Table 4. Retinol Concentration in Chicken Liver Of Brand B, Subjected to Different Cooking Methods and Their Losses. Each Line Value Corresponds to the Average ± Standard Deviation Of Triplicate. * Means Differs Significantly (P < 0.05) from the Raw

Cooking Methods	Retinol (µg/100g)	% Loss
Roasted at 200°C, 35 min	6,915.8 ± 818.2*	39.9
Cooked at (26 to 100)°C, 40 min	10,802.9 ± 881.2	6.1
Fried at 180°C, 5 min	10,424.1 ± 863.0	9.4
Raw at 3 ºC	11,510.6 ± 245.7	

Cooking methods of meat products are designed to be more palatable, soft, and digestible products, besides ensure the food safety because heat-sensitive microorganisms are eliminated or reduced [39].

Roasted foods are healthy and practical, but take longer to cook, which allows the heat contact with the food, causing losses of certain nutrients such as vitamin A, sensitive to excess heat, besides turning the food dry.

Foods after roasted continue to cook inside, even after its being removed from the oven; it occurs because the heat continues to transfer energy from outside to inside of the food [39].

3.3. Vitamin A in Different Defrost Methods

Changes in vitamin A values after diverse defrost methods are shown in Table 5.

Table 5. Retinol Concentration in Chicken Liver of Brand A, Subjected to Different Defrost Methods and Their Losses. Each Line Value Corresponds to the Average ± Standard Deviation of Triplicate. * Means Differs Significantly (P < 0.01) from the Raw

Defrost	Retinol (µg/100g)	% Loss
Fridge 3°C, 8h	4,663.9 ± 347.0	7.9
Bench 23°C, 2h	5,133.2 ± 410.7	
Submersion in Water 25°C, 35 min	4,424.2 ± 75.9	12.6
Microwave for 1 min	3,733.6 ± 241.2*	26.2
Raw 3°C	5,062.2 ± 481.9	

The lack of information on the correct defrost method of food is one of the major mistakes that compromise the food safety. Studies show that the relationship between the socioeconomic and educational status is directly linked to erroneous practices in food preparation [17,18].

According to Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA), there are safe methods to make the defrost, which guarantee the nutritional quality and safety of food at the end of the process, such as defrost in the fridge, in cold running water, and microwave [34].

Though it may be a safety and fast method [41], the defrost in microwave had a significant decrease (Table 5) in the physical and chemical quality of the food, especially in vitamin A, when compared to concentration of vitamin A in fresh chicken liver.

A study evaluating the effect of cow's milk heating in a microwave, on vitamins A, E, B1, B2, and B6, showed a significant decrease of vitamin A [42].

Reference [43] studied the effect of breast milk defrost in microwave, on vitamin A values, and observed a significant decrease of 34 %.

Another study showed a significant loss of carotenoids around 57 % in papaya purees submitted to thermal processes using microwave [44].

Microwaves are characterized by their emission frequency between 300 MHz and 300 GHz, with a wavelength ranging from 1 cm to 1 mm, and associated energy from 1.2×10^{-12} to 1.2×10^{-7} eV. Its energy is converted into heat when absorbed by matter, where the water is the main component absorbing energy, and generating heat to the food. Microwaves easily penetrate the food, generating heat around the entire food uniformly and quickly. One of the mechanisms of heat generation by microwaves is dipoles rotating, favored in food with a high moisture level, which generates friction and molecular shock, increasing the kinetic energy, and consequently, the food temperature. Besides water, sugar and ethanol are components interacting with the microwaves by dipoles rotating, thereby increasing the heat supplied to the food, and consequently, increasing the loss of labile vitamins [45].

Thus, although it is stable to heat, vitamin A was lowered significantly. In addition, it is a vitamin sensitive to light and subject to oxygen actions, which helps in the significant losses.

Chicken liver is a great food, given its accessibility, good palatability, low cost, a good source of vitamin A, thus minimizing cases of vitamin A deficiency in developing countries [46].

3.4. Chicken Liver as a Source of Vitamin A

Considering the amount of retinol in a normal serving, chicken liver is considered an excellent source of vitamin A, as one serving exceeds 20% of the DRI.

Acute or chronic toxicity or hypervitaminosis A are reported when retinol levels are greater than 100 µg/dL. Signs of this toxicity are: hair loss, mucosal dryness, chapped skin, double vision, headache, hepatic fibrosis, ascites, and fractures [12,47]. [48] describe chronic toxicity resulting from the ingestion of large amounts of

previtamins, for months or years, where the quantities of this ingestion may vary accordingly with the individualities of the organism.

Some authors presented possible risks of congenital anomalies in babies born to mothers who took more than 3000 µg of vitamin A per day in the form of supplements during the gestation period. According to the study, about 1 infant in 57 had a malformation attributable to the supplement [49]

Although it is necessary for bone growth, evidence indicates that vitamin A intake above 1500 µg/day is associated with decreased bone density and an increased risk of fractures [50].

4. Conclusions

Chicken liver showed significantly different retinol levels among the different brands. This difference could signify that livers originating from different farms and distinct breeds of chicken with contrasting diets, and should be investigated.

A significantly reduction was found in concentration of vitamin A, in values of 39.9 % and 26.2 %, in chicken livers subjected to roast in oven at 200 ºC for 40 minutes, and defrost by microwave for 1 minute. However, chicken livers have a high amount of vitamin A, meeting the daily needs and proving to be a food of excellent source of vitamin A, being a great alternative against the deficiency of this vitamin. It is highlighted that in populations with a good nutrition of vitamin A, the consumption of this food shall be moderate, considering the toxic and teratogenic effects of a high ingestion can cause.

Acknowledgements

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