International Journal of Nutrition and Food Sciences
2025, Vol. 14, No. 4, pp. 199-210
https://doi.org/10.11648/j.ijnfs.20251404.13

Research Article

Hypolipidemic and Antioxidant Potential of Fermented Beverages Made from Tamarind (*Tamarindus indica*), Ginger (*Zingiber officinale*), and Turmeric (*Curcuma longa* L.) in Rabbits

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Abstract

Beverages are important components of diet as they facilitate hydration. However, their high sugar content is often implicated in metabolic diseases development. Tamarind (*Tamarindus indica*), turmeric (*Curcuma longa* L.), and ginger (*Zingiber officinale*) may help address this issue due to their antioxidant properties. A study was therefore conducted to assess the antioxidant properties of a tamarind-based beverage prepared with 10% turmeric and ginger. The beverages were subjected to spontaneous fermentation for 36 hours at 37 °C. Subsequent analyses were performed to evaluate vitamin C content, total phenolic compounds, flavonoids, and beverage antioxidant potential. The *in vivo* hypolipidemic and antioxidant properties were also tested on healthy 2-month-old rabbits. Results revealed that the combination of tamarind (90%), turmeric (5%), and ginger (5%) led to a significant increase in vitamin C and phenolic compound levels, rising from 27.14 to 42.95 mg/L and from 1.498 to 1.514 mg GAE/L, respectively. In tamarind-turmeric-ginger beverage, flavonoid levels increased from 0.132 ±0.02 to 0.164 ±0.01 mg QE/L. DPPH 50% inhibitory concentrations (IC_{50}) of fermented tamarind-turmeric and tamarind-turmeric-ginger beverages were 2.14 and 2.23 µg/mL, respectively, revealing antiradical activities of 37.74 and 44.84 µmol of reduced DPPH. The tamarind-turmeric-ginger combination had a hypolipidemic effect after the fattening phase, reducing triglycerides from 2.24 to 1.81 mg/dL, LDL cholesterol from 5.87 to 0.26 mg/dL, and total cholesterol from 6.02 to 0.42 mg/dL. However, it increased HDL cholesterol from 0.35 to 1.54 mg/dL. Regarding antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, tamarind-turmeric-ginger blend led to increases after the fattening phase from 4.44 to 10.29 U/mL for superoxide dismutase and from 12.02 to 193.16 U/mL for glutathione peroxidase. Beverages prepared with tamarind, turmeric, and ginger demonstrated substantial antioxidant potential, stimulating increased antioxidant enzyme activity that may help mitigate metabolic diseases.

Keywords

Tamarind, Ginger, Turmeric, Fermentation, Antioxidant Potential

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Received: 11 June 2025; **Accepted:** 25 June 2025; **Published:** 16 July 2025

1. Introduction

Metabolic diseases, diabetes, and dyslipidemia are among the leading causes of mortality worldwide due to their high prevalence and associated complications [1]. While cholesterol is essential for the formation of the lipid cell membrane, elevated levels—especially when poorly distributed throughout the body and combined with prolonged exposure to high glucose concentrations—stimulate pathways that increase the production of free radicals (ROS: reactive oxygen species) and oxidative stress [2]. Free radicals are produced for various reasons, including aging, environmental factors, and poor dietary habits such as the consumption of fatty or sugary foods and beverages. However, these free radicals can disrupt bodily functions and contribute to various pathologies development, including diabetes and metabolic syndromes [3].

To combat the effects of free radicals, the consumption of fruits and vegetables is recommended, as they contain phenolic compounds and phytosterols with antioxidant and cholesterol-lowering properties [4]. Among these plants, tamarind has laxative, antioxidant, hypotensive, hypoglycemic, and hypolipidemic properties that can help fight against metabolic diseases.

Tamarind (*Tamarindus indica*) is widely appreciated and consumed by Ivorian population, primarily in the form of juice. However, traditional juices often have the problem of added sugar, typically adjusted to producer taste. Therefore, improving juice production process would be beneficial in order to offer a healthy, antioxidant-rich product that could help reduce the rise of metabolic diseases. Several approaches can be used for this purpose, such as fermentation and the addition of antioxidant spices.

Fermentation is a process used to extend the shelf life of food while improving health and safety by inhibiting the growth of potentially harmful microorganisms and enhancing the nutritional and organoleptic properties of food [5, 6]. Antioxidant spices like ginger and turmeric have been shown to possess antioxidant potential that helps neutralize free radicals [7, 8], particularly hydroxyl and superoxide anions, thereby exerting a protective effect against free radical-induced damage to lipids and DNA [9].

Some authors [10] have reported that incorporating ginger (*Zingiber officinale*) and turmeric (*Curcuma longa* L.) into tamarind increases the antioxidant potential of the unfermented beverage. Another study indicated that tamarind fruit pulp has antioxidant effects and may offer protective benefits against diet-induced hypercholesterolemia [11]. Furthermore, a substantial body of research has highlighted the health benefits of fermented foods [12], as well as the enhanced nutritional value provided by fermentation (e.g., phenolic compounds, B vitamins, essential amino acids) in fruits and vegetables [13], and their ability to reduce the LDL/HDL cholesterol ratio—a widely accepted indicator of cardiovascular risk [14]. However, these studies have not addressed the cumulative effect of fermentation and antioxidant spices on improving tamarind juice.

The present study aims to fill this gap by evaluating the effects of fermented and non-fermented beverages on lipid profiles (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol) and antioxidant enzymes (SOD, GPX) in rabbits. To achieve this, *in vitro* antioxidant potential will first be assessed, followed *in vivo* hypolipidemic and antioxidant parameters evaluation.

2. Materials and Methods

2.1. Biological Material

Plant materials used in this study were tamarind pods (*Tamarindus indica*), turmeric (*Curcuma longa* L.), and ginger (*Zingiber officinale*). Tamarind pods were harvested in Lagnonkaha, a village in Korhogo department of the PORO region, located in northern Côte d'Ivoire, approximately at 600 kilometers from Abidjan, the economic capital. Turmeric and ginger rhizomes were collected in Divo, in the LÔH-DJIBOUA region, in southwestern Côte d'Ivoire, at approximately 187.9 km from Abidjan.

Animal materials consisted of 30 Hyplus rabbits, aged two months, with body weights ranging from 600 to 1800 grams. The rabbits were acclimatized for one week in cages and received daily water and pellets (IVO-GRAINS). They were divided into 11 groups of 3 rabbits and received treatments over a period of 28 days. All rabbits were used for *in vivo* tests, and the experimental protocol and animal handling procedures were conducted in accordance with the guidelines of the Ethics Committee of Nangui Abrogoua University (Côte d'Ivoire).

2.2. Methodology

2.2.1. Beverage Fermentation

Tamarind beverage was prepared by adding 800 mL of water to 250 g of tamarind. Tamarind-turmeric and tamarind-ginger beverages were prepared by mixing 800 mL of water with 90% tamarind (225 g) and 10% turmeric or ginger (25 g), respectively. The tamarind-turmeric-ginger beverage was prepared by mixing 800 mL of water with 90% tamarind, 5% turmeric, and 5% ginger. The mixtures were transferred into sterilized glass jars, sealed hermetically, and 20 g of salt was added as a preservative and as a nutrient for bacterial growth. Samples of each beverage were taken at time 0 (T0H), representing the non-fermented beverages. The remaining mixtures underwent spontaneous fermentation for 36 hours at 37 ℃, maintaining a pH below 3 for each beverage. Samples were then taken every 12 hours for analysis. However, for *in*

vivo analysis, only T0H and T36H samples were used.

2.2.2. Determination of Biochemical Parameters and *In Vitro* Antioxidant Activity

Vitamin C content was determined according to the method described in [15]. Carotenoids were analyzed following [16]. Total polyphenols were measured using the method of [17], and flavonoids were quantified according to [18]. Antiradical activity using DPPH was determined using the method of [19]. The IC_{50} (half maximal inhibitory concentration for DPPH) was determined from the graph and used to calculate the antioxidant power following the method described in [20], using the following equation:

$$ARP = \frac{\text{Concentration of DPPH solution (mg}\frac{\text{sample}}{\mu\text{mol DPPH}}\text{ reduced)}}{\text{CI50 x 10}-3\ (\mu g/ml)} \quad (1)$$

2.2.3. Induction of Hyperlipidemia and Beverage Administration

Hyperlipidemia was induced over a two-week period using VITALAC fattening pellets and water. Ten groups of rabbits were fed VITALAC, while one group received IVOGRAINS, the regular pellet feed. Induction of hyperlipidemia was then confirmed through blood analysis. Following this phase, the groups received beverages or water as follows: The group fed with IVOGRAINS continued to receive IVOGRAINS and water for the next two weeks and was considered as negative control group. Group 2, which had been given VITALAC, continued with VITALAC and water and served as positive control group. The other groups were switched to IVOGRAINS for the next 14 days. Additionally, Group 3 received vitamin C and served as a reference group for antioxidant enzyme testing. The remaining groups (4 to 10) received the following beverages:

Non-fermented beverages:

1) Tamarind only (A100%)
2) Tamarind 90% + Turmeric 10% (AC10%)
3) Tamarind 90% + Ginger 10% (AG10%)
4) Tamarind 90% + Turmeric 5% + Ginger 5% (ACG10%)

Fermented beverages:

1) Tamarind only (D100%)
2) Tamarind 90% + Turmeric 10% (DC10%)
3) Tamarind 90% + Ginger 10% (DG10%)
4) Tamarind 90% + Turmeric 5% + Ginger 5% (DCG10%)

Each group received 7.5 mL of beverage diluted in 742.5 mL of water daily, to make a final beverage volume of 750 mL.

2.2.4. Blood Sampling

Blood samples collection was performed by puncturing the marginal ear vein using a syringe after hyperlipidaemia induction. Blood was taken at the begining (J0), at J14 and at J28 (at the end of the experience). Blood was collected in dry red tubes and subjected to centrifugation at 4000 rpm for 10 minutes. Serum was collected and used for triglycerides and cholesterols analysis. It was also stored at -20 ℃ for determination of antioxidant enzymes.

2.2.5. Determination of Triglycerides, Total Cholesterol, HDL and LDL Cholesterol

Triglycerides, total cholesterol and HDL cholesterol were determined using Reckon diagnostic kit from India. The wavelengths were 500 nm for triglycerides, 505 nm for total cholesterol and HDL cholesterol. LDL cholesterol was performed using the following formula:

$$\text{LDL cholesterol} = \text{total cholesterol} - (\text{HDL cholesterol} + (\text{TRIG}/5) \quad (2)$$

2.2.6. Determination of in Vivo Antioxidant Activity

i) Superoxide Dismutase (SOD)

Assay was carried out according to protocol BC0170 of the Superoxide Dismutase (SOD) Activity Assay Kit from SOLARBIO (China). The spectrophotometer was preheated for 30 minutes, adjusted to 560 nm wavelength and set to zero with distilled water. Then, to 90 µL sample, 240 µL of Reagent I, 60 µLof Reagent II working solution. 180 µL of Reagent III, 400 µL of distilled water and 30 µL ofReagent IV working solution were added. The mixture was then thoroughly mixed and incubated at 37 ℃ for 30 minutes. The mixture was finally put into 1mL glass cuve and absorbance value was detected at 560 nm.

ii) Determination of Glutathione Peroxidase

Assay was carried out according to protocol BC1195 of the "Glutathione Peroxidase Assay" kit from SOLARBIO (China). Spectrophotometer/microplate reader was preheating for 30 minutes. The wavelength was adjuted to 412 nm andspectrophotometer counter was set to zero with distilled water. A quantity of 80 µmol /mL of standard solution was diluted with diluent to 0.08 µmol/mL. The standard solution was prepared when the solution was ready to be used. A quantity of 20 µL Reagent I working solution was added to 20 µL of sample supernatant. The mixture was mixted and preheated 5 min at 37 ℃ Then, 5 ml of Reagent II working solution was added and let to react at 37 ℃ with the mixture. After this, 200 µL of Reagent III were added. All the mixture was centrifuged at 4000 rpm at room temperature for 5 minutes and 100 µL of supernatant, added with 100 µL of Reagent IV and 25 µL of Reagent V was taken into EP tube or 96-well plate. The mixture was well stirred, react 15 min at room temperature and the absorbance was measured at 412 nm. every minute for 5 minutes.

2.3. Statistical Analysis

The results were statistically analysed using Statistica 7.1 software. A variance analysis (ANOVA) followed by a Dunnet post hoc test was used to compare the means at the 5% significance level (p=0.05). DPPH antioxidant activities graphic representations were made with Graph Pad Prism 5.0 (Microsoft U. S. A).

3. Results

3.1. Evolution of Vitamin C and Phenolic Compounds During Fermentation

3.1.1. Evolution of Vitamin C Rates During Fermentation

Table 1. *Evolution of vitamin C in unfermented and fermented beverages.*

Beverages	**Vitamin C (mg/L)**			
Times	**T100%**	**TC10%**	**TG10%**	**TCG10%**
T0H	27.61 ±0.0^{b}	19.82 ±0.0^{a}	21.24 ±0.0^{a}	27.14 ±0.0^{b}
T12H	21.47 ±0.0^{b}	21.47 ±0.0^{a}	20.53 ±0.0^{a}	27.61 ±0.0^{b}
T24H	27.61 ±0.0^{b}	25.96 ±0.0^{b}	21.24 ±0.0^{a}	28.32 ±0.0^{b}
T36H	21.95 ±0.0^{a}	24.78 ±0.0^{b}	23.36 ±0.0^{a}	42.96 ±0.0^{b}

T100%: beverage prepared using a blend of tamarind (100%) TC10%: beverage prepared using a blend of tamarind (90%) and turmeric (10%); TG10%: beverage prepared using a blend of tamarind (90%) and ginger (10%); TCG10%: beverage prepared using a blend of tamarind (90%) and turmeric (5%) and ginger (5%). It is noteworthy that values in a given column that are followed by the same letter do not differ significantly at a 0.05 Dunnet p level.

Table 1 shows the changes in vitamin C concentration during fermentation of the beverages from T0h to T36h. A drop was observed in the T100% beverage, from 27.61 mg/L at T0h to 21.47 mg/L at T36h. Conversely, vitamin C levels increased in the TC10% (from 19.82 to 25.96 mg/L), TG10% (from 20.53 to 23.36 mg/L) and TCG10% (from 20.53 to 23.36 mg/L) beverages.

3.1.2. Evolution of Total Phenolic Compounds During Fermentation

As shown in Figure 1, total phenolic compounds content generally increased from T0h to T36h during beverage fermentation. In fact, the level of total phenolic compounds increased from 1.331 to 1.528 mg EAG/L (in T100% beverage) and from 1.305 to 2.011 mg EAG/L (in TC10% beverage). However, there were peaks in values at T12H for TG10% beverage (1.326 mg EAG/L) and at T24H for TCG10% beverage (1.499 mg EAG/L).

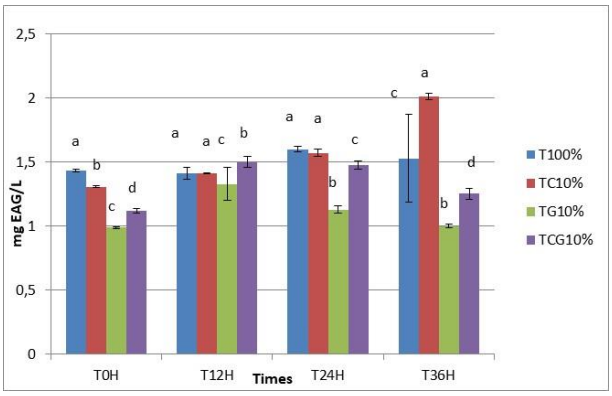

Figure 1. *Total phenolic compounds evolution in unfermented and fermented beverages.*

T100%: beverage prepared using a blend of tamarind (100%); TC10%: beverage prepared using a blend of tamarind (90%) and turmeric (10%); TG10%: beverage prepared using a blend of tamarind (90%) and ginger (10%); TCG10%: beverage prepared using a blend of tamarind (90%) and turmeric (5%) and ginger (5%).

3.1.3. Evolution of Flavonoid Content During Fermentation

Table 2 illustrates evolution of flavonoid content during the beverage fermentation process. From T0H to T36H, a peak was observed at T24H followed by a decrease from T24H to T36H in T100%, TG10% and TCG10% beverages, ranging from 0.131 to 0.068 mg EQ/L for T100% beverage, then from 0.148 to 0.073 mg EQ/L for TG10% beverage and then from 0.134 to 0.076 mg EQ/L for TCG10% beverage. However, for TC10% beverage, a peak is observed at T12H and was followed by a decrease from T12H to T36H ranging from 0.194 to 0.073 mg EQ/L.

Table 2. *Flavonoid in unfermented and fermented beverages.*

Beverages	**Flavonoides (mg EQ/L)**			
Times	**T100%**	**TC10%**	**TG10%**	**TCG10%**
T0H	0.119 ± 0.01^{a}	0.140 ± 0.02^{a}	0.127 ± 0.01^{a}	0.132 ± 0.02^{a}
T12H	0.119 ± 0.01^{a}	0.194 ± 0.1^{b}	0.108 ± 0.01^{a}	0.131 ± 0.02^{a}
T24H	0.131 ± 0.01^{a}	0.148 ± 0.01^{a}	0.134 ± 0.02^{a}	0.164 ± 0.01^{a}
T36H	0.068 ± 0.01^{a}	0.073 ± 0.02^{a}	0.076 ± 0.01^{a}	0.079 ± 0.01^{a}

T100%: beverage prepared using a blend of tamarind (100%), TC10%: beverage prepared using a blend of tamarind (90%) and turmeric (10%); TG10%: beverage prepared using a blend of tamarind (90%) and ginger (10%); TCG10%: beverage prepared using a blend of tamarind (90%) and turmeric (5%) and ginger (5%). It is noteworthy that values in a given column that are followed by the same letter do not differ significantly at a 0.05 Dunnet p level.

3.2. Evolution of Antiradical Activity and Antioxidant Power of Beverages

Table 3. *Inhibitory concentration (IC_{50}) of non-fermented and fermented beverages.*

Time	**Vitamin C**	**IC50 (µg/ml) Beverages**			
		T100%	**TC10%**	**TG10%**	**TCG10%**
T_{0H}	1.61	3.11 ± 0.1^{a}	1.82 ± 0.1^{a}	4.86 ± 0.2^{c}	2.65 ± 0.1^{b}
T_{12H}	1.61	4.51 ± 0.1^{c}	2.32 ± 0.2^{c}	4.86 ± 0.2^{c}	6.08 ± 0.2^{e}
T_{24H}	1.61	6.35 ± 0.2^{j}	2.46 ± 0.2^{j}	4.07 ± 0.2^{b}	3.18 ± 0.2^{j}
T_{36H}	1.61	6.3 ± 0.2^{j}	2.14 ± 0.1^{b}	3.5 ± 0.2^{a}	2.23 ± 0.1^{a}

T100%: beverage prepared from 100% tamarind; TC10%: beverage prepared from 90% tamarind and 10% turmeric; TG10%: beverage prepared from 90% tamarind and 10% ginger; TCG10%: beverage prepared from 90% tamarind, 5% turmeric, and 5% ginger. Note: In any given column, values followed by the same letter do not differ significantly at the p = 0.05 level, according to Dunnett's test.

DPPH free radical scavenging activity of vitamin C and the beverages increased with concentration (Figure 2). The 50% inhibitory concentration (IC_{50}) of vitamin C was approximately 1.61 µg/mL, indicating an antiradical power (ARP) of 62.11 µmol. mL/µg of reduced DPPH. Among the beverages, only the tamarind-turmeric beverage (TC10%) at T0H had an IC_{50} value (1.82 µg/mL) close to that of vitamin C (Table 3), revealing an ARP of 54.95 µmol mL/µg. However, this value decreased after fermentation to 46.73 µmol. mL/µg at T36H. From T0H to T36H, there was an increase in antioxidant activity in TG10% and TCG10% beverages. This increase was characterized by a reduction in IC_{50} from: 4.86 to 3.5 µg/mL for TG10%, 2.65 to 2.23 µg/mL for TCG10%, And a corresponding increase in ARP from: 20.58 to 28.57 µmol. mL/µg (TG10%), 37.74 to 44.84 µmol. mL/µg (TCG10%) (Tables 3 and 4). In contrast, the ARP of

T100% decreased from 32.15 to 15.87 µmol. mL/µg after 36 hours of fermentation.

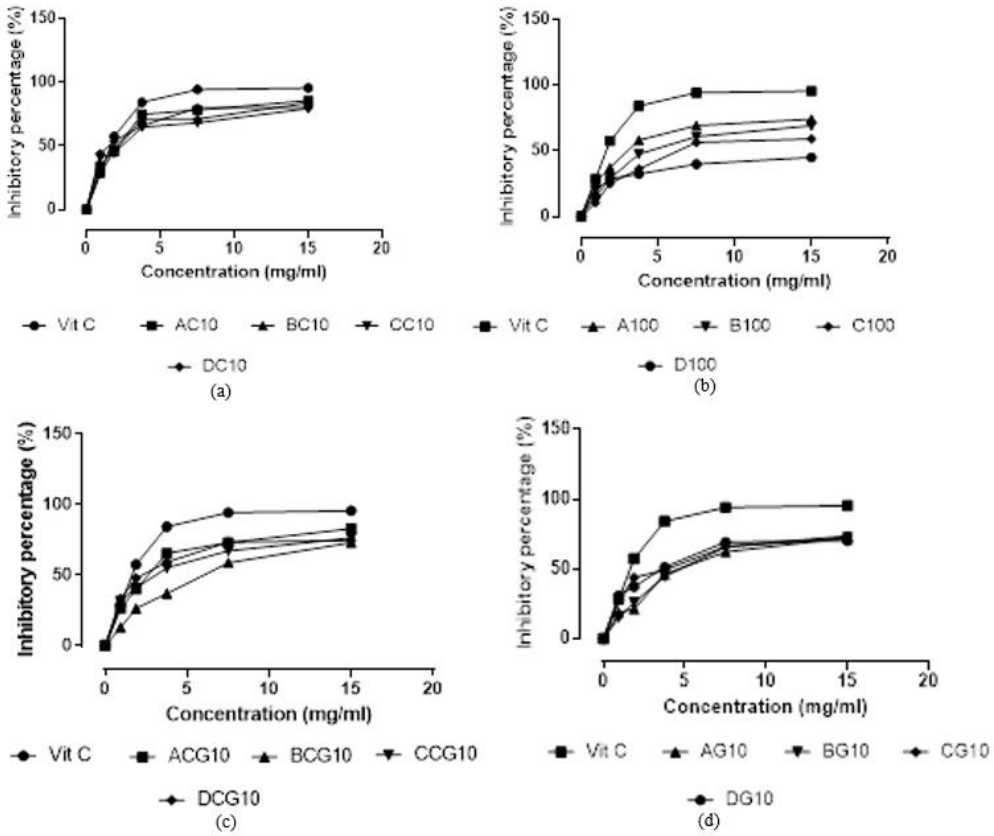

Figure 2. *Evolution of the Percentage of Inhibition of Beverages and Vitamin C as a Function of Concentration:(a) tamarind-based beverage, (b) tamarind-turmeric beverage, (c) tamarind-ginger beverage, (d) tamarind-turmeric-ginger beverage.*

A100: Tamarind at T0; B100: Tamarind at T12; C100: Tamarind at T24; D100: Tamarind at T36; AC10: Tamarind-turmeric at T0; BC10: Tamarind-turmeric at T12; CC10: Tamarind-turmeric at T24; DC10: Tamarind-turmeric at T36; AG10: Tamarind-ginger at T0; BG10: Tamarind-ginger at T12; CG10: Tamarind-ginger at T24 DG10: Tamarind-ginger at T36; ACG10: Tamarind-turmeric-ginger at T0; BCG10: Tamarind-turmeric-ginger at T12; CCG10: Tamarind-turmeric-ginger at T24; DCG10: Tamarind-turmeric-ginger at T36.

Table 4. *Evolution of anti-radical power in non-fermented and fermented beverages.*

		ARP (µmol. ml/µg)			
Time	**Vitamine C**	**Beverages**			
		T100%	**TC10%**	**TG10%**	**TCG10%**
T0H	62.11 ±0.2^{a}	32.15 ±0.2^{d}	54.95 ±0.2^{e}	20.58 ±0.1^{a}	37.74 ±0.2^{d}
T12H	62.11 ±0.2^{a}	22.17 ±0.1^{b}	43.10 ±0.1^{c}	20.58 ±0.1^{a}	16.45 ±0.1^{a}
T24H	62.11 ±0.2^{a}	15.75 ±0.1^{a}	40.65 ±0.1^{b}	24.57 ±0.2^{c}	31.45 ±0.1^{b}
T36H	62.11 ±0.1^{a}	15.87 ±0.1^{a}	46.73 ±0.2^{d}	28.57 ±0.2^{d}	44.84 ±0.2^{e}

T100%: beverage prepared from 100% tamarind; TC10%: beverage prepared from 90% tamarind and 10% turmeric; TG10%: beverage prepared from 90% tamarind and 10% ginger; TCG10%: beverage prepared from 90% tamarind, 5% turmeric, and 5% ginger. Note: In any given column, values followed by the same letter do not differ significantly at the p = 0.05 level, according to Dunnett's test.

3.3. Evolution of Cholesterols and Triglycerides in Rabbits

3.3.1. Evolution of Total Cholesterol Levels in Rabits

Total cholesterol levels in rabbits were low (ranging from 0.82 to 0.83 mg/dL) in those receiving only water and IVOGRAINS pellets (negative control). However, VITALAC fattening pellets led to an increase in total cholesterol levels within 14 days. The highest recorded value was 6.16 mg/dL in rabbits administered with non-fermented tamarind-turmeric-ginger beverage. In rabbits that received only water and VITALAC, total cholesterol increased from 1.3 mg/dL (Day 0) to 5.9 mg/dL (Day 28). Following the induction of hypercholesterolemia, total cholesterol levels decreased from Day 14 to Day 28 in all other groups receiving either non-fermented or fermented beverages. The reduction ranged from 1.19 mg/dL (in rabbits receiving the non-fermented tamarind beverage) to 0.38 mg/dL (in rabbits receiving the fermented tamarind-turmeric-ginger beverage) (Figure 3).

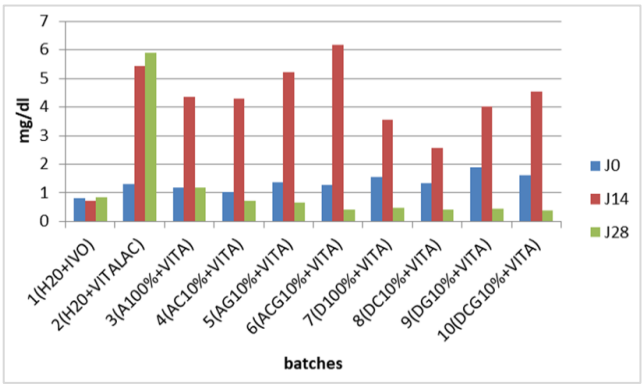

Figure 3. *Evolution of total cholesterol levels in rabbit serum.*

H_2O: water; IVO: Ivograins pellets; VITALAC (VITA): Vitalac pellets; A100: Tamarind at T0; AC10: Tamarind-curcuma at T0; AG10: Tamarind-ginger at T0; ACG10: Tamarind-curcuma-ginger at T0; D100: Tamarind at T36; DC10: Tamarind-curcuma at T36; DG10: Tamarind-ginger at T36; DCG10: Tamarind-curcuma-ginger at T36; J0: First day before fattening; J14: End of fattening period; J28: End of beverage consumption period.

3.3.2. Evolution of HDL Cholesterol Levels in Rabits

HDL cholesterol levels in rabbits did not significantly change (ranging from 0.38 to 0.40 mg/dl) in those receiving only water and Ivograins pellets (negative control) during the experiment. Consumption of VITALAC pellets decreased the HDL cholesterol levels in rabbits over 14 days from 0.26 mg/dl to 0.09 mg/dl, respectively, in the groups receiving fermented tamarind-curcuma-ginger beverage and non-fermented tamarind beverage. After induction of hypercholesterolemia, HDL cholesterol levels increased from day 14 to day 28 in groups receiving both fermented and non-fermented beverages. The highest values were observed in rabbits receiving non-fermented tamarind-curcuma beverage (1.74 mg/dl) and non-fermented tamarind-ginger beverage (1.89 mg/dl) (Figure 4).

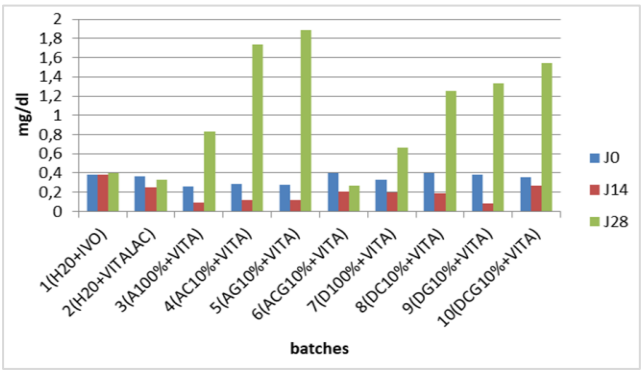

Figure 4. *Evolution of HDL Cholesterol levels in rabbit serrum.*

H_2O: water; IVO: Ivograins pellets; VITALAC (VITA): Vitalac pellets; A100: Tamarind at T0; AC10: Tamarind-curcuma at T0; AG10: Tamarind-ginger at T0; ACG10: Tamarind-curcuma-ginger at T0; D100: Tamarind at T36; DC10: Tamarind-curcuma at T36; DG10: Tamarind-ginger at T36; DCG10: Tamarind-curcuma-ginger at T36; J0: First day before fattening; J14: End of fattening period; J28: End of beverage consumption period.

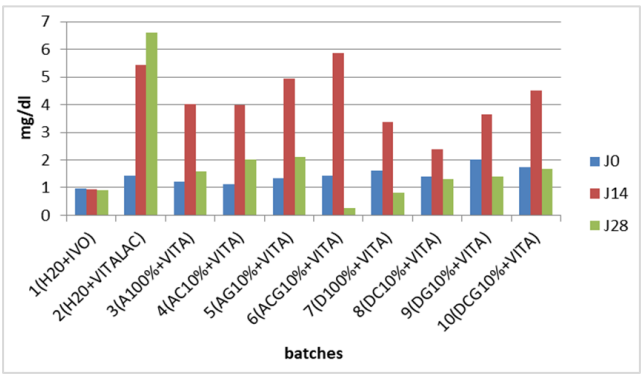

Figure 5. *Evolution of LDL Cholesterol levels in rabbit serum.*

H_2O: water; IVO: Ivograins pellets; VITALAC (VITA): Vitalac pellets; A100: Tamarind at T0; AC10: Tamarind-curcuma at T0; AG10: Tamarind-ginger at T0; ACG10: Tamarind-curcuma-ginger at T0; D100: Tamarind at T36; DC10: Tamarind-curcuma at T36; DG10: Tamarind-ginger at T36; DCG10: Tamarind-curcuma-ginger at T36; J0: First day before fattening; J14: End of fattening period; J28: End of beverage consumption period.

3.3.3. Evolution of LDL Cholesterol Levels in Rabbits

A slight decrease in LDL cholesterol was observed in rabbits receiving only water and Ivograins pellets (from 0.97 to 0.90 mg/dl). VITALAC pellets increased the LDL cholesterol

levels in rabbits over 14 days. The highest value (5.97 mg/dl) was recorded in rabbits that received a non-fermented tamarind-curcuma-ginger beverage. In rabbits receiving only water and VITALAC, total cholesterol rose from 1.42 mg/dl (J0) to 6.61 mg/dl (J28). After induction of hypercholesterolemia, LDL cholesterol levels decreased from day 14 to day 28 in the groups receiving either fermented or non-fermented beverages. The decrease ranged from: 2.12 mg/dl in rabbits receiving non-fermented tamarind-ginger beverage, to 0.26 mg/dl in rabbits receiving non-fermented tamarind-curcuma-ginger beverage (Figure 5).

3.3.4. Evolution of Triglycerides Levels in Rabbits

Triglyceride content increased in both the negative and positive control groups, rising respectively from 1.17 to 1.63 mg/dl and from 1.20 to 2.10 mg/dl. During the fattening phase, triglyceride levels increased up to 2.49 mg/dl in rabbits receiving non-fermented tamarind-curcuma-ginger beverages. No change in triglyceride levels was observed between day 14 and day 28 in rabbits receiving non-fermented tamarind or tamarind-curcuma beverages. However, all fermented beverages led to a decrease in triglyceride levels (Figure 6).

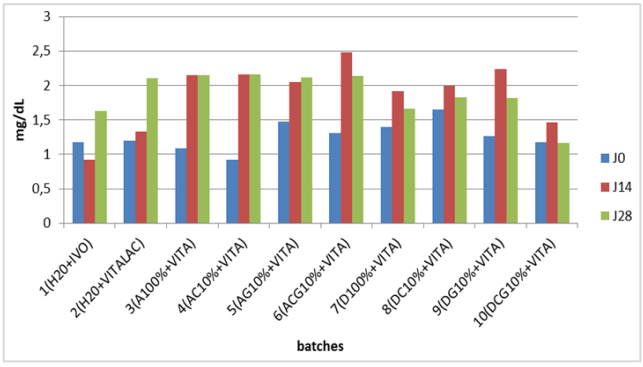

Figure 6. *Evolution of triglyceride levels in rabbit serum.*

H_2O: water; IVO: Ivograins pellets; VITALAC (VITA): Vitalac pellets; A100: Tamarind at T0; AC10: Tamarind-curcuma at T0; AG10: Tamarind-ginger at T0; ACG10: Tamarind-curcuma-ginger at T0; D100: Tamarind at T36; DC10: Tamarind-curcuma at T36; DG10: Tamarind-ginger at T36; DCG10: Tamarind-curcuma-ginger at T36; J0: First day before fattening; J14: End of fattening period; J28: End of beverage consumption period.

3.4. Evolution of *in Vivo* Antioxidant Potential

3.4.1. Evolution of Super Oxyde Dismutase (SOD) Levels in Rabbits

Superoxide dismutase (SOD) levels decreased in all rabbit groups during the induction of hyperlipidemia. However, during beverage administration, SOD levels increased in rabbits. In fact, fermented beverages raised SOD levels from 7.41 U/ml (with fermented tamarind) to 10.29 U/ml (with fermented tamarind-curcuma-ginger), which is higher than the level induced by vitamin C (6.84 U/ml) (Figure 7).

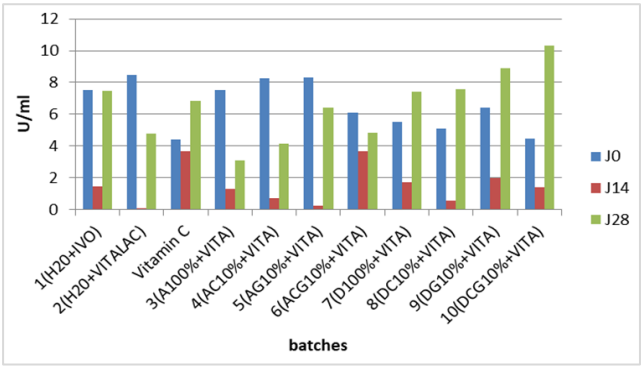

Figure 7. *Evolution of SOD in rabbit serum.*

H_2O: water; IVO: Ivograins pellets; VITALAC (VITA): Vitalac pellets; A100: Tamarind at T0; AC10: Tamarind-curcuma at T0; AG10: Tamarind-ginger at T0; ACG10: Tamarind-curcuma-ginger at T0; D100: Tamarind at T36; DC10: Tamarind-curcuma at T36; DG10: Tamarind-ginger at T36; DCG10: Tamarind-curcuma-ginger at T36; J0: First day before fattening; J14: End of fattening period; J28: End of beverage consumption period.

3.4.2. Evolution of Glutathione Peroxidase (GPx) Levels in Rabbits

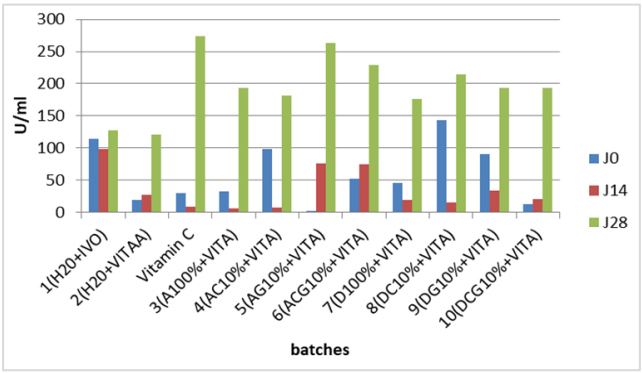

Figure 8. *Evolution GP_X in rabbit serum.*

H_2O: water; IVO: Ivograins pellets; VITALAC (VITA): Vitalac pellets; A100: Tamarind at T0; AC10: Tamarind-curcuma at T0; AG10: Tamarind-ginger at T0; ACG10: Tamarind-curcuma-ginger at T0; D100: Tamarind at T36; DC10: Tamarind-curcuma at T36; DG10: Tamarind-ginger at T36; DCG10: Tamarind-curcuma-ginger at T36; J0: First day before fattening; J14: End of fattening period; J28: End of beverage consumption period.

Glutathione peroxidase levels decreased during the fattening phase in all rabbit groups. However, an increase in GPx levels was observed after the consumption of the beverages. GPx levels rose to 274.25 U/ml in rabbits given vitamin C (used as a control). They also increased to 263.07 U/ml in rabbits receiving non-fermented tamarind-ginger beverage,

and to 229.26 U/ml in those receiving non-fermented tamarind-curcuma-ginger beverage. As for the fermented beverages, GPx levels were approximately 214.64 U/ml for tamarind-curcuma, and 173.17 U/ml for both tamarind-ginger and tamarind-curcuma-ginger beverages (Figure 8).

4. Discussion

Vitamin C content in pure tamarind beverages decreased during fermentation. However, in mixed beverages, vitamin C content increased during fermentation process. This may be attributed to the addition of spices and the lowering of pH (acidification) due to fermentation. In fact, an alkaline pH and high temperatures have been associated with vitamin C losses. A study on sterilized fermented carrot juice [21] showed that up to 50% of vitamin C loss was mainly due to sterilization, not fermentation. Furthermore, lactic fermentation of juices such as pomegranate, carrot, bean, and zucchini has been shown to enhance vitamin C content, phenolic compounds, and antioxidant activity [22, 23]. Phenolic compounds increased during beverages fermentation. According to [24], this rise may be attributed to microbial activity that facilitated the release of phenolic compounds during fermentation. Other authors [25, 26] also reported a rise in total phenolics in fermented kale juice. However, a decrease in polyphenols was observed in the tamarind-ginger beverage after 12 hours of fermentation. This decline may be due to interactions between polyphenols and nutrients, including hydrolysis and oxidation reactions, condensation and polymerization, or adsorption of phenolic compounds on yeast cells [27]. The results also showed an increase in flavonoid content from T0H to T12H or T24H in the beverages, followed by a decrease up to T36H. This reduction could be attributed to temperature and bacterial activity. In fact, [27] also observed up to a 50% reduction in flavonoids during the fermentation of pomegranate juice. The antioxidant potential decreased in tamarind juice but increased in Tamarind-ginger beverage (T0H to T36H), Tamarind-curcuma juice (T24H to T36H), and Tamarind-curcuma-ginger juice (T12H to T36H). This increase could be due to curcuma and ginger, whose antioxidant activities are enhanced through fermentation, making their compounds (such as curcuminoids and gingerol) more bioavailable [28, 29]. As a result, tamarind pulp, phenolic compounds from curcuma and ginger, and flavonoids demonstrated strong antioxidant properties, along with hepatoprotective and hypolipidemic effects, giving them promising antioxidant potential and anti-hyperlipidemic activity [30, 31]. The consumption of the beverages led to a decrease in total cholesterol, LDL cholesterol, and triglycerides. This could be explained by the total phenolic content and phytosterols, which are involved in reducing intestinal cholesterol absorption by lowering its solubility in micelles and increasing fecal excretion [32]. Additionally, [33] revealed that curcuma and ginger may contribute to lowering lipid profiles by stimulating bile production and enhancing lipid digestion. Moreover, [34] showed that consuming fermented tea leaves reduced triglycerides by 24.3%, non-esterified fatty acids by 24.6%, and liver cholesterol by 43.6% in hamsters. On the other hand, tamarind-based beverages administered to rabbits after the fattening phase resulted in increased HDL cholesterol levels. This increase may be due to an adaptive mechanism, reflecting a need to enhance reverse cholesterol transport in response to a high-cholesterol diet [11]. This finding is consistent with prior studies in humans and hamsters, where high-cholesterol diets led to an increase in HDL cholesterol [35]. These beverages also caused an increase in antioxidant enzymes SOD and GPx. Regarding SOD activity, the increase may be due to both the protective antioxidant effect and the hypolipidemic effect of tamarind constituents found in the extracts [36]. This is supported by [11], who showed that administration of *T. indica* fruit pulp to hypercholesterolemic hamsters enhanced antioxidant enzyme activities such as CAT, SOD, and GPx. An increase in SOD implies an improved capacity to detoxify superoxide anions (O_2^-), resulting in higher H_2O_2 production. This rise is also linked to the incorporation of curcuma and ginger and the impact of fermentation [37]. In fact, ginger and curcuma are rich sources of antioxidants, particularly gingerol, zingiberene (the major active compound of ginger), and curcumin (the main active compound in curcuma) [38]. These compounds help to scavenge and neutralize free radicals. GPx activity decreased during fattening but increased after beverage consumption.

The decline may be due to an increase in free radicals, impairing GPx's ability to inhibit oxidative stress and detoxify toxic metabolites at the cellular level [39, 40]. However, the increase in GPx activity following beverage consumption could be attributed to their antioxidant capacity, which may activate a cytosolic transcription factor responsible for up-regulating γ-glutamyl cysteine synthase (γ-GCS), the enzyme involved in glutathione synthesis [41]. Thus, superoxide dismutase (SOD) and glutathione peroxidase (GPx) are key antioxidant enzymes in the body's defense system, helping to combat oxidative damage.

5. Conclusion

This study demonstrates that tamarind-based beverages, enriched with ginger and curcuma, and fermented, exhibit significant free radical inhibition, thereby conferring considerable antioxidant potential.

This potential enables the beverages to increase the activity of antioxidant enzymes SOD and GPx in rabbit serum. Such enhancement positively impacts hyperlipidemia and may be useful in the prevention of other metabolic diseases such as diabetes.

Abbreviations

IC_{50}	Half Maximal Inhibitory Concentration
ROS	Reactive Oxygen Species
DNA	Deoxyribonucleic Acid
LDL	Low-Density Lipoprotein
HDL	High-Density Lipoprotein
SOD	Superoxide Dismutase
GP_X	Glutathione Peroxidase

Acknowledgments

The authours express their sincere thanks to the Nutrition and Food Safety Laboratory and the Animal Production Laboratory of Nangui Abrogoua University.

Author Contributions

Anoh Ettien Raïssa Inès Stéphanie: Data curation, Funding acquisition, Investigation, Methodology, Software, Writing - original draft

Agbo Adouko Edith: Conceptualization, Data curation, Methodology, Supervision, Validation, Visualization

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Brou Kouakou: Supervision, Validation, Visualization

Funding

This work was carried out using the authors' own resources, without any specific funding.

Conflicts of Interest

The authors declare no conflicts of interest.

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