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Research Article

Stability Study of a Formulation Based on Pineapple Juice and *Hibiscus sabdariffa* Concentrated Extract for the Preparation of Food Supplements

Kakwokpo Clémence N'GUESSAN-GNAMAN *, Nakognon Awa TUO-KOUASSI, Sandrine AKA- ANY-GRAH, Apo Laurette ANIN, Ismael DALLY

Laboratoire des Sciences du Médicament, Sciences Analytiques et Santé Publique, Unité de formation et de recherche des sciences pharmaceutiques et biologiques, Université Felix Houphouët-Boigny, Abidjan, Côte d'Ivoire

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*Address for Correspondence:

Dr N'GUESSAN-GNAMAN Kakwokpo Clémence, Laboratoire des Sciences du Médicament, Sciences Analytiques et Santé Publique, Unité de formation et de recherche des sciences pharmaceutiques et biologiques, Université Felix Houphouët-Boigny, Abidjan, Côte d'Ivoire

Abstract

The aim of this study was to evaluate the influence of additives on the stability of a beverage based on pineapple juice and *Hibiscus sabdariffa* concentrated extract at room temperature. This beverage was intended to be used to formulate food supplements. Acidifier (citric acid), antioxidant (ascorbic acid) and antimicrobial (sodium benzoate and potassium sorbate) were added alone or in combination. Organoleptic, physicochemical (pH, titratable acidity and Brix degree) and microbiological characteristics were observed over a 21-day period. Beverages containing potassium sorbate (1 g/L) and sodium benzoate (0.5 g/L) were more stable over 21 days.

Keywords: dietary supplements, storage, bissap, extract

1. INTRODUCTION

A project to develop food supplement formulations in the form of fruit juice-based drinks has been initiated. Because fruit juices provide hydration, quench thirst and provide tasting pleasure. These drinks, combined with other more specific nutrient concentrates, are intended for patients over 3 years of age and elderly people, with specific nutritional needs in case of undernutrition or risk of undernutrition. The choice of pineapple, a local fruit, and bissap, a local plant, was chosen to give these formulations a delicious taste. *Hibiscus sabdariffa* is an herbaceous plant grown mainly in tropical and subtropical regions [1]. Its calyxes, known as "bissap" in Côte d'Ivoire, are rich in vitamins C, B2 and B3, glucose, fructose, sucrose and organic acids [2]. Their mineral composition essentially refers to the presence of iron, phosphorus, calcium, sodium and potassium [3], as well as trace elements such as copper and chromium [2]. They are also rich in anthocyanins and antioxidants [4]. Pineapples (*Ananas Comosus*) are rich in vitamins and minerals, and are much appreciated around the world for their sweet, juicy taste. Drinks based on pineapple and *H. sabdariffa* concentrated extract are therefore a vehicle for administering specific nutrients as a dietary supplement. A dietary supplement is a foodstuff made up of one or more nutrients (vitamins, minerals, trace elements) or substances (plant extracts, fibers, amino acids, etc.), usually in capsule, tablet, powder, syrup, lozenge, pill, stick, drink or ampoule form. Their purpose is to supplement a normal diet with these concentrates of substances with a nutritional or physiological effect. However, given all the possible alterations with pineapple and the possible instabilities of *H. sabdariffa* anthocyanins, it was important to stabilize these pineapple and *H. sabdariffa* concentrated extract drinks at room temperature.

2. MATERIALS AND METHODS

2.1 Materials

Pineapple was from Bonoua, a town in Côte d'Ivoire. The dried red calyxes of *Hibiscus sabdariffa* originated in Burkina Faso, but were sold on the market in Côte d'Ivoire. Analytical grade citric acid, ascorbic acid, sodium hydroxide, sodium benzoate and potassium sorbate were all supplied by Sigma aldrich. Culture media for the microbiological test were supplied by the national public health laboratory of Côte d'Ivoire.

2.2. **Methods**

2.2.1. Beverage formulation

The pineapple was pressed to obtain the juice and pasteurized. The dried red calyxes of *Hibiscus sabdariffa* were ground, decocted, filtered and concentrated. The raw beverage contained 90% pineapple juice and 10% concentrated bissap extract. Chemical additives such as citric acid (acidifier) at concentrations of 1.5g/l and 3g/l, ascorbic acid (antioxidant) at a concentration of 300mg/l, sodium benzoate (antimicrobial) at concentrations of 0.5 g/l and 1 g/L and potassium sorbate (antimicrobial) at concentrations of 1 g/L and 2 g/L were added to the raw beverage. The formulas obtained are shown in Table I.

Table I: Beverage formulas

Codes	Composition of each formula
M	Raw beverage
MC1	Beverage + citric acid (1.5 g/L)
MC2	Beverage + citric acid (3g/L)
MB1	Beverage + sodium benzoate (0.5g/L)
MB2	Beverage + sodium benzoate (1 g/L)
MA	Beverage + ascorbic acid (300 mg/l)
MS1	Beverage + potassium sorbate (1 g/L)
MS2	Beverage + potassium sorbate (2 g/L)
MAC1	Beverage + ascorbic acid (300 mg/l) + citric acid (1.5 g/L)
MAC2	Beverage +ascorbic acid (300 mg/l) +citric acid (3g/L)
MAS1	Beverage + ascorbic acid (300 mg/l) + potassium sorbate (1 g/L)
MAS2	Beverage + ascorbic acid (300 mg/l) + potassium sorbate (2 g/L)
MC1B1	Beverage +citric acid (1.5 g/L) +sodium benzoate (0.5g/L)
MC1B2	Beverage + citric acid (1.5 g/L) + citric acid (3g/L)
MC2B1	Beverage + citric acid (3g/L) + sodium benzoate (0.5g/L)
MC2B2	Beverage + citric acid (3g/L) + sodium benzoate (1 g/L)

2.2.2. Organoleptic characteristics

Odor, color and appearance were observed over 21 days.

2.2.3. Assessment of beverage physico-chemical stability

pH was monitored using a HANNA pH meter with dual display (temperature and pH).

The Brix level was monitored using a HANNA refractometer (0 to 85%) with display (refractive index and Brix level), Titratable acidity was monitored using a burette.

Controls were performed at days D0, D7, D14 and D21.

2.2.4. Evaluation of beverage microbiological stability

Controls were performed at D0, D7, D14 and D21. Germs tested included aerobic mesophilic germs, total coliforms, thermotolerant coliforms, yeasts and molds, sulfito-reducing anaerobic germs, *Staphylococcus aureus* and salmonella. Only formulations with good physico-chemical stability were included in this evaluation.

3. RESULTS AND DISCUSSION

Chemical additives were selected for beverage stabilization on the basis of their role, their authorization for use under the *Codex Alimentarius* and their frequency of use in agri-food products.

3.1 Organoleptic stability

Formulas were prepared and organoleptic characteristics observed. They were all red in color, as if dominated by the color of bissap, with the odor of pineapple and bissap and a fluid appearance. The following day, some preparations showed changes in color, odor or appearance (Table II). N for normal and AN for abnormal.

Table II: Organoleptic characteristics of beverages over 7 days

	Color					Odor					Aspect					
	D0	D1	D2	D3	D7	D0	D1	D2	D3	D7	D0	D1	D2	D3	D7	
M	N	AN	A	AN	AN	N	A	A	AN	AN	N	A	AN	A	AN	Fermentation
MC1	N	AN	A	AN	AN	N	A	A	AN	AN	N	A	AN	A	AN	Fermentation
MC2	N	AN	A	AN	AN	N	A	A	AN	AN	N	A	AN	A	AN	Fermentation
MB1	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	**Stable at D**
MB2	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	**Stable at D**
MA	N	AN	A	AN	AN	N	A	A	AN	AN	N	A	AN	A	AN	Fermentation
AC1	N	AN	A	AN	AN	N	A	A	AN	AN	N	A	AN	A	AN	Fermentation
AC2	N	AN	A	AN	AN	N	A	A	AN	AN	N	A	AN	A	AN	Fermentation
C1B1	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**Stable at D**
C1B2	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**Stable at D**
C2B1	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**Stable at D**
C2B2	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**Stable at D**
MS1	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	**Stable at D**
MS2	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	**Stable at D**
MAS1	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	**Stable at D**
MAS2	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	N	**N**	**N**	**N**	**N**	**Stable at D**

These organoleptic characteristics are important indicators in the evaluation of beverage stability. According to Meledje et al, organoleptic characteristics are very important criteria for beverage analysis, and play an almost immediate role in consumer acceptance or rejection of the product [5].

Beverages containing citric acid and ascorbic acid were altered at D1. These two additives were unable to prevent the proliferation of germs responsible for beverage fermentation. Their use was therefore ineffective in preserving beverages at room temperature.

Beverages containing at least sodium benzoate or potassium sorbate showed organoleptic stability for 7 days. Sodium benzoate and potassium sorbate prevented the proliferation of germs responsible for beverage fermentation. In fact, sodium benzoate and potassium sorbate have an optimal action on bacteria and fungi when the beverage pH is below 4.5[6]. Sodium benzoate and potassium sorbate are therefore effective treatment techniques for preserving beverages at room temperature. The formulas containing these preservatives and other additives were therefore abandoned for the remainder of the study.

3.2. Physico-chemical stability

The physico-chemical stability of beverages containing sodium benzoate at concentrations of 0.5 g/L and 1 g/L or potassium sorbate at concentrations of 1 g/L and 2 g/L was evaluated. pH, titratable acidity and sugar content (Brix level) were monitored. Beverage pH values (2.52-2.68) were satisfactory, as according to CODEX-STAN 243-2003, beverage pH should be below 4.5. The values recorded are close to those reported in other studies, where the pH of the beverages studied ranged from 2.66 to 2.77 [5]. This acidic character is a criterion for beverage authentication, as the red calyxes of *Hibiscus sabdariffa* are known to have a high content of organic acids, mineral acids and amino acids [7]. This acidity is a medical disadvantage for people suffering from gastric diseases (gastric ulcers) [8].

The pH values of beverages containing sodium benzoate (0.5 g/L and 1 g/L) and potassium sorbate (1 g/L and 2 g/L) for 21 days did not vary significantly, and no change in color, appearance or odor was observed, in contrast to the raw beverage (without additives) where an increase in pH with a change in color, odor and appearance was observed. According to several studies carried out on the stability of anthocyanin in *Hibiscus sabdariffa*, changes in color, odor, appearance and pH increase are indicators of instability in bissap beverages [9].

Titratable acidity values for beverages containing sodium benzoate (0.5 g/L and 1 g/L) of 1.792 g/L and 1.856g/L respectively, and potassium sorbate (1 g/L and 2 g/L) of 1.792 g/L and 1.856g/L respectively, remained constant for 21 days. As for the crude beverage, the titratable acidity value of 1.792 g/L on day 0 decreased to 1.6 g/L from day 7e onwards. This decrease could be due to the proliferation of micro-organisms [10]. The Brix value of beverages containing sodium benzoate (0.5 g/L and 1 g/L) and potassium sorbate (1 g/L and 2 g/L) remained constant at 10.4°B for 21 days. As for the raw beverage, the Brix value of 10.4°B on day 0 decreased to 6.9°B from day 7e. This decrease in Brix level could be due to the partial degradation of sugars. This hypothesis is confirmed by the work carried out by Echeverria [11]. Beverages containing sodium benzoate at 0.5 g/L and 1 g/L and potassium sorbate at 1 g/L and 2 g/L have shown physicochemical stability.

Table III: Changes in physico-chemical stability

	pH				BRIX DEGREE (°B)				Titratable acidity (g/L)			
	D0	D7	D14	D21	D0	D7	D14	D21	D0	D7	D14	D21
M	2.53	2.62	2.63	2.63	10.4	6.9	6.9	6.9	1.79	1.60	1.60	1.60
MB1	2.51	2.52	2.53	2.53	10.4	10.4	10.4	10.4	1.79	1.79	1.79	1.79
MB2	2.55	2.55	2.57	2.57	10.4	10.4	10.4	10.4	1.85	1.85	1.85	1.85
MS1	2.60	2.60	2.61	2.61	10.4	10.4	10.4	10.4	1.79	1.79	1.79	1.79
MS2	2.67	2.66	2.66	2.68	10.4	10.4	10.4	10.4	1.85	1.85	1.85	1.85

3.3. Microbiological stability

Germs of interest in food hygiene and those responsible for food spoilage were sought. Indicator microorganisms such as thermotolerant coliforms and salmonella, pathogenic germs likely to cause food poisoning, were also sought.

The results of microbiological analysis at D0 of beverages containing sodium benzoate at 0.5 g/L, potassium sorbate at 1 g/L, and the raw beverage (without additive) show an absence of Staphylococcus aureus and salmonella, and a microbial load of less than 10 CFU/ml for all germs enumerated, with the exception of aerobic mesophilic germs with a microbial load of 50 CFU/ml. All formulations therefore comply with the Ivorian standard. These results are similar to those of another study carried out on bissap, which revealed an absence of all germs with the exception of total mesophilic flora, where a load of 38 to 240 CFU/ml was observed [12]. In a study on the influence of manufacturing processes on the microbiological quality of bissap beverages, it was reported that the absence of certain germs such as yeasts and moulds was due to the type of extraction [13]. Indeed, extraction by decoction results in a lower level of fungal flora than extraction by maceration. In addition, the pineapple juice was pasteurized.

From day 7e onwards, the raw beverage showed a microbial load of less than 10 CFU/ml for all germs counted, except for aerobic germs, where a growth rate of 3.103 CFU/100ml was observed. This germ proliferation indicates the deterioration of this beverage. These results correlate with the physicochemical analyses carried out (decrease in Brix level, increase in pH and decrease in titratable acidity) and organoleptic analyses (appearance=cloudy, color=brown and unpleasant odor). Other studies show mesophilic flora growth in excess of 3.103 CFU/100ml for degraded bissap beverages [14].

Beverages containing 0.5 g/L sodium benzoate and 1 g/L potassium sorbate had a microbial load of less than 10 CFU/ml for all germs counted up to 21 days. It was deduced that these formulations are of satisfactory microbiological quality and therefore comply with Ivorian standards. These results correlate with the physicochemical (Brix level, pH and titratable acidity constant) and organoleptic analyses carried out. These results demonstrate the effectiveness of treatment techniques using sodium benzoate at 0.5 g/L and potassium sorbate at 1 g/L for beverage preservation at room temperature.

Beverages containing 0.5 g/L sodium benzoate and 1 g/L potassium sorbate have shown microbiological stability.

Table IV: Microbiological stability trends

PARAMETRES	M				MB1				MS1				
	D0	**D7**	**D14**	**D21**	**D0**	**D7**	**D14**	**D21**	**D0**	**D7**	**D14**	**D21**	**NORME**
mesophilic aerobic germs	**50**	**3.10^3**	**3.10^3**	**3.10^3**	20	< 10	< 10	< 10	50	< 10	< 10	< 10	< 10^2 CFU / 100 ml
total coliform germs	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10 CFU / ml
thermotolerant coliforms	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10 CFU / ml
yeasts and moulds	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10 CFU / ml
Anaerobic sulfite-reducing germs (ASR)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10 CFU / ml
Staphylococcus aureus	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABSENCE (ABS)
Salmonella testing	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABS	ABSENCE (ABS)

CONCLUSION

Formulations based on the mixture of pineapple juice and concentrated extract of *H. sabdariffa* for use as a base for dietary supplement drinks were produced with the addition of additives to improve stability. Organoleptic, physicochemical and microbiological stability were assessed. The use of citric acid and ascorbic acid at different concentrations did not improve the shelf life of the beverages at room temperature. On the other hand, the use of sodium benzoate and potassium sorbate resulted in beverages that could be stored at room temperature for 21 days.

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