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**Research/Technical Note**

# Washing-Disinfectant Product Synthesis Tested During the Production of the "Speb-Pebe" - Spicy Product Energy Booster Characterized by Established Titration Procedures

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**Abstract:** A washing-disinfectant product was synthesized with citric acid which was a readily available raw material on the chemical markets. Following-up the established procedure to use this product reflected its efficiency to eliminate, to remove, to kill and to decrease significantly the binary fission of various germs and micro-organisms not only on raw materials but also on materials and containers that could cause consumers illness. Indeed, the quantifications of these micro-organisms on a product "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)" at the "Institut Pasteur de Madagascar" whose analyzes were accredited by COFRAC © reflected that firstly there were any *Salmonella spp.* and any *Listeria monocytogenes*; secondly its quantities of *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus* respected the European standardization for food products; thirdly this "speb-pebe" product decreased significantly the binary fission speed of these previous micro-organisms in order 4.7 to 80 times less speed than its speeds deduced by a reference and/or by its generation time; and fourth the product "speb-pebe" best-before dates during a conservation at 303.15 (°K), natural ambient temperature, were calculated from each micro-organism's binary fission speed. Then, two procedures titrations consisting in titration of the "speb-pebe" - spicy product energy booster's equivalent-citric acid molecules in each level of its packaging using NaOH-0.0557N and in titration of the "speb-pebe" - spicy product energy booster's alkenes-$C^{=}$ organic functions (on surface and on structure-texture) in each level of its packaging using HF-0.0026N were established and allowed to appreciate the "speb-pebe" quality in each level of its packaging and also to control its packaging-quality. It was noticed that the concentration of alkenes-$C^{=}$ organic functions on structure and on texture per grams on the Level-high-packaging were 5.9 times less than those on Level-medium-packaging 1.23E-1 [$mol \times l^{-1} \times g^{-1}$] and on Level-low-packaging 1.16E-1 [$mol \times l^{-1} \times g^{-1}$] confirming in return the highest value of the concentration of alkenes-$C^{=}$ organic functions on surface per grams of "speb-pebe" observed at Level-high-packaging sample (4.805E-3 [$mol \times l^{-1} \times g^{-1}$]) compared with Level-middle-packaging sample (1.72E-3 [$mol \times l^{-1} \times g^{-1}$]) and with Level-low-packaging sample (2.19E-3 [$mol \times l^{-1} \times g^{-1}$]). Another results, the dispersion of the equivalent-citric acids molecules on total alkenes-$C^{=}$ organic functions noted dispersion ($Ac/C^{=}$) or dispersion $_{([Ac]/[C=])}$ for the Level-high-packaging sample, the Level-middle-packaging sample and the Level-low-packaging sample were respectively 0.2813, 0.0250 and 0.0714 and confirmed the useful role of citric acids molecules quantities and their protons $H^{+}$ capacities and activities to catalyze some chemistry reactions and responsible of this "speb-pebe" - spicy product energy booster's acidity-pH=2.62, flavors and tastes.

**Keywords:** Micro-organisms, Disinfectant, Binary Fission Speed, Citric Acid, "Speb-Pebe", NaOH, HF, Alkenes-$C^{=}$

# 1. Introduction

In the first time, synthesis of a washing-disinfectant product based on concentrated citric acid solution was done in order to eliminate, to remove, to kill and to decrease the binary fission of various germs with micro-organisms that could cause consumers illness not only on materials, containers but also on raw materials necessary to synthesis alimentary products including the "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)". To evaluate the effectiveness of this cleaning-disinfection-sterilization using the previous disinfectant,, some pathogenic micro-organisms such as "presumptive Enterobacteriaceae", "presumptive *Bacillus cereus*", "positive *Staphylococci coagulase*", "*Salmonella spp."* and "*Listeria monocytogenes* (research)" in the spicy product energy booster (speb-pebe) synthesized by using these utensils and raw materials disinfected with this disinfectant were quantified at the "Institut Pasteur de Madagascar" whose analyzes were accredited by COFRAC ©.

Then, to control the "speb-pebe" - spicy product energy booster quality in each level of its packaging and also to control the packaging-quality which depended principally on the dispersion of the equivalent-citric acids molecules on total alkenes-C= organic functions, two titration procedures were established such as the first titration procedure consisted on the titration of the "speb-pebe" - spicy product energy booster's equivalent-citric acid molecules in each level of its packaging using NaOH-0.0557N and the second titration procedure consisted on the titration of the "speb-pebe" - spicy product energy booster's alkenes-C= organic functions in each level of its packaging using HF-0.0026N.

Laboratory materials and chemicals used during these experimentations were beaker-250ml, magnetic stirrer-Fischer scientific, stirring rod, precision balance KERN, test tube-100ml, test tube-50ml, burette-50ml, separating funnel, helianthine color indicator, bromophenol blue color indicator, dichloromethane, citric acid.

# 2. Synthesis of a Disinfectant Product Based on Citric Acid

Materials, containers and raw materials necessary for the synthesis of the "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)" could contain various pathogenic and alteration germs with micro-organism that could cause consumers illness. Bibliographies presented several methods and procedures to eliminate, to remove, to kill or to deactivate them including heat, chemicals, irradiation, high pressure and filtration [1-8]. Seeing that the minimum growth pH and growth temperature of all pathogenic and alteration germs with micro-organism are

respectively, inferior to 2 for only molds but superior to 3 for all over germs with micro-organisms, and 268.15 (°K) to 305.15 (°K) [9]; a disinfectant with pH equal to 2.14 synthesized with tri-carboxylic acids-citric acid molecules was used to eliminate and deactivated them from materials, containers and raw materials necessary for the synthesis of the "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)". The effectiveness of these different cleaning and disinfection using this disinfectant was evaluated and controlled by analyzing and quantifying the rate of some typical dangerous pathogenic and alteration germs in the synthesized "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)" at IPM-Institut Pasteur of Madagascar. Thereafter, the results of these analyses made it possible to evaluate the speed of scission-multiplication of some typical dangerous pathogenic and alteration germs and to deduce the maximum and minimum best-before dates of the pebe-speb according to the maximum thresholds fixed by the competent authorities among others the European level Regulation (CE) n°2073/2005 [10] as a reference for this study.

### *2.1. Disinfectant Synthesis Procedure*

This disinfectant used high temperature and low pH with eventually reactions between citric acids' tri-carboxylic acids or alcohol functions and micro-organisms accompanied with hydrogen bonds links [11-13] to deactivate and to eliminate pathogenic and altered germs. In the other words, it was used at temperature in the vicinity of water ebullition temperature. Thus, it was practice to synthesize and/or to boil this disinfectant just before utilization such as boil 1 (l) of water; weigh 41 [g] of citric acid and dissolve it in the previous boiled water. Shake the solution until citric acid molecules were totally dissolved. At the end, a clear homogeneous concentrated citric acid hot solution 2.1341E-1 ($mol.l^{-1}$) with calculated pH=2.14 was obtained which must be used immediately not only to disinfect all necessary raw materials but also all materials, containers and utensils to synthesize the "speb-pebe".

### *2.2. Disinfectant Utilization Procedure*

The "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)" was a 100% biological product composed principally with spices, lemons which synthesis required materials, containers and utensils. First of all, spices and lemons were sorted by removing blackened spices, inedible spices as well as all spices stalks. Then, wash fresh spices with cold water and put them in a colander. Now, rinse two to three times with the disinfectant the washed fresh spices and at the end immerse them in the hot disinfectant solution during 30 seconds to 60 seconds; allow to air dry before use. Concerning the disinfection of materials, containers and utensils, they were immersed in the hot disinfectant and then rinsed with it if necessary at each use.

The evaluation of the effectiveness of the cleaning-sterilization of the utensils and raw materials was carried out by analyzing and quantifying the contents of some pathogenic micro-organisms in the "spice-speb-synthesized" product at the Pasteur Institute of Madagascar.

### *2.3. Spicy Product Energy Booster (Speb) Pathogenic Micro-organisms anAlyzing*

As said previously, some pathogenic micro-organisms in the spicy product energy booster (speb) were quantified such as "presumptive *Enterobacteriaceae*, "presumptive *Bacillus cereus",* "positive *Staphylococci coagulase*", *"Salmonella spp."* et "*Listeria monocytogenes"*. The results of these analyzes done at "Institut Pasteur de Madagascar" was accredited by COFRAC © and shown in the following table 1.

***Table 1.*** *Pathogenic micro-organisms quantities in the spicy product energy booster (speb).*

| Pathogenic micro-organisms | Quantities | Procedure-IPM |
|---|---|---|
| Salmonella spp. (research) © | Absence/25g | BIO 12/16-09/05 |
| *Listeria* monocytogenes (research) © | Absence/25g | BIO 12/11-03/04 |
| *Bacillus cereus* at 30 (°C) – 303.15 (°K) © | 3000 (cfu/g) | NF EN ISO 7932 |
| Presumptive *Enterobacteriaceae* at 30 (°C) – 303.15 (°K) © | 14.000 (cfu/g) | NF V 08-054 |
| Positive *Staphylococci coagulase* at 37 (°C) – 310.15 (°K) © | 10 (cfu/g) | NF EN ISO 6888-2 |

In microbiology, "cfu" (colony forming unit) is a unit used to estimate the number of only viable micro-organisms or fungal cells in a sample. And, viability is defined as the ability of micro-organisms to multiply via binary fission under controlled conditions [14-16]. To evaluate the effectiveness of the cleaning-sterilization of the utensils using the previous disinfectant and procedures (§2.1. and §2.2.), interpretations and discussions of the results on table 1 will be provided in the next paragraph-§2.4., but in general the non-existence of *Salmonella spp.* (research) ©, *Listeria monocytogenes* (research) © and Positive *Staphylococci coagulase* at 37 (°C) – 310.15 (°K) inferior to 10 (cfu/g) indicated that this disinfection method was very effective.

### *2.4. Synthesized Spicy Product Energy Booster (Speb) Micro-organisms Analyzing Results Interpretations and Discussions*

First of all, noticed that the speb-sample for analysis was synthesized on October 26$^{th}$, 2020 at seventeen hours. It had been sealed in a sterile bag and then carried out to "Institut Pasteur de Madagascar" under refrigeration in a cooler at temperature below 6 (°C)-279.15 (°K) on October, 27$^{th}$ 2020. And, it was analyzed on 28$^{th}$ 2020. Thus, the estimated elapsed time between synthesis-preservation and analysis was 41 (h). Assuming the sample micro-organisms right estimated rate just after disinfection, it was possible to evaluate the estimated average speed of binary fission of these micro-organisms on the "spicy product energy booster-speb" during these 41 (h) and/or during the incubation time described by the laboratory procedure analysis. Consequently, it was possible to deduce the product-speb best-before dates based to the maximum acceptable quantities of each micro-organism according to a standardization.

*Spicy product energy booster-speb Salmonella spp. (research) rate results interpretations and discussions*

The absence of *Salmonella spp.* (research) in the "speb" confirmed that its consumption doesn't risk contamination with *Salmonellosis*, especially for children and vulnerable people including pregnant women [17, 18]. Also, this result confirmed that the minimum parameters for growth of the main pathogenic micro-organisms including salmonella such as storage temperature, pH, activity water-$a_w$ [12, 19] were respected by the speb, particularly its pH equals to 2.62. Those explained not only the efficacy of the cleaning-disinfection product and procedure of raw materials and utensils described previously (§2.1. and §2.2.) but also the non-presence with non-development of salmonella spp. during these 41 (h) before analysis and during analysis.

*Spicy product energy booster-speb Listeria monocytogenes (research) rate results interpretations and discussions*

The absence of *Listeria monocytogenes* (research) in the "speb" confirmed that its consumption doesn't risk contamination with *Listeria monocytogenes* and listeriosis disease which consequences for pregnant women, fetuses, babies and immune-compromised adults or young were significant [20]. Also, this result confirmed that the minimum parameters for growth of the main pathogenic micro-organisms including salmonella such as storage temperature, pH, activity water-$a_w$ [12, 19] were respected by the speb, particularly its pH equals to 2.62. Those explained not only the efficacy of the cleaning-disinfection product and procedure of raw materials and utensils described previously (§2.1. and §2.2.) but also the non-presence with non-development of *Listeria monocytogenes* during these 41 (h) before analysis and during analysis.

*Spicy product energy booster-speb Bacillus cereus at 30 (°C) – 303.15 (°K) rate results interpretations and discussions*

The *Bacillus cereus* amount in the "speb" quantified at 30°[C]-303.15 (°K) according to the normalization NF EN ISO 7932 was 3,000 (cfu/g). *Bacillus cereus* is a highly resistant bacterium present in dry-dehydrated spices and other vegetables or soil crops. They are killed during normal cooking but spores are more resistant. Indeed, in wet heat, spores require more than 5 minutes at 394.15 (°K) at the coldest spot to be destroyed. In dry heat, 393.15 (°K) in a sterilizer for 1 hour works for rice, for instance [21]. For reference, the *Bacillus cereus* quantities in soil is in the range of 10,000 (cfu/g) to 100.000 (cfu/g) and the minimum and maximum acceptable amount in spices and spice mixtures for food products under European standardization are respectively 1,000 (cfu/g) and 10,000 (cfu/g) [10]. Referring to these bibliographic studies, the effectiveness and efficiency of the cleaning-disinfection conditions-procedure described previously (§2.1. and §2.2.) not only for the raw materials but also for the utensils using the washing-disinfection solution was further confirmed. In fact, assuming that the initial *Bacillus cereus* content of raw

materials-spices was in the average equal to 55,000 (cfu/g), then the efficiency of the cleaning-disinfection procedure using the washing-disinfection solution explained above (§2.1. and §2.2.) was equal to 94.55% assuming that the *Bacillus cereus* quantities after washing-disinfection was equal to that of the "spicy product energy booster-speb" 3,000 (cfu/g).

In addition, assuming that the evaluated *Bacillus cereus* quantities after washing-disinfection was approximately equal to 810 (cfu/g) slightly less than 1,000 (cfu/g) (The minimum quantity accepted by the food products under European standardization) and also assuming that test incubation time was 48 (h), then the estimated average binary fission speed of these *Bacillus cereus* on "speb" was equal to 0.0127 (cfu/g/s) or 0.76 (cfu/g/mn) or 45.63 (cfu/g/h]. Noticed that this "speb" binary fission speed was very slow compared to milk's *Bacillus cereus* estimated average binary fission speed of these *Bacillus cereus* on "speb" in the order of 3.57 (cfu/g/mn), value calculated by bibliographies datas [22, 23]. This result confirmed and affirmed that not only this disinfectant and its utilization procedure were efficiency to limit micro-organism development including *Bacillus cereus* but also the "spicy product energy booster-speb" was a product which can limit micro-organism development including *Bacillus cereus.*

The dose of cereulide sufficient to cause emetic symptoms would be in the range of 5 [µg/kg] to 10 [µg/kg] body weight, based on monkey trials and analysis of foods involved in human food poisoning [24]. This quantity of cereulide can be found in foods when its *Bacillus cereus* strain reaches a concentration greater than $10^6$ (cfu/g). Consequently, an evaluation of the "speb" best-before dates during a conservation at 303.15 (°K) (and/or at natural ambient temperature) based on this *Bacillus cereus* binary fission speed was equals to 21,897.65505 (hours) equivalent to 2 (years)16 (months)1 (day)0 (hours)8 (mn)16 (s). In the other words, based on this *Bacillus cereus* binary fission speed, this product "speb" well packaged in a sealed container could be conserved for a long time in a freezer and/or non-infected refrigerator; and in the order of two years at room temperature on the shelves of various points of sale, shelves of disinfected supermarkets and at home.

*Spicy product energy booster-speb presumptive Enterobacteriaceae at 30 (°C) – 303.15 (°K) rate results interpretations and discussions*

The *presumptive Enterobacteriaceae* amount in the "speb" quantified at 30 (°C)-303.15 (°K) according to the normalization NF V 08-054 was 14,000 (cfu/g). *Enterobacteria* (*Enterobacteriacea* family) are very ubiquitous Gram-negative bacilli found everywhere in soil, water and especially in the intestines of humans and animals. They constituted one of the most important families of bacteria, both quantitatively (includes more than 40 genera and more than 100 species) and qualitatively [25]. Indeed, many of the more familiar pathogens are frequently encountered in infectious pathology such as *Salmonella, Escherichia coli, Klebsiella* and *Shigella* and others in bio-industries (cheese and dairy products fermentation, alcohols production, supplementary medical treatments, toxins production for cosmetic use, manufacture of antiviral agents for pharmaceutical industry). Thus, only strict pathogenic *Enterobacteriaceae* could have sufficient pathogenicity to cause disease in a healthy host. This isn't the case for opportunistic pathogenic *Enterobacteriaceae* which are susceptible to trigger an infection only in an immunocompromised subject. There are also commensal enterobacteria which are animals and humans hosts and reside mainly in the intestine where they contribute to food residues degradations and intestinal gases production. On the other hand, the *Escherichia coli* species plays a preponderant role because of its presence and large predominance against other *Enterobacteriaceae* species: it constitute 80% of the aerobic flora with a concentration close to $10^8$ E. coli/g of terminal stool. However, it's a minority flora compared with anaerobic flora. These commensal *Enterobacteriaceae* were also located in the oral cavity, skin moist areas particularly the perineum, nasal cavity and female genital tract.

The European standardization stipulated that the *Escherichia coli* in spices should be between 100 (cfu/g) and 1,000 (cfu/g) [10]. Referring to these previous bibliographies, the marked-up value of the *Escherichia coli* in the "speb" would be $\frac{14{,}000}{40} = 350\,(cfu/g)$ which respected the European standardization [10].

In pathology, the pathogenic *Escherichia coli* could cause gastroenteritis, urinary tract infections, meningitis or sepsis. But until now, the consumption of the "speb" didn't lead to any of this pathologies which allowed to affirm that this *Escherichia coli* estimated value based to the "speb" presumptive *Enterobacteriaceae* at 303.15 (°K) quantification was acceptable.

Then, assuming that the initial amount of *Escherichia coli* and *Enterobacteriaceae* on the "speb"-raw materials-spices were respectively $\frac{10{,}000}{40} = 250[\text{cfu/g}]$ and 10,000 (cfu/g) which corresponded to raw meats, sausages, meat sausages, raw cooked meats *Enterobacteriaceae* quantities [10], the efficiency of the cleaning-disinfection procedure using the washing-disinfection solution explained above (§2.1. and §2.2.) was respectively equal to 65.00 (%) and 96.5 (%) compared to respectively *Enterobacteriaceae* and *Escherichia coli.* In addition, seeing that for the quantification of presumptive *Enterobacteriaceae* at 30 (°C) – 303.15 (°K) (NF V 08-054), the incubation time is 24 (h), the estimated average binary fission speed of these *Escherichia coli* and *Enterobacteriaceae* on "speb" was respectively equal to 2.6042E-3 (cfu/g/s)/0.1389 (cfu/g/s) or 0.1563 (cfu/g/mn)/8.3333 (cfu/g/mn) or 9.375 (cfu/g/h]/500 (cfu/g/h].

Consequently, an evaluation of the "speb" best-before dates during a conservation at 303.15 (°K) (and/or at natural ambient temperature) based on this *Escherichia coli* and presumptive *Enterobacteriaceae* binary fission speed was respectively equal to 93.3333 (hours)/76 (hours) equivalent respectively to 3 (days)21 (h)20 (mn)/ 3 (days)4 (h). In the other words, based on this *Escherichia coli* binary fission

speed, this product "speb" well packaged in a sealed container could be conserved for a long time in a freezer and/or non-infected refrigerator; and in the order of 4 days at room temperature on the shelves of various points of sale, shelves of disinfected supermarkets and at home. But, in any case, the consummation tests confirmed that after 4 months, the "speb" stayed consumable without losing any of its flavors and any of its energy virtues without pathologies apparitions on consumers.

In addition, seeing that the generation time of the *Escherichia coli* is 20 (mn), the average speed of its fission on this experimental conditions must be

$\frac{(500-250)}{20} = 12.5\ (cfu/g/mn)$ i.e. 0.2083 (cfu/g/s).

But, as previously calculated, the real speed fission of the *Escherichia coli* on the "speb" was 1.1574E-3 (cfu/g/s), approximately 80 twice less speed than the correspondent deduced by generation time. This result confirmed enough that not only this disinfectant and its utilization procedure described previously (§2.1. and §2.2.) were efficiency to limit micro-organism development including *Escherichia coli* but also the "spicy product energy booster-speb" was a product which can limit micro-organism development including *Escherichia coli.*

*Spicy product energy booster-speb positive Staphylococci coagulase at 37 (°C) – 310.15 (°K) rate results interpretations and discussions*

The positive *Staphylococci coagulase* amount in the "speb" quantified at 37°[C]-310.15 (°K) according to the normalization NF EN ISO 6888-2 was inferior to 10 (cfu/g). *Staphylococcus aureus* is the best known of the coagulase-positive staphylococci (CPS) belonging to the genus Staphylococcus [26] which is responsible of food poisoning, localized suppurated infections and in extreme cases potentially fatal infections (immunocompromised patients, sepsis, endocarditis, osteomyelitis, pneumonia, and toxemias of the gastrointestinal and reproductive tracts, cardiac disease) [27]. Its pathogenicity results from several secretions, in particular the secretion of various enzymes like:

1) Coagulase or staphylocoagulase, an enzyme apt to coagulate blood plasma [28].
2) Phosphatase which remove a phosphated group from a simple molecule or a biological macromolecule by hydrolysis [29].
3) Hyaluronidases which degrade hyaluronic acids. In cosmetics as well as in aesthetic medicine, hyaluronic acid remains the leading molecule for smoothing dehydrated and wrinkled skin, giving it a plump texture and a more youthful appearance [30]. In addition, by catalyzing the hydrolysis of hyaluronic acids major constituents of extracellular matrix, hyaluronidases decrease their viscosity and thus increase the permeability of tissues, it contributes significantly to cell proliferation and migration, and also may be involved in the progression of some malignant tumors [31].
4) Deoxyribonuclease (or DNAase or DNAse) is an enzyme which catalyzed deoxyribonucleic acids into nucleotides or polynucleotides. It hydrolyzed the phosphodiester bonds.
5) Peptidases (or proteases or proteolytic enzymes) are enzymes which break the peptide bonds of proteins, also called proteolytic cleavage or proteolysis. This process classified as hydrolases involves the use of a water molecule. The proteases biological functions are varied including the maturation of proteins, in the digestion of food, in blood coagulation, in the remodeling of tissues during the development of the organism and in wound healing [32]. However, some proteases are toxins such as the botulinum toxin [33] the "miracle poison" because of its use for the treatment of numerous disorders of spasticity and a host of other conditions [34]; and the caspases [35].

This amount of positive *Staphylococci coagulase* inferior to 10 (cfu/g) in the "speb" showed the efficiency of the cleaning-disinfection procedure using the washing-disinfection solution explained above (§2.1. and §2.2.) in the order of 99.10 (%) if the initial amount of *Staphylococci coagulase* on the "speb"-raw materials-spices were 1.000 (ufc/g) the maximum content tolerated by the European standard.

In addition, seeing that for the quantification of positive *Staphylococci coagulase* at 37 (°C) – 310.15 (°K) (NF EN ISO 6888-2), the incubation time for their counting was 48 (h) and assuming that the initial amount of positive *Staphylococci coagulase* just after the washing-disinfection procedure was in the vicinity of 1 equal to 1.05 (cfu/g) and assuming also that the positive *Staphylococci coagulase* amount in the "speb" quantified at 37° [C]-310.15 (°K) was equal to 9 (cfu/g), consequently the estimated average binary fission speed of these *Staphylococcus aureus* on "speb" was respectively equal to 4.6E-5 (cfu/g/s) or 2.76E-3 (cfu/g/mn) or 0.1656 (cfu/g/h]. Consequently, an evaluation of the "speb-pebe" best-before dates during a conservation at 303.15 (°K) (and/or at natural ambient temperature) based on this *Staphylococcus aureus* binary fission speed was equal to 6,032.3068 (hours) or approximately 251 (days) 8 (hours) 18 (mn) 24 (s) equivalent to 8 (months) 11 (days) 8 (hours) 18 (mn) 24 (s). In the other words, based on this *Staphylococcus aureus* binary fission speed, this product "speb" well packaged in a sealed container could be conserved for a long time in a freezer and/or non-infected refrigerator; and in the order of maximally 8 (months) at room temperature on the shelves of various points of sale, shelves of disinfected supermarkets and at home. But, in any case, the consummation tests confirmed that after 4 months, the "speb" stayed consumable without losing any of its flavors and any of its energy virtues without pathologies apparitions on consumers.

The generation time for the general *Staphylococcus aureus* is 30 minutes, the average speed of its fission on this experimental conditions must be $\frac{(2.1-1.05)}{30} = 0.035\ (cfu/g/mn)$ i.e. 5.8333E-4 (cfu/g/s).

But, as previously calculated, the real speed fission of the *Staphylococcus aureus* on the "speb" was 4.6E-5 (cfu/g/s), approximately 12.6812 twice less speed than the correspondent deduced by generation time. This result confirmed enough that not only this disinfectant and its utilization procedure described previously (§2.1. and §2.2.) were efficiency to limit micro-organism development including *Staphylococcus aureus* but also the "spicy product energy booster-speb" was a product which can limit micro-organism development including *Staphylococcus aureus.*

***Table 2.*** *Summary table of the results of the analysis of microorganisms in the spicy product energy booster (speb-pebe) at the Pasteur Institute of Madagascar and their interpretations.*

| **Pathogenic micro-organisms** | **Estimated raw materials' micro-organisms quantities before washing-disinfection procedure (cfu/g) (1)** | **"spicy product energy booster (speb-pebe)" micro-organisms quantities after ipm analysis (cfu/g) (cf. Table 1) (2)** | **Minimum the efficiency of the cleaning-disinfection procedure** $(3) = \frac{(1)-(2)}{(1)} \times 100$ | **evaluated raw materials' micro-organisms quantities after washing-disinfection procedure (cfu/g) (4)** | **Incubation time (h) © (5)** |
|---|---|---|---|---|---|
| *Salmonella spp.* (research) © | very low [37] | Absence/25g | 100 | Absence | - |
| *Listeria monocytogenes* (research) © | <100 [38] | Absence/25g | 100 | Absence | - |
| *Bacillus cereus* at 30 (°C) – 303.15 (°K) © | 55,000 | 3,000 | 94.55 | 810 | 48 |
| *Presumptive of all Enterobacteriaceae at 30 (°C) – 303.15 (°K) ©* | 40,000$^{maxi}$ [10] | 14,000 | 65 | = (100$^{mini}$ [10]×40)/2 =2.000 | 24 |
| *Escherichia coli-estimated by Enterobacteriaceae quantities* | 10,000 | 350 | 96.5 | = (250$^{mini}$ [10]/2) =125 | 24 |
| *Positive Staphylococci coagulase-staphylococcus aureus at 37 (°C) – 310.15 (°K) ©* | 1,000$^{maxi}$ [10] | 9 (<10) | 99.10 | 1.05 | 48 |

***Table 2.*** *Continued.*

| **Pathogenic micro-organisms** | **estimated average binary fission speed of each micro-organisms on "speb-pebe" (cfu/g/h]** $(6) = \frac{(2)-(4)}{(5)}$ | **estimated average binary fission speed of each micro-organisms (cfu/g/h] (7)** | **Binary fission speed ratio of each micro-organism on a reference and on "speb-pebe"** $(8) = \frac{(7)}{(6)}$ | **Maximum accepted value for each micro-organism according references (9) (cfu/g)** | **Estimated "speb-pebe" best-before dates during a conservation at 303.15 (°K) (and/or at natural ambient temperature) according each micro-organism** $(10) = \frac{(9)-(4)}{(6)}$ |
|---|---|---|---|---|---|
| *Salmonella spp.* (research) © | - | - | Absence | <100 [37] | infinity |
| *Listeria monocytogenes* (research) © | - | - | Absence | <100 [38] | infinity |
| *Bacillus cereus* at 30 (°C) – 303.15 (°K) © | 45.63 | 214.2§2.4.3 | 4.6948 | 1E6 [24] | 2 (years)<br>16 (months)<br>1 (day)<br>0 (hours)<br>8 (mn)16 (s) |
| *Presumptive of all Enterobacteriaceae at 30 (°C) – 303.15 (°K) ©* | 500 | - | - | 40,000$^{maxi}$ [10] | 3 (days)4 (h) |
| *Escherichia coli-estimated by Enterobacteriaceae quantities* | 9.375 | 750 | 80 | 1000$^{mini}$ [10] | 3 (days)21 (h)<br>20 (mn) |
| *Positive Staphylococci coagulase-staphylococcus aureus at 37 (°C) – 310.15 (°K) ©* | 0.1656 | 2.1 | 12.6812 | 1000$^{mini}$ [10] | 8 (months)<br>11 (days) 8 (hours)<br>18 (mn)24 (s) |

In fact, these previous results indicated that firstly, the cleaning-disinfection procedure using the washing-disinfection solution explained above (§2.1. and §2.2.) was efficient both for raw materials and utensils used during "speb" synthesis. Secondly, the spicy product energy booster-"speb" is an efficient bio-natural consumer product apt to decrease the binary fission speed of various micro-organisms including *Salmonella spp.*, *Listeria* monocytogenes, *Bacillus cereus, Enterobacteriaceae* and *Staphylococci coagulase-Staphylococcus aureus.* Consequently, as described previously on paragraph §2.4.1. to paragraph §2.4.5. many diseases and pathologies could be avoided by consuming the spicy product energy booster-"speb" within its best-before dates. Questionnaire with response points and rating scales was given to be answered by people who took regularly the spicy product energy booster-"speb" during one month. The results will be described subsequently but it was ascertained that they not only affirmed to gain strength and vigour but also they regain their youthful shape. Noticed that the total sugars of principle fresh raw materials on "speb-pebe" were respectively from 198 to 1543 (mg/100g) (sucrose+glucose+fructose) [39] and

18 (g/100g) (gluicides) [40-47] which indicated that there were small amounts of sugars fundamentals food for micro-organisms development in the "speb-pebe". That explained probably why, in any case, the consummation tests confirmed that after 4 months, the "speb" stayed consumable without losing any of its flavors and any of its energy virtues without pathologies apparitions on consumers. In all cases, according to the precautionary principle, the product "speb-pebe" should be stored at room temperature, away from humid air and sunlight, in order to reduce as much as possible the development of these two main micro-organisms whose binary fission speed was the highest such as *Enterobacteriaceae* and *Escherichia coli* and increasing certainly the "speb-pebe" best-before dates as noticed previously. In addition, it was possible to increase its best-before dates not only to use freezer without *Enterobacteriaceae* and *Escherichia coli* products contaminations [10] but also to choose adequate product design and adequate product packaging.

Generally, the efficiency of this spicy product energy booster-"speb-pebe" resided not only on its citric acids' protons $H^+$ capacities and activities to catalyze some chemistry reactions and responsible of its acidity-pH=2.62 [11-13, 36] but also their dispersions within the "speb" [12-36] which certainly increased the efficiency of "speb" raw materials' active molecules including spices bioactive molecules [12-36] as shown in the following Table 3.

***Table 3.*** *"speb-pebe"- spicy product energy booster characteristics and raw materials.*

| "speb-pebe" - spicy product energy booster characteristics | Values |
|---|---|
| *Capsicum chinense* | - |
| *Capsicum frutescens* | - |
| Other "speb-pebe's " confidential raw mwterials | - |
| Calculated-Citric acid [moles] | 0.000252111 |
| Calculated moles of $H^+$ (pH2.6) | 2.52111E-05 |
| Calculated-Total $C^=$ [moles] | 0.059236915 |
| Ratio ($C^=$/citric acid) | 234.963299 |
| pH | 2.6 |
| dispersion $_{[molAc/molC=]}$ - $V_{totalconstant}$ | 0.004255984 |
| Dispersion $H^+$actif [$mol_{H+}/mol_{C=}$] - $V_{totalconstant}$ | 4.26E-04 |
| Initial Concentration $C^=$ $[C^=]_{initial}$ | 3.39 |

But, experiences and experimentations were carried out whose objective was to determine the citric acids molecules equivalent distributions on the alkenes-$C^=$ organic functions on three levels of the "speb-pebe" product in its packaging such as level-high, level-middle and level-low after doing these levels titration with NaOH-0.0557N and titration with HF-0.0026N inspired by bibliographic-titration [48] according to procedures described below to determine respectively the citric acid molecules equivalent and the alkenes-$C^=$ organic functions on each level. Results showed in brief that these distributions influenced certainly the "speb-pebe" best-before dates and influenced its efficiency by staying consumable without losing any of its flavors and its energy virtues without pathologies apparitions as noticed previously.

### *2.5. Citric Acids Molecules Equivalent Distributions on the Alkenes-$C^=$ Organic Functions on Three Levels of the "speb-pebe" Product in Its Packaging*

#### *2.5.1. Titration Procedure of the "speb-pebe" - Citric acid Molecules Equivalent in Each Level of Its Packaging Using NaOH-0.0557N*

Take three samples of the spicy product energy booster - "speb-pebe" such as a sample from the level-high, a sample from the level-middle and a last sample from level-low of its packaging. Make sure that each sample weighed in the vicinity of 200 (mg) ($M_s$ [g]). Dilute each sample with 30 (ml) of distillated water in a beaker-250 (ml) and mix two to three minutes to dissolve citric acids molecules and its derivatives soluble in distilled water until having a heterogeneous solution.. Then, add 15 (ml) of dichloromethane in this last beaker-250 (ml) to extract citric acids molecules and its polar derivatives soluble in dichloromethane. Allow to decant for 15 (mn) then recover the organic phase at the bottom in another beaker-250 (ml). Then, add 30 (ml) of distillated water in this beaker-250 (ml) and mix carefully for at least two minutes to extract citric acid molecules and its derivatives soluble in the distillated water. Allow to decant during 15 (mn) using a separating funnel and recover the aqueous phase at the top in a measuring cylinder within paying attention to record its volume ($V_e$ (ml)). Transfer this solution into a beaker-250 (ml), add 2 to 3 drops of helianthine color indicator and insert a stirring rod. Take this beaker-250 (ml) on a magnetic stirrer and begin to mix slowly. The solution turned to red. Begin to titrate by opening the burette containing the prepared titration solution NaOH-0.557N. When, the solution turned to yellow-orange, close the burette and record the titration solution NaOH-0.557N volume ($V_q$ (ml)) which correspond to the equivalent-point volume.

Finally, it could be deduced the:

1) Total moles of equivalent-citric acids (eca) - (1)

$$eca\ [moles] = 0.6622 \times ([NaOH] \times V_q \times 10^{-3})$$

2) Equivalent-citric acids concentrations [eca] – (2)

$$[eca]\ [mol.l^{-1}] = \frac{eca\ [moles]}{(V_e \times 10^{-3})}$$

3) Equivalent-citric acids concentrations per grams of sample [eca] $_{per\ grams}$ – (3)

$$[eca]_{per\ grams}\ [mol.l^{-1}.g^{-1}] = \frac{[eca]\ [mol.l^{-1}]}{M_s}$$

The following table 4 showed these results of "speb-pebe" - spicy product energy booster's citric acid molecules equivalent in each level of its packaging using NaOH-0.0557N titration.

***Table 4.*** *Results of "speb-pebe" - spicy product energy booster's citric acid molecules equivalent in each level of its packaging using NaOH-0.0557N titration.*

| Results | Level-high-packaging sample | Level-middle-packaging sample | Level-low-packaging sample |
|---|---|---|---|
| Equivalent-citric acids concentrations [eca] [mol×l$^{-1}$] | 2.17E-04 | 9.27E-05 | 2.53E-04 |
| Equivalent-citric acids concentrations per grams of sample [eca] $_{per\ grams}$ [mol×l$^{-1}$×g$^{-1}$] | 7.23E-03 | 3.12E-03 | 8.44E-03 |

### *2.5.2. Titration Procedure of the "Speb-Pebe" - Spicy Product Energy Booster's Alkenes-C$^{=}$ in Each Level of Its Packaging Using HF-0.0026N*

Take three samples of the spicy product energy booster - "speb-pebe" such as a sample from the level-high, a sample from the level-middle and a last sample from level-low of its packaging. Make sure that each sample weighed in the vicinity of 0.100 [g] ($M_s$ [g]). Dilute each sample with 30 (ml)- ($V_e$ (ml)) of distillated water in a beaker-250 (ml). Then, add 2 to 3 drops of bromophenol blue color indicator and insert a stirring rod. Take this beaker-250 (ml) on a magnetic stirrer and begin to mix slowly. The solution turned to blue. Begin to titrate by opening the burette containing the prepared titration solution HF-0.0026N. When, the solution turned firstly to transparent, close the burette and record the titration solution HF-0.0026N volume ($V_{q1}$ (ml)) which correspond to the titrated alkenes-C$^{=}$ organic functions localized on the surface of the "speb-pebe" - spicy product energy booster samples. Afterwards, open the burette and continue the titration until the solution turned secondly to yellow-transparent. Thus, close the burette and record the titration solution HF-0.0026N volume ($V_{q2}$ (ml)) which correspond to the titrated alkenes-C$^{=}$ organic functions localized not only on surface but also on the structure and texture of the "speb-pebe" - spicy product energy booster samples. In the other words, the second volume ($V_{q2}$ (ml)) corresponded to the total titrated alkenes-C$^{=}$ organic functions on the "speb-pebe" - spicy product energy booster samples at the equivalent-point of titration.

Finally, it could be deduced the:

1) Total moles of alkenes-C$^{=}$ organic functions on surface of sample titrated (4)

$$Total_{C=surface}\ [moles] = [HF] \times V_{q1} \times 10^{-3}$$

2) Total moles of alkenes-C$^{=}$ organic functions on surface per grams of sample titrated (5)

$$Total_{C=\ surface\ per\ grams}[mol.g^{-1}] = \frac{Total_{C=surface}}{M_s}$$

3) Concentration of alkenes-C$^{=}$ organic functions on surface per grams of sample titrated (6)

$$[C^{=}]_{surface\ per\ grams}[mol.g^{-1}.l^{-1}] = \frac{Total_{C=\ surface\ per\ grams}}{(V_e \times 10^{-3})}$$

4) Total moles of alkenes-C$^{=}$ organic functions on structure and on texture of sample titrated (7)

$$Total_{C=structure-texture} = [HF] \times\ (V_{q2} - V_{q1}) \times 10^{-3}$$

5) Total moles of alkenes-C$^{=}$ organic functions on structure and on texture per grams of sample titrated (8)

$$Total_{C=\ structure-texture\ per\ grams}[mol.g^{-1}] = \frac{Total_{C=structure-texture}}{M_s}$$

6) Concentration of alkenes-C$^{=}$ organic functions on structure and on texture per grams of sample titrated (9)

$$[C^{=}]_{structure-texture\ per\ grams}\ [mol.g^{-1}.l^{-1}] = \frac{Total_{C=\ structure-texture\ per\ grams}}{(V_e \times 10^{-3})}$$

The following table 5 showed these results of "speb-pebe" - spicy product energy booster's alkenes-C$^{=}$ organic functions in each level of its packaging using HF-0.0026N.

***Table 5.*** *Results of "speb-pebe" - spicy product energy booster's alkenes-C$^{=}$ organic functions in each level of its packaging using HF-0.0026N.*

| Results | Level-high-packaging sample | Level-middle-packaging sample | Level-low-packaging sample |
|---|---|---|---|
| Total moles of alkenes-C$^{=}$ organic functions on surface per grams [mol×g$^{-1}$] | 1.44E-4 | 5.16E-5 | 6.57E-5 |
| (1) Concentration of alkenes-C$^{=}$ organic functions on surface per grams [mol×l$^{-1}$×g$^{-1}$] | 4.805E-3 | 1.72E-3 | 2.19E-3 |
| Total moles of alkenes-C$^{=}$ organic functions on structure and on texture per grams [mol×g$^{-1}$] | 6.28E-4 | 3.70E-3 | 3.48E-3 |
| (2) Concentration of alkenes-C$^{=}$ organic functions on structure and on | 2.09E-2 | 1.23E-1 | 1.16E-1 |

| Results | Level-high-packaging sample | Level-middle-packaging sample | Level-low-packaging sample |
|---|---|---|---|
| texture per grams [mol×l$^{-1}$×g$^{-1}$] (3)= (2)+ (1) Total concentration of alkenes-C$^{=}$ organic functions per grams [mol×l$^{-1}$×g$^{-1}$] | 2.57E-2 | 1.25E-1 | 1.18E-1 |

By definition, the dispersion of the equivalent-citric acids molecules on total alkenes-C$^{=}$ organic functions noted $dispersion(Ac/C^{=})$ was equal to the ratio between the equivalent-citric acids molecules concentration and total alkenes-C$^{=}$ organic functions concentration which was deduced by the sum between the total moles of alkenes-C$^{=}$ organic functions on structure and on texture and total moles of alkenes-C$^{=}$ organic functions on surface divided by ($V_e$ (ml)). In the other words, the dispersion deduced by the table 4 and table 5 equals to (10):

$$dispersion(Ac/C^{=}) = \frac{[eca]\ per\ grams}{([C^{=}]_{surface\ per\ grams} + [C^{=}]_{structure-texture\ per\ grams})}$$

The following table 6 showed these results.

***Table 6.*** *Results of "speb-pebe" - spicy product energy booster's dispersions in each level of its packaging.*

| Results | Level-high-packaging sample | Level-middle-packaging sample | Level-low-packaging sample |
|---|---|---|---|
| Dispersion $dispersion(Ac/C^{=})$ | 0.2813 | 0.0250 | 0.0714 |

### *2.5.3. Interpretations and Discussions*

Alkenes-C$^{=}$ organic functions on surface came not only from alkenes of dense and less dense raw materials' molecules extracted by esterification with citric acid molecules [11, 12] but also from citric acids' monomers and polymers synthesized as described by bibliographies [12]. These later compounds were unsaturated oxygenated compounds with oxygen bridges [12] and confirmed not only the high concentrations per grams of equivalent-citric acids in Level-high-packaging sample and in Level-low-packaging sample (Table 4) but also the low dispersions results of "speb-pebe" - spicy product energy booster in the

Level-middle-packaging sample and the Level-low-packaging sample. Thus, the white products as fin films observed mainly on Level-high-packaging sample was probably composed by these previous unsaturated oxygenated compounds [12] which were responsible for the protection of the "speb-pebe" flavors and tastes. Indeed, the highest value of the concentration of alkenes-C$^{=}$ organic functions on surface per grams of "speb-pebe" (Table 5) were observed at Level-high-packaging sample (4.805E-3 [mol×l$^{-1}$×g$^{-1}$]) compared with Level-middle-packaging sample (1.72E-3 [mol×l$^{-1}$×g$^{-1}$]) and Level-low-packaging sample (2.19E-3 [mol×l$^{-1}$×g$^{-1}$]) (Table 5).

Seeing that the "speb-pebe" - spicy product energy booster contained also peanut oil and colza oil, trans-esterification with citric acids' alcohol functions could occurred with formations of esters [36] less dense extracted with dichloromethane as strong yellow color solution during the realization of the citric acid equivalent titrations for the Level-high-packaging sample and decreased appreciably to light yellow until Level-low-packaging sample. These results confirmed that trans-esterification evolved positively with dispersions [36].

These previous results confirmed also the effectiveness of all titrations procedures described on §2.5.1. and §2.5.2. to control the "speb-pebe" - spicy product energy booster quality in each level of its packaging and also to control the packaging-quality.

# 3. Conclusion

Following-up the established procedure for use, this synthesized washing-disinfectant was efficiency to eliminate 100% of *Salmonella spp.* and *Listeria monocytogenes*; 94.55% of *Bacillus cereus*, 65% of all presumptive *Enterobacteriaceae*, 96.5% of *Escherichia coli* and 99.10% of

Positive *staphylococcus aureus.* Also, it was noticed that this product "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)" was apt to decrease significantly the binary fission speed of these previous micro-organisms in order 4.7 to 80 times less speed than its speeds deduced by a reference and/or by its generation time. The shortest "speb-pebe's'' best-before dates during its conservation at 303.15 (°K) (and/or at natural ambient temperature) was 3 (days) 4 (h) calculated from all presumptive *Enterobacteriaceae* binary fission speed; and the longest "speb-pebe's'' best-before dates at the same condition was 2 (years) 16 (months) 1 (day) 0 (hours) 8 (mn) 16 (s) calculated from *Bacillus cereus* binary fission speed seeing that in any cases, the consummation tests confirmed that after 4 months, the "speb" stayed consumable without losing any of its flavors and any of its energy virtues without pathologies apparitions on consumers. Finally, the two established procedures titrations consisting in titration of the "speb"- spicy product energy booster's equivalent-citric acid molecules in each level of its packaging using NaOH-0.0557N and in titration of the "speb-pebe" - spicy product energy booster's alkenes-C$^{=}$ organic functions (on surface – on structure and on texture) in each level of its packaging using HF-0.0026N allowed in fine to calculate the dispersion of the equivalent-citric acids molecules on total alkenes-C$^{=}$ organic functions noted $dispersion(Ac/C^{=}) = dispersion_{([Ac]/[C^{=}])}$ for the Level-high-packaging sample, the Level-middle-packaging sample and the Level-low-packaging sample which was a

fundamental indicator to understand and evaluate not only the "speb-pebe" - spicy product energy booster quality in each level of its packaging, to understand and to evaluate the packaging-quality but also to understand and to evaluate the useful role of citric acids molecules quantities and their protons $H^+$ capacities and activities to catalyze some chemistry reactions and responsible of this "speb-pebe" - spicy product energy booster's acidity-pH=2.62, flavors and tastes. In the other words, these established titrations and the interpretations of their results may be used to characterize the quality of products composed with citric acid and alkenes organic function as this "spicy product energy booster (speb)" – "produit épicé boosteur d'énergie (pebe)". Also, analysis and following-up of the micro-organisms evolutions in products-output may be used to evaluate the efficiency not only the washing procedure of ustensils and raw materials as this synthesized washing-desinfectant product but also the product-output capacities to limit micro-organisms binary fission speed as this "spicy product energy booster (speb-pebe).

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