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SACHA INCHI OIL (*PLUKENETIA VOLUBILIS*) STABILIZED WITH ANTIOXIDANTS FOR ADDITION IN FRESH CHEESE

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ABSTRACT

Sacha Inchi (*Plukenetia volubilis*) is a nut that has been grown in the Amazon Rainforest and the high Andes Mountains of Peru for countless centuries. The oil of this nut, natural source of omega 3, 6 and 9, has been recognized by its high antioxidant capacity in humans. In this work, oil from Sacha Inchi was fortified with two commercial antioxidants (Ecoprol 2020 and tocopherol) in order to prepare a fresh cheese from cow's milk. The antioxidant capacities of Sacha Inchi and commercial antioxidants were used as preservatives with the purpose to increase the shelf-life of fresh cheese besides nutritional content**.** The factorial method was necessary to prepare seven formulations in order to find the optimal concentration of the antioxidants added to Sacha Inchi oil and the addition of this oil to the fresh cheese. A sensory analysis was performed to choose the best formulation**.** The results showed that an oil formulation (F4) with tocopherol (150 mg/kg of oil) and Ecoprol 2020 (1000 mg/kg of oil) displayed the lowest peroxide values (PI: 2.6 ± 0.1 meq O_2/kg of oil, $p < 0.001$) and it was able to reduce approximately 50% of fatty acid oxidation in Sacha Inchi oil in relation to the PI control. Then, F4 was used to elaborate further nine formulations (F'1 – F'9), enriched with Sacha Inchi oil (1 to 4%) to prepare the fresh cheese. Microbiological analysis for all formulations were performed (limits of mold, yeasts, coliforms, salmonella, and bacteria) in order to meet the legal requirements of health and safety in Peru. The cheese taste acceptability was determined through the sensorial evaluation, which reached 7.2 according to the 9-hedonic scale for F'5. Thus, an optimum fresh cheese was obtained from the formulation (F'5) with 22.5g/L of salt and 2.5% of Sacha Inchi oil enriched with 150 mg/kg of tocopherol and 1000 mg/kg of Ecoprol 2020. The cheese shelf-life was also evaluated, increasing it up from 7 days to 16 days in refrigeration.

Key words: cheese, antioxidant, Sacha Inchi, shelf-life, Ecoprol 2020, tocopherol

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INTRODUCTION

Actually, there is an interest in developing healthy foods for the human body, promoting the search for natural products that contain low levels of saturated fat and that prevent diseases. In this context, Sacha Inchi oil has beneficial properties for health, since it is a natural product[1].Sacha Inchi (*Plukenetia volubilis)*, has been grown in the Amazon Rainforest and the Peruvian high Andes Mountains, and it was part of the Inca diet for 3000 years[2]. The Sacha Inchi plant is a rainforest vine, with star-shaped seed pods, meaning that these Inca peanuts are technically seeds[3,4]. Sacha Inchi is known as one of the world's healthiest superfoods. It is a great source of omega-3 (48% of total fatty acids), -6 (36% of total fatty acids) and -9 (9% of total fatty acids), alpha-tocopherol, Vitamin E, carotenoids (Vitamin A), and antioxidants.[2–4]. Omega–3 and omega–6 polyunsaturated fatty acids are essential fatty acids beneficial to human health. The omega–3 (PUFAs) decrease inflammatory markers, blood pressure and triglycerides, which are risk factors for cardiovascular disease[5,6].

Antioxidants are present in many foodstuffs; they play a major part in ensuring that our foodstuffs keep their taste and color and remain edible over a longer period. Their use is particularly important for avoiding oxidation of fat-containing products [7]. Oxidative stability is an important factor in oil quality[8]. The vegetable oil stability against oxidation depending on the fatty acid composition and the content and composition of antioxidants[9]. When antioxidants are thoroughly mixed with fat or oil, the onset of the final stages of autoxidation when rancidity development of unpleasant off-flavors and odors- becomes evident, is delayed.

The use of antioxidants in Sacha Inchi are used to prevent its degradation, such as, some studies on the antioxidant effects of tocopherols in vegetable oil stability[8]. Tocopherol has been used as a preservative because it acts on polyunsaturated fatty acids and modifies the chain reaction of lipid peroxidation, therefore, it can inhibit food degradation[6]. Ecoprol 2020, a commercial antioxidant (ascorbyl palmitate and propyl gallate) has been used as a preservative in the food industry to avoid rancidity for example in butter [7] and soybean oil [8].

The fat present in milk contains a large quantity of short chain fatty acids, which are very susceptible to oxidation, when this happens, there are undesirable original flavors and aromas in products such as milk, butter, but in certain varieties of cheese[10]. The way to make an improved cheese includes changes in the production techniques[11]. The replacement of milk fat by vegetable oils is feasible also in fresh cheeses, as evidenced by the research carried out by Saransig *et al.* [12], who partially replaced milk fat by Sacha Inchi oil. They proposed a methodology for the production of low-fat cream cheese with partial substitution of dairy fat for vegetable oils such as Sacha Inchi oil and olive oil, in which cream cheese was first prepared, after the fermentation process. The vegetable oil was added by mechanical stirring to avoid oil coalescence[13].

In this work, the aim was to prepare a fresh cheese enriched with Sacha Inchi oil stabilized with a mixture of commercial antioxidants, in order to obtain a good quality product, improved shelf-life with good sensory acceptability.

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MATERIALS AND METHODS

The Sacha Inchi oil was purchased from Nutriomega® (Perú). This oil contained 48.61% of ω3 and 36.80% of ω6 according to specifications. Tocopherol was purchased from Sigma Aldrich® (Germany) and the commercial supplement Ecoprol 2020 (propyl gallate and ascorbyl palmitate) was purchased from Somerex® (Perú).

Sacha Inchi oil stabilization with antioxidants: Optimal formulation from different concentrations of tocopherol and Ecoprol 2020 in samples of Sacha Inchi oil were evaluated by a factorial method. The tocopherol concentrations were 10, 20, 42, 84, 150 and 300 mg/kg, taken as reference by Kim *et al.*[14], who worked in the 10-84 mg/kg range. The values of 150 and 300 mg/kg were taken from the average and maximum value given by the Codex standard[15]. The Ecoprol 2020 concentrations were 500, 625, 750, 875 and 1000 mg/kg, whose range was recommended by the supplier. The factorial method was carried out in order to obtain seven different formulations (n=7) with the different concentrations of antioxidants and a control (Sacha Inchi oil without tocopherol and without Ecoprol 2020). (Table 1). Statgraphics Centurion XVI® software could generate the seven formulations from the ranges of each antioxidant. In Table 1, three values of the range studied in the tocopherol peroxide index (10, 150 and 300 mg/kg) were taken, evaluated due to the minimum, intermediate and maximum value of this study (Figure 1). Similarly, three values were taken from the range studied in the peroxide index of Ecoprol 2020 (500, 750 and 1000 mg/kg), evaluated due to the minimum, intermediate and maximum value recommended by the supplier (Figure 2). Tocopherol has been used as a preservative because it acts on polyunsaturated fatty acids and modifies the chain reaction of lipid peroxidation of low density lipoproteins[16].

Ecoprol 2020, a commercial antioxidant (ascorbyl palmitate and propyl gallate) has been used as a preservative in the food industry to avoid rancidity in butter [7], and soybean oil [8].

Peroxide index (PI): The peroxide index is an indicator of the antioxidant capacity of a compound. A lower PI indicates decreased lipid peroxidation of fatty acids, which increases the shelf-life of the food [9]. The PI was measured according to the AOAC 965.33 – Peroxide Value of Oils and Fats [10], by titration with 0.1 mol L^{-1} sodium thiosulfate. The following equation (1) was used to calculate PI values:

$$PI = \frac{(A - A_1) \times C \times 1000}{M} \quad (1)$$

Where A: sodium thiosulfate used in the titration (mL), A_1: sodium thiosulphate used in the titration of blank (mL), C: equivalent concentration of sodium thiosulphate in meq/L and M: sample mass (g). The concentration of PI is expressed in terms of milliequivalents of active oxygen per kg of oil.

Cheese Preparation: The preparation followed established procedures [17] with some modifications since Sacha Inchi was incorporated into the formulation. Thirty liters of previously pasteurized (65 °C, 10 minutes) filtered cow milk was cooled down to 50 °C

before calcium chloride addition (0.2g/L) and it was maintained at 39 °C. Sacha Inchi oil was added as shown in Table 2. Then, 0.65 g of chymosin was added, and the mixture further stirred for 1 minute and left to rest for 80 minutes at 39 °C. Finally, the process of draining was performed [17], and the fresh cheese was stored between 0- 4 °C. The salt content added to the cheese was varied to obtain a cheese with optimum sensory acceptability. The evaluated salt range was taken as a reference of a previous study of a sensory analysis of fresh cheese[18]. The control concentration was 17.5, because it is the lowest value in the studied range.

Microbiological analysis: The microbiological analysis was performed according to described protocols described in [18], for to ascertain compliance with standards of cheese. The samples were compared and analyzed according to parameters established in the Peruvian technical standards NTP 202.195 2004 [19]. According to the Peruvian standards, the microbiological requirements should be: total coliforms (10^2 – 10^3 CFU/g), *Staphylococcus aureus* (10-10^2 CFU/g) and *Salmonella* (absent) [19].

Sensorial analysis: Sensorial analysis was performed with consumers of the product. One hundred people not trained in this analysis were selected (50 men and 50 women) [8,10]. About 30 g of cheese were evaluated by each person (n =9). Each of them answered a questionnaire with alternatives of acceptability level for three sensory attributes, the taste of salt, appearance, and texture, according to the 9-hedonic scale [20]. The general appreciation value for each formulation was obtained from the sum of the acceptability of the respondents, divided by the number of people (100) [20].

Statistical analysis: Each experimental test was validated by analysis of variance (ANOVA). Peroxide index (PI) and sensorial analysis, were performed using the Holm-Sidak *post hoc* test. Analyses were performed using statistical software Sigma Stat for Windows Version 3.10 and the graphics with Statgraphics Centurion XVI® software. All statistical tests were run with a 5% level of significance.

RESULTS AND DISCUSSION

A factorial method was carried out with formulations adequate for the preparation of cheese with Sacha Inchi, according to the nutritional requirement [12]. The peroxide index (PI), for different concentrations of tocopherol and Ecoprol and the seven formulations, are described in the bar diagrams of Figure 1-3. A concentration range between 10 and 300 mg/kg was evaluated because the Codex Alimentarius[15] suggests using as an oil additive, tocopherol, at a maximum level of 300 mg/kg and the intermediate values were referenced by Kim *et al*.[14]. Therefore, six serial concentration of tocopherol between 10 to 300 mg/kg of oil identified as T-10 to T-300 and a control (without tocopherol) were necessary to evaluate PI values in Sacha Inchi oil (Figure 1), after 20 days of storage at 8 ° C. According to the NTP 151.400 2009[19], the maximum peroxide index that the extra virgin Sacha Inchi oil must have is 10 meq O_2/Kg of oil. The peroxide value for the control is around 6 meq O_2/Kg, while with the presence of tocopherol the values were lower. For a concentration of 300 mg/kg of tocopherol, the PI was higher (6.65 meq O_2/kg) than other concentrations with significant differences, even higher than the control value. (Figure 1, $p < 0.05$). This indicates that an excess of

tocopherol would not be favorable in the formulation. Therefore, the appropriate concentrations were between T-10 to T-150. Tocopherol, also known as vitamin E, is an important antioxidant lipophilic compound, also found in Sacha Inchi[21,22]. This work uses α-tocopherol such as in some investigations, where they add α-tocopherol up to a level of 0.2%, increasing the oxidative stability of refined olive oil[8]. According to Blekas *et al*. [23], α-tocopherol acted as antioxidant at all levels of addition evaluated, although the antioxidant effect was greater at 100 ppm than at higher concentrations.

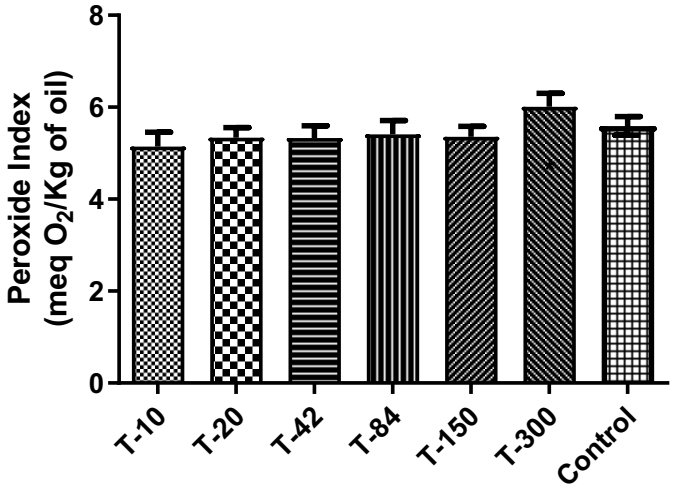

Figure 1: Peroxide index (meq O_2/kg oil) in control sample and in samples of Sacha Inchi oil with different concentrations of tocopherol (10, 20, 42, 84, 150, 300 mg/kg oil), identified as T-10 to T-300. (* p <0.05)

Furthermore, PI values were determined in concentration between 500 to 1000 mg/kg of Ecoprol in oil, whose range was recommended by the supplier and a control (without Ecoprol) (Figure 2). The peroxide value for the control is around 6 meq O_2/Kg, while with the presence of Ecoprol the values were significantly lower. The highest concentration used (E-1000) displayed lower PIs (3.8 ± 0.1, $p < 0.001$), it means that E-1000 had more effective antioxidant capacity. These antioxidant proprieties can inhibit food degradation as a result of a decrease in lipid peroxidation of fatty acid, therefore increasing the shelf-life [24]. Hawrysh *et al*. [25], evaluated the effects of propyl gallate (PG) with and without ascorbyl palmitate (AP) on the stability of canola-oil, showing that after 12 days of storage, the additions of PG + AP to oils at 200/200 ppm and 100/200 ppm were most effective in retarding oil deterioration. Lu *et al*. [26], stabilized flaxseed oil with an antioxidants mix. Flaxseed oil containing 80 mg/kg tocopherol, 40 mg/kg AP, 40 mg/kg phytic acid (PA), and 240 mg/kg tea polyphenol palmitate TPP was found to be more stable to oxidation.

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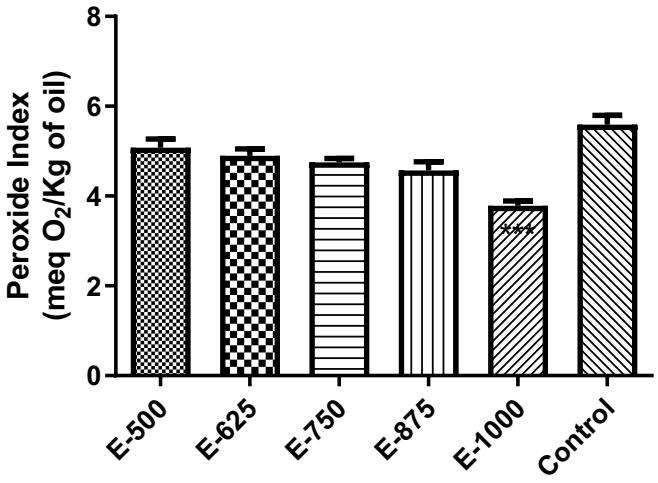

Figure 2: **Peroxide index (meq O_2/kg of oil) in control and in Sacha Inchi oil with different concentrations of Ecoprol 2020 (500, 625, 750, 875, 1000 mg/kg of oil), identified as E-500 to E-1000. (*** p <0.001)**

The peroxide indexes were also determined from the mixture of antioxidants tocopherol and Ecoprol 2020 (Table 1 and Figure 3). Formulation 4 (F4: 150 mg/kg of tocopherol and 1000 mg/kg of Ecoprol 2020) displayed the lowest values (2.6 ± 0.1 meq O_2/kg oil, p <0.001) even this formulation was able to reduce c.a. 50% of fatty acid oxidation in Sacha Inchi oil in relation to the PI control.

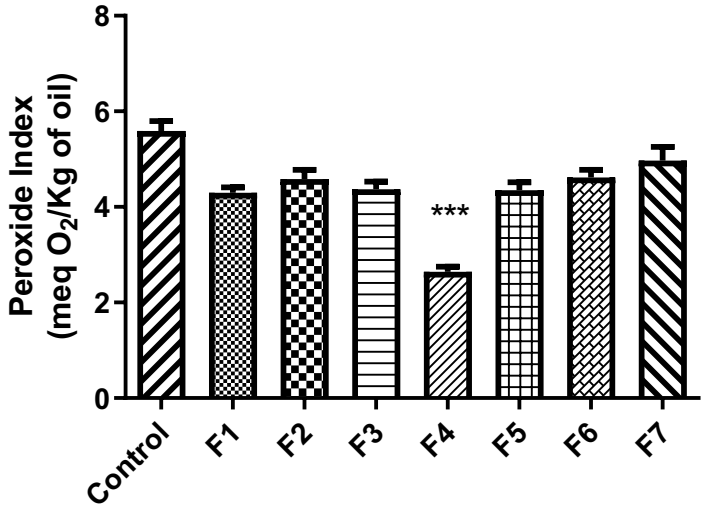

Figure 3: **Peroxide index (meq O_2/kg of oil) in Sacha Inchi oil of different formulations (1 to 7), by mixing the antioxidants tocopherol and Ecoprol 2020. (*** p <0.001)**

After determining the adequate formulation of antioxidants (F4) with the lowest PI value in the Sacha Inchi oil, this was used to elaborate nine formulations (F'1 – F'9) by the factorial method, considering different concentration of Sacha Inchi oil (1 to 4%) and salt (Table 2) to prepare the fresh cheese.

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Table 3 shows the microbiological analysis of the fresh cheeses prepared (F'1 – F'9) for 18 days stored between 0- 4 ° C. According to the Peruvian legal parameters for fresh cheese, low rates of molds and yeasts (10 - 10^3 CFU /g), total coliforms (10^2 – 10^3 CFU/g), *Staphylococcus aureus* (10-10^2 CFU/g) and *Salmonella* (absent) should be found. The content of aerobic mesophiles was followed to indicate the degree of contamination experienced by the cheese during the storage period. Thus, low concentrations of aerobes, molds and yeasts (57 x 10 CFU/g and <10 CFU/g, respectively) were found to all formulations. The concentration of aerobes after 18 days was >92 x 10^6 CFU/g, indicating that it is a much higher value than at the beginning of the evaluation. In addition, the number of total coliforms was also below the reference values, as well as the amounts of *Staphylococcus aureus* and *Salmonella sp*. These results suggest that the fresh cheese enriched with oil and antioxidants could preserve the properties required by Peruvian regulation [9]. After 18 days of microbiological analysis of the cheeses fortified with Sacha Inchi oil stabilized with antioxidants displayed higher levels of mesophiles, molds, yeasts, and total coliforms, however, levels of bacteria and Salmonella remained constant. After the period of 18 days, the cheese lost its properties and was rendered unsuitable for consumption. Other studies have shown that the effect of high pressure on storage can extend the shelf life of cheeses from 7 to 14 days in refrigeration [17]. In addition, the modification of the packing atmosphere of the fresh cheeses with nitrogen can also extend the shelf life up to 14 days stored at 4 ° C [18]. Thus, the use of antioxidants in Sacha Inchi could increase the shelf life of cheeses from 7 to 16 days stored in refrigeration. In other words, without the need to modify storage conditions or packaging under specials conditions, a stable product can be obtained and production costs reduced.

Finally, nine formulations of fresh cheese developed through different concentration of antioxidant and Sacha Inchi were evaluated by 100 untrained individuals, to verify which of these formulations would be more acceptable to the public, besides presenting all physical and microbiological characteristics previously reported. **Table 4** shows the acceptance values according to the hedonic scale for nine formulations of fresh cheese prepared from adequate concentrations of salt, antioxidants and Sacha Inchi oil. The optimum formulation (F'5) was determined through the sensorial evaluation, which reached 7.2 according to the 9-hedonic scale. That formulation displayed concentration of Sacha Inchi oil (2.5%) enriched with tocopherol (150 mg/kg of oil) and Ecoprol 2020 (1000 mg/kg of oil), and 22.5 g/L of salt. F'5 was the optimal formulation, because it has a higher percentage of Sacha Inchi compared to F'4, which although its general appreciation was 7.3, its percentage of Sacha Inchi was 1%.

Previous work was performed with Peruvian natural product, tara gun, to extending the shelf-life of fresh cheese sensory acceptability of 8.3 was reported according to 9-hedonic scale with 14 days of shelf-life stored at 4 °C [27]. Furthermore, despite the fact that Sacha Inchi can increase the nutritional properties in fresh cheese with omega-3, -6 and vitamin E, it can also extend its shelf-life [2].

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CONCLUSION

The incorporation of Sacha Inchi oil stabilized with antioxidants into the formulation of fresh cheese was suitable. The optimum percentage Sacha Inchi oil in the preparation of fresh cheese was 2.5%, which contained tocopherol 150 mg/kg of oil and Ecoprol 2020 1000 mg/kg of oil. The salt concentration was 22.5 g/L. This formulation was able to extend the shelf-life of fortified cheese from 7 to 16 days between 0-4 °C. Finally, sensory analysis obtained acceptance of 7.2 (means) in a 9-hedonic scale. This formulation in the preparation of fresh cheese may propose an alternative manufacturing for artisan and industrial producers, to improve the storage conditions.

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Table 1: Proportion of antioxidants tocopherol and Ecoprol 2020 in Sacha Inchi oil (SI)

Formulation	Tocopherol (mg/Kg of oil)	Ecoprol 2020 (mg/Kg of oil)
F1	10	500
F2	10	750
F3	10	1000
F4	150	1000
F5	150	750
F6	300	500
F7	150	500
Control	0	0

Table 2: Formulations for preparation of fresh cheese with Sacha Inchi oil fortified with antioxidants, (1 to 4% oil) and salt

Formulation	Salt (g/L)	% Sacha Inchi Oil
F'1	20.0	4.0
F'2	22.5	4.0
F'3	20.0	1.0
F'4	22.5	1.0
F'5	22.5	2.5
F'6	17.5	1.0
F'7	20.0	2.5
F'8	17.5	2.5
F'9	17.5	4.0
Control	17.5	0.0

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Table 3: Microbiological analysis of fresh cheese (control) versus cheese enriched with Sacha Inchi oil

Sample	Viable Mesophile aerobes (CFU/g)	Molds and yeasts (CFU /g)	Total coliforms (NMP/g)	*Staphylococcus aureus*(CFU/g)	*Salmonella sp*. in 25 g
Control	not evaluated	not evaluated	5×10^2	$10 - 10^2$	Absent
Cheese with oil fortified	57 x 10	not evaluated	90	<10	Absent
After 18 days	$>92 \times 10^6$	<10	$>11 \times 10^2$	<10	Absent

Table 4: **Sensorial analysis of fresh cheese with Sacha Inchi oil**

Formulation	General appreciation (GA)
F'1	6.7
F'2	6.7
F'3	7.0
F'4	7.3
F'5	7.2
F'6	6.2
F'7	6.3
F'8	6.0
F'9	5.4
Control	5.4

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