Blueberry-based gummies with partial substitution of unflavored gelatin for cushuro (*Nostoc commune* Vauch.) flour

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Abstract*– *Cushuro (Nostoc commune Vauch.) is an alga resistant to adverse weather conditions and has been consumed since ancient times. However, in recent years, more research has been carried out to revalue this product since it has beneficial functional and nutritional characteristics. In the present study, cushuro and blueberry (Vaccinium myrtillus Var. Biloxi) pulp were proposed as an alternative to improve gummies, a traditional and highly consumed treat. For this, partial substitutions of unflavored gelatin for cushuro flour were performed at 35% (T1), 45% (T2), 55% (T3). As a result, it was obtained that the higher the percentage of cushuro used, the protein and carbohydrate content increased reaching values of 3.85 ± 0.03 % and 91.58 ± 0.64% in dry basis, and the fat content decreased until 4.13 ± 1.15% in dry basis. On the other hand, by using cushuro flour and blueberry pulp, the antioxidant capacity of the gummies was increased (reaching > 60% inhibition of the DPPH radical). Finally, changes in the texture and color of the gummies were found by using cushuro flour. In conclusion, the use of raw materials little-explored can present a great alternative to improve the quality of traditional gummies.

Keywords-- Nostoc commune Vauch., gummies, algae, cushuro flour, nutritional properties.

I. Introduction

Traditional edible jelly beans or gummies are candies with confectionery gelling agents, which are identified as being highly concentrated carbohydrate solutions, containing acids, colorants, flavorings, texturizing and stabilizing agents [1]. These products use a gelling agent such as gelatin (hydrolysate collagen) that gives it its characteristic elastic texture, which allows deformation and recovery of its shape by putting pressure on any part of the product. The demand for this type of product is increasingly growing, mainly in children and adolescents. However, the traditional composition does not make these products attractive from a nutritional point of view as they contain a high content of sugars, colorants and flavorings which trigger malnutrition problems in the population. That is why both academia, industry and consumers are looking for alternatives to produce, obtain and consume healthier products.

Cushuro (*Nostoc commune* Vauch.) is a raw material that, although consumed since ancient times, has recently been studied and valued [2-4]. The cushuro corresponds to the genus *Nostoc*, a type of algae (cyanobacteria) that prevails for millions of years due to its ability to adapt to changes. A great diversity of cyanobacteria have been found in places with extreme climates from deserts to colder parts of the world [5]. So far 70 species have been discovered with taxonomic information (Chacova et, 2007). The *Nostoc Commune* Vauch. form colonies that in their natural environment resist dryness and can easily restore their metabolism after being rehydrated because cells produce large amounts of protective polysaccharides [6]. Cushuro has an attractive nutritional content, Table I shows the composition in 100 g of dehydrated *Nostoc* [7]. In addition, *Nostoc* is source of bioactive peptides [5]. Therefore, cushuro is a source of valuable nutrients such as protein, calcium and iron as well as bioactive compounds.

TABLE I
COMPOSITION OF DEHYDRATED CUSHURO PER 100 G OF SAMPLE

Compound	**Content**	**Unit**
Water	15.1	g
Protein	29	g
Carbohydrate (total)	46.9	g
Fat (total)	0.5	g
Ash	8.5	g
Calcium	147	mg
Phosphorus	64	mg
Iron	83.6	mg
Thiamin	0.20	mg
Riboflavin	0.41	mg

Source: Tablas peruanas de composición de los alimentos [7].

Considering the above, the aim of the present work was to evaluate the effect of the partial substitution of unflavored

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gelatin for cushuro flour (*Nostoc commune* Vauch.) on the nutritional characteristics (proteins, ashes, fats, carbohydrates, calories and antioxidant capacity) and physical (instrumental color and texture) of blueberry (*Vaccinium myrtillus* Var. Biloxi) based edible gummies.

II. MATERIAL AND METHODS

A. Raw material

Cushuro (*Nostoc commune* Vauch.) (Fig. 1) was obtained from the Chinchaycocha lagoon, Mantaro Valley, Junín, Peru, in this study it was used 10 kg of cushuro. In addition, 5 kg of blueberry (*Vaccinium myrtillus* Var. Biloxi) obtained from Trujillo, Peru, was used.

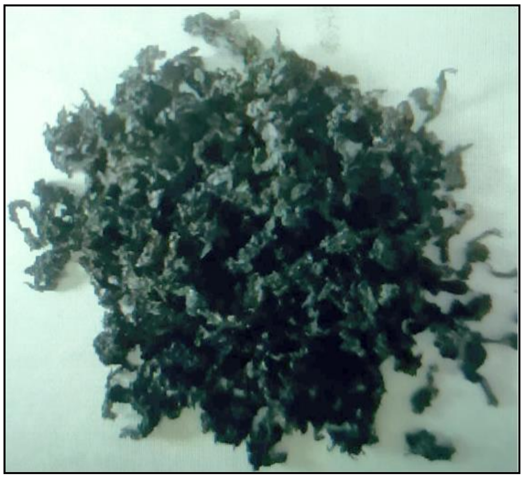

Fig. 1 Sample of dry cushuro (*Nostoc commune* Vauch.)

B. Obtaining cushuro flour

Algae free of fungi or other contaminants were selected, the algae with signs of decomposition were discarded. The selected algae were subjected to a washing process using potable water and chlorine at a concentration of 50 mg/kg.

The drying was carried out on metal trays using an oven at 40 °C until reaching constant humidity. Once the material was dry, it was crushed and hydrated in a 1:30 (alga:water) ratio.

The mixture was cooked at 90 °C for 3 hours with constant stirring. Subsequently, the mixture was poured into buckets and they were frozen at -45 °C. The trays containing the frozen mixture were placed in the freeze-drying equipment (Labconco, Modelo 7806030, Serie 120558491D). The lyophilisate presented 9.47 ± 1% moisture content and was stored in hermetic containers stored at low humidity and protected from light. In addition, the gel force of cushuro flour was measured, obtaining 0.75 ± 0.01 N.

C. Obtaining blueberry pulp

The blueberry was selected discarding the green fruit, the excessively ripe and those that presented bumps and rottenness. The selected fruit was subjected to a washing process using a chlorine water solution at a concentration of 50 mg/kg.

The fruit was placed in water at a temperature of 60 °C for 20 minutes, to facilitate the extraction of the pulp and its juice. Subsequently, the blanched blueberry was placed in an industrial pulper until the pulp was extracted. The remains of seeds and shells were separated from the pulp. It was pasteurized at 90 °C for 5 min, finally, the pulp was stored in a disinfected and hermetically sealed container, which was refrigerated for later use. The initial characteristics of blueberry pulp were pH of 3.98 ± 0.01, instrumental color values of L* (24.32 ± 0.15), a* (2.32 ± 0.15), b* (-0.27 ± 0.032), C* (2.34 ± 0.14) and ΔE (2.96 ± 0.11), and 73.46 ± 0.39% of DPPH inhibition.

D. Gummies elaboration

Gummies formulations consist of sugar 31.77%, glucose 38.64%, blueberry pulp 12.74%, water 11.16%, citric acid 0.11% and unflavored gelatin 5.58%. Where, on the amount of gelatin, the partial substitution with cushuro flour was carried out considering 35%, 45% and 55% substitution. Therefore, the amounts of the other ingredients, except for the unflavored gelatin, were kept constant.

To elaborate the gummies, the blueberry pulp was mixed with the sugar and glucose, cooking until it reached 110 °C, then it was cooled to 60 °C while maintaining constant stirring. On the other hand, the unflavored gelatin and lyophilized cushuro flour were weighed according to the formulations proposed (35%, 45% and 55% substitution), it was hydrated with the water and with constant stirring it was heated (45-50 °C). Subsequently, the blueberry jelly was mixed with the paste (containing the different formulation of unflavored gelatin and cushuro flour) and citric acid until its complete homogenization, later it was brought to 95 °C. The mixture was poured into molds, allowed to cool at room temperature for 2 to 3 h until completely hardened, subsequently unmold, and were stored in 100g samples.

Therefore, gummies with different percentages of cushuro flour were obtained, hereinafter called T1 (treatment with 35% substitution), T2 (treatment with 45% substitution) and T3 (treatment with 55% substitution).

E. Proximal analysis and antioxidant activity

1) Protein determination: It was carried out by the Kjeldahl method according to N.T.P. 205.005/79. For this, 1-2 g of sample was weighed and 10 mL of H_2SO_4 (98%) with 8 g of catalyst were added. The digestion was carried out in three steps, with a gradual increase in temperature from (150 °C to 400 °C), until a translucent liquid was obtained. The digestion was carried out in three steps, with a gradual increase in temperature from (150 °C to 400 °C), until a translucent liquid was obtained. Once the process was complete, it was cooled, and 15 mL of distilled water was added slowly. Subsequently, the distillation stage was carried out by adding NaOH and using boric acid in the collected liquid, then % of proteins was calculated.

2) Ash determination: It was carried out by the calcination of organic matter according to Reyes Sánchez and Mendieta Araica [8]. For this, 2 g of sample was weighed into crucibles, they were placed inside a muffle at 600 °C for two hours. Subsequently, it was cooled in desiccators and the weights were recorded, then percentage of ash was calculated.

3) Fat determination: Soxhlet equipment according to NTP 205.006/80 was used. For this, 3g of sample was weighed into filter cartridges, then, the moisture was removed. Subsequently, the samples were placed in the siphon of the equipment, it was used two siphons of petroleum ether and left at 80 °C for 4 hours. Subsequently, it was cooled, and the percentage of fat was calculated.

4) Moisture determination: The gravimetric technique was used according to Reyes Sánchez and Mendieta Araica [8].

5) *Determination of carbohydrates:* The carbohydrate percentage (C%) was determined by difference concerning the content in percentage of protein, water, fat and ash [9] according to the following equation.

$$C(\%) = 100 - (\%protein + \%water + \%fat + \%ash)$$

6) Determination of antioxidant capacity (AC): It was determined following the method of decolorization of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) described by Brand-Williams, et al. [10], which is based on the radical absorbance reduction measured at 517 nm. The result was expressed as inhibition % of the DPPH radical.

F. Physical characteristics: texture and instrumental color

1) Determination of texture: It was performed according to Casas-Alencáster and Pardo-García [11] by compression test using a INSTRON texturometer, Model 3342 coupled to 3.5 mm diameter probe, which crossed the samples (1 cm height) at a speed of 1 mm/s.

2) Color determination: The instrumental color of samples was measured using a Konica Minolta colorimeter, model CR-400 according to Cano-Lamadrid, et al. [12]. The CIE (Commission Internationale *d'Eclairage*) color scale was used, where parameters of L* (Lightness), a* (green to red) and b* (blue to yellow), C* (Saturation) and ΔE (total color difference) were measured.

G. Experimental design and statistical analyses

A completely randomized design (CRD) was conducted. All analyses were performed at least three times. The one-way ANOVA was used, all were carried out with a significance level of 5%. To determine statistical differences among means of treatments, Tukey test was used. Statistical analyses were determined using the IBM SPSS Statistics 23 software (IBM SPSS, USA).

III. RESULTS AND DISCUSSION

A. Effect of substitution on the proximal composition and antioxidant activity of gummies

After the gummies were obtained, the moisture was determined for each treatment. The moisture in wet basis (g/100 g sample) was 12.24 ± 1.21%, 13.43 ± 2.64%, and 10.15 ± 1.11% for treatments T1, T2 and T3, respectively. As observed, moisture values were slightly different among treatments. Therefore, to avoid that this amount of water contained in the samples affects the interpretation of results, the quantity of proteins, ashes, fats, and carbohydrates were calculated on a dry basis, that is, in grams per 100 grams of dry matter (g/100 g d.m) (Table II).

In Table II is observed that as the substitution percentage increase, the gummies composition was modified. The protein and carbohydrate content increases, the fat content decreases, and ash content did not change. Cushuro is composed mainly of water (~ 98%) and the other two largest compounds are proteins (from 34% to 47.71% in dry basis), and Carbohydrates (~ 34.4% in dry basis) [7, 13, 14]. Therefore, the increase of protein and carbohydrates as the amount of used cushuro increased makes sense.

TABLE II
GUMMIES COMPOSITION FOR THE DIFFERENT SUBSTITUTION TREATMENTS. DIFFERENT SUPERSCRIPT LETTERS IN THE COLUMNS INDICATE SIGNIFICANT DIFFERENCES (P < 0.05) AMONG TREATMENTS.

Treatment	Gummies composition (g/100 g d.m)			
	Proteins (%)	Ash (%)	Fat (%)	Carbohydrates (%)
T1 (35% substitution)	3.58 ± 0.01^{c}	0.17 ± 0.12^{a}	11.43 ± 0.96^{a}	84.83 ± 0.31^{b}
T2 (45% substitution)	3.67 ± 0.01^{b}	0.09 ± 0.11^{a}	5.64 ± 0.20^{b}	90.59 ± 3.15^{a}
T3 (55% substitution)	3.85 ± 0.03^{a}	0.44 ± 0.58^{a}	4.13 ± 1.15^{b}	91.58 ± 0.64^{a}

On the other hand, fat content decrease at a higher percentage of cushuro substitution. According to Reyes García, et al. [7] cushuro fat content is about 0.6% in dry basis, being a low value. Therefore, as the unflavored gelatin was replaced by cushuro, the amount of fat in the gummies was decreased. Finally, there was no difference in ash (consisting mainly of minerals, salts and inorganic compounds). In fact, cushuro contains mostly minerals such as iron, calcium, and phosphorus [15]. Therefore, probably the ash levels in both unflavored gelatin and cushuro are equivalent resulting in no significant changes with the different substitutions.

In addition, Tafur Medina and Obregón Dionicio [3] elaborated functional gummies with cushuro. Compared to traditional products, which provide mostly carbohydrates, the gummies that include cushuro cover the daily requirements of schoolchildren regarding protein (13,53%) and iron (34,25%). In fact, by considering the caloric value of protein (4 kcal/g), fat (9 kcal/g) and carbohydrates (4 kcal/g) [16], the composition showed in Table II result in 456.46 ± 9.90 kcal, 427.85 ± 14.49 kcal, 418.87 ± 13.00 kcal for treatments T1, T2 and T3, respectively. Therefore, from the nutritional point of view, the inclusion of cushuro to elaborate gummies is a good alternative to improve the quality of traditional gummies, helping to reduce malnutrition, especially in children.

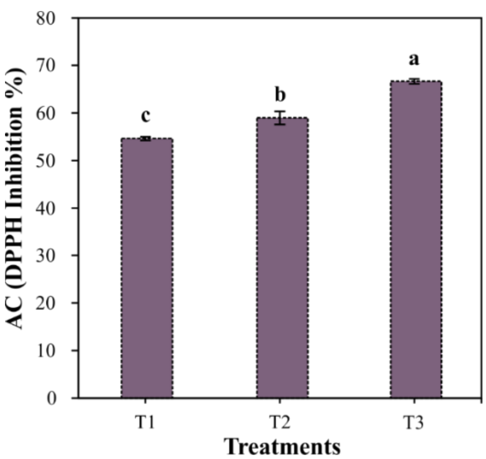

Fig. 2 Antioxidant capacity (AC, expressed as DPPH inhibition %) of gummies with the different substitution treatments. *different letters indicate significant differences (p<0.05) among treatments.

Fig. 2 shows the antioxidant capacity of gummies, as the percentage of substitution increases, an increase in the antioxidant capacity is observed in the gummies. In fact, in previous studies, it was demonstrated that cushuro (*Nostoc commune* Vauch.) compared to other algae (such *Nostoc flagelliforme, Nostoc sphaeroides Kütz, Spirulina platensis, Chlorella pyrenoidosa* and *Dunaliella salina*) presented stronger antioxidative activity, being its O^{2+} radical scavenging capacity more than 54.8% higher [4, 17]. In addition, the water-soluble polysaccharides of *Nostoc commune* showed high hydroxyl radical scavenging activity (92.71%) and reducing capacity (0.445) at the concentration of 10 mg/mL (Wang, 2014). Not only the cushuro contributes to the antioxidant capacity, but also the blueberry pulp used in the production of the gummies, whose antioxidant capacity was 73.46 ± 0.39% of DPPH inhibition. Therefore, the incorporation of cushuro in blueberry-based gummies is beneficial from the point of view of increasing their antioxidant capacity, reaching levels greater than 60% of DPPH inhibition (T3).

B. Effect of substitution on the physical characteristics: texture and color of gummies

Figure 3 shows that as the percentage of cushuro increases, there is a decrease in the compression force (firmness indicator) in the gummies. The firmness of the gummies is directly dependent on the amount of gelatin used.

Since the amount of gelatin decreased to 3.63%, 3.07% and 2.51% with the substitution treatments T1, T2 and T3 respectively, a gradual decrease in hardness is to be expected, as observed in Fig. 3. According to Pang, et al. [18] at low gelatin concentrations (< 2.5%) the gel formed is soft increasing fracturability. Probably, this could be improved if only hydrocolloids extracted from cushuro were used, in fact, the rheological properties of hydrocolloids extracted from cushuro have been compared with other thickeners such as carboxymethylcellulose [2]. In addition, the ultrasound-assisted extract of *Nostoc commune* Vauch. polysaccharides (UAP) microcapsules with alginate/chitosan as packaging materials were tested by texture profile analysis. Through measurement, UAP microcapsules displayed hardness (1.33 ± 0.299 N), springiness (0.07 ± 0.0383 mm), and adhesiveness (0.0258 ± 0.00744 mJ) [19]. Results in this study show that the gelling power of cushuro flour is not comparable to the gelling power of gelatin.

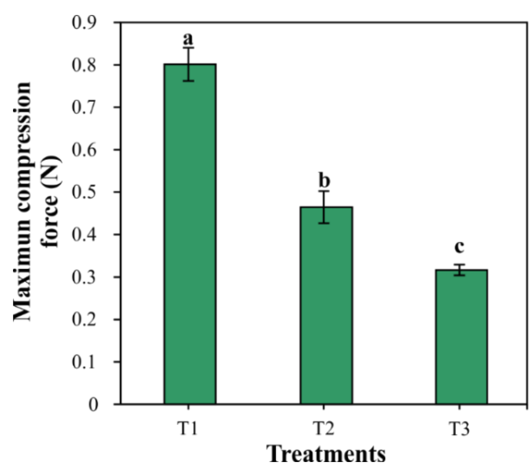

Fig. 3 Compression force (N) of gummies with the different substitution treatments. *different letters indicate significant differences (p<0.05) among treatments.

As the percentage of substitution increases, a decrease in the lightness (L*) value, a decrease of a* value, and a decrease in the value of b* were observed (Table III). It means that the addition of cushuro, rich in pigments such as chlorophyll [20], changed the color of gummies.

Therefore, the lightness (L*) decreased with pre-treatments being more pronounced in samples with 55% substitution (T3) (Table III). The L* value is correlated with the characteristics of the sample surface where more colored samples absorbed more light, and then decreasing the L* values. Regarding the a* parameter, the a* value decrease with treatments. This is expected, once a decrease in a* means a change towards greenish colors, due to cushuro pigments.

On the other hand, the b* values increased with cushuro addition, which means the color of the gummies tends towards more bluish colors. Probably not only the cushuro pigments but the combination with the blueberry pigments influenced the observed color changes. Regarding the C* value, it indicates the color saturation level of gummies, the treatment with higher saturation was the T3 (55% substitution). In addition, the total color difference (ΔE) values indicate how different the color of the samples is compared to the standard, the samples with a more noticeable difference are the samples from the T3 treatment containing the highest substitution percentage.

TABLE III
GUMMIES COLOR PARAMETERS FOR THE DIFFERENT SUBSTITUTION TREATMENTS. DIFFERENT SUPERSCRIPT LETTERS IN THE COLUMNS INDICATE SIGNIFICANT DIFFERENCES ($P < 0.05$) AMONG TREATMENTS.

Color parameters	Standard	Treatments		
		T1 (35% substitution)	T2 (45% substitution)	T3 (55% substitution)
L*	33.87	33.71 ± 0.09 ^{a}	33.71 ± 0.09 ^{a}	28.48 ± 0.94 ^{b}
a*	0.8	0.85 ± 0.03 ^{a}	0.45 ± 0.01 ^{c}	0.70 ± 0.03 ^{b}
b*	0.02	0.01 ± 0.00 ^{b}	0.12 ± 0.02 ^{b}	0.77 ± 0.08 ^{a}
C*	0.8	0.85 ± 0.03 ^{b}	0.46 ± 0.02 ^{c}	1.04 ± 0.04 ^{a}
ΔE	1.8	1.85 ± 0.03 ^{b}	1.43 ± 0.03 ^{c}	2.24 ± 0.04 ^{a}

IV. CONCLUSION

It was studied the effect of the partial substitution of unflavored gelatin for cushuro flour in blueberry-based gummies. Substitution treatments were studied at 35% (T1), 45% (T2) and 55% (T3) on the proximal composition, antioxidant activity, and physical characteristics (texture and instrumental color). Among the most outstanding results, it was found that the protein content increases from 3.58 to 3.85% for treatments T1 and T3, respectively. Similarly, an increase in carbohydrate content was observed and, conversely, a decrease in the amount of fat. The antioxidant capacity increased proportionally with the percentage of cushuro flour used. A decrease in firmness was observed with an increase in the percentage of cushuro, as well as changes in the instrumental color due to the pigments present in the cushuro flour. Finally, the use of cushuro flour and cranberry paste showed an improvement in the nutritional characteristics, while regarding the physical characteristics it is recommended to use less than 45% of cushuro flour to avoid possible negative effects on the texture and color of the gummies.

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