ASSESSMENT OF UTILIZING ANNATTO SEEDS POWDER AS A NATURAL FOOD INGREDIENT FOR CHEDDAR CHEESE

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Abstract

Food products with desirable sensory qualities, safety, and nutrition are in high demand in industrialized countries. Annatto (*Bixa orellana L.*) is a small tree cultivated in tropical and subtropical America and is appreciated for the pigmented seeds that come from which the yellow-orange achiote or annatto natural food color is made. This study investigated the effect of the annatto seeds powder on the phytochemical and physicochemical properties as well as the textural and color of the value-added cheddar cheese. Two types of cheddar cheese were produced containing different concentrations of annatto seeds powder, 0.25 and 0.5% (ASP0.25, ASP0.5), and a control (ASP0). Based on the results, annatto seed powder appeared to show good antioxidant activity (79.432 ±1.015%). The addition of annatto seed powder positively influenced the textural and color characteristics of the value-added cheese. The obtained samples highlighted the satisfactory content in phytochemicals. The sensory analysis showed that the addition of annatto seed powder had no detrimental effects on the cheese's general acceptance, the improved color being appreciated. Therefore, annatto seed powder might be a good source of natural antioxidants for the production of dairy products being a natural alternative to synthetic food coloring ingredients.

Key words: Annatto seeds, antioxidant activity, pigments, food ingredients, cheddar cheese

One of the most significant aspects of food product that affects consumer preference, taste perception, and ultimately purchasing decision is color (Sukkwai S. *et al*, 2018).

The pericarp of the seeds of the tropical tree *Bixa orellana L*. (Bixacea), which is native to the woods of Central and South America, is used to produce the red-orange-yellow natural colorant known as annatto (achiote). It is widely grown throughout the world's tropical regions, particularly in Mexico, Colombia, Ecuador, and the Peruvian Andes. The name "annatto" refers to the crude pigment extract derived from achiote that contains bixin, norbixin, and other carotenoids in varying amounts. Achote is now one of the most intriguing plant sources of vegetable colorants due to the possibility of acquiring both water-soluble (norbixin) and oil-soluble (bixin) colorants depending on the kind of extraction, the solvent, and the temperature utilized (Smith J., 2006).

The seeds contain 4.5–5.5% pigment, which about 80% of the total carotenoids in the color made from achiote are represented by bixin. Norbixin, bixin dimethyl ester, and by-products of lycopene breakdown are additional carotenoids that are also present but at smaller levels. The carotenoid 9′-cis-bixin is the main coloring agent in the oily soluble annatto extract, while the carotenoid 9′-cis-norbixin is the main coloring agent in the alkaline aqueous annatto extract (Viuda M. *et al*, 2012). According to research by Chiste R.C. *et al* (2011), achiote includes significant levels of tocotrienols, tocopherols (vitamin E), terpenes, and flavonoids in both the seeds and the leaves.

Due to the emergence of degenerative diseases, the use of some artificial food colors, (carmoisine, Ponceau 4R), has been prohibited in the USA and Europe. Instead, the use of natural colorants, such as the dye that comes from the surface of the seeds of *B. orellana L.* (E 160b, annatto extract), has been suggested. Because it doesn't change flavor and is mostly non-toxic, annatto extract has a significant economic impact on the entire world and is one of the natural colorants used most frequently in the food, cosmetic, and pharmaceutical industries (Lourido P.H., Martınez S.G., 2010).

Some bioactive substances extracted from annatto seeds have demonstrated antioxidant and antibacterial properties of special significance for food product manufacturing. In fact, because of their coloring ability, these extracts have been used in food matrices like dairy, meat, and baked goods.

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Many different food products, including butter, margarine, cheese, beverages (soft drinks and juices), ice creams, poultry, breakfast cereals, several desserts and confectionary, use annatto seeds as a natural color (Zhang *et al*., 2018). Researchers reported that 0.065 mg/kg body weight/day of bixin is an appropriate consumption for people and that feeding experimental animal diets high in bixin and norbixin did not have any harmful or carcinogenic effects (Lauro G., Francis J., 2000).

The aim of the present study was to obtain value-added cheddar cheese. The cheese's physicochemical, phytochemical and color qualities, as well as their textural and sensory analyses, were examined in order to show the added value of the products.

MATERIAL AND METHOD

Material and method. The cow's milk (200 L) was donated by the University of Life Sciences' Rediu Iași Research Station. Annatto seeds (moisture content of 10.11±0.87 %) were ground using a blender (Blender Nutribullet Original). Hexane, acetone, ethanol, methanol, sodium carbonate, 2,2-diphenyl1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, gallic acid solution, sodium hydroxide were purchased from Sigma Aldrich (Schnelldorf, Germany).

Extraction of bioactives from annatto seeds powder (ASP). The ultrasound-assisted extraction method described by Quintero Quiroz J. *et al* (2019) was utilized to extract the bioactive components from ASP with a few minor modifications. 1.0 g of ASP were mixed with 10 mL of a solvent mixture of 3:1 n-hexane:acetone or 70% ethanol (for the extraction of total polyphenols) before being treated with an ultrasound bath (Elmasonic S 180 H, Elma, Germany) for 30 minutes at 25°C and a frequency of 37 kHz. The resulting extract was then recovered, and it was centrifuged for 10 minutes at 6000 rpm and 10°C. After that, the ASP supernatant was examined for total carotenoids, total flavonoids, and total polyphenols.

The determination of carotenoids, phenolic compounds and antioxidant activity of annatto seeds powder (ASP) extract.

Total carotenoid content. Spectrophotometric analysis was used to measure and determine the total carotenoids concentration as described by Mihalcea *et al*. (2018). A UV–VIS Spectrophotometer, Analytik Jena - Specord 210 Plus, Germany was used to measure the absorbance at 450 nm for total carotenoids content of the extract. The results were reported as mg/100 g of dry weight (d.w.).

Total flavonoid content. The total flavonoid content values of ASP extract were determined using the aluminum chloride spectrophotometric technique (Dewanto V. *et al,* 2002). Briefly, 0.075 mL of 5% sodium nitrite ($NaNO_2$) and 0.25 mL of the extract were combined with 2 mL of distilled water. 0.15 mL of aluminum chloride (AlCl3) was added to the mixture after 5 minutes. 0.5 mL of sodium hydroxide (NaOH) 1 M was added to the mixture after 6 minutes, and the mixture was then measured at 510 nm. A calibration curve for catechin as a standard was employed and the results were expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g d.w.).

Total polyphenolic content. The Folin-Ciocalteu method was used to spectrophotometrically measure the ASP extract total polyphenolic contents (Cheok *et al*. 2013). The Folin-Ciocalteau reagent, 15.8 mL of distilled water, and 200 µL of the extract were carefully combined. 3 mL of NaCO3 20% was added to the mixture after 10 minutes. After 60 minutes of dark storage at room temperature, the resultant combination was measured at 765 nm. The results were expressed as milligrams of Gallic acid equivalents per gram of dry weight (mg GAE/g d.w.) and a standard curve for Gallic acid was used.

Antioxidant activity (DPPH). The DPPH technique was used to measure the antioxidant activity, and the results were expressed as µmol of Trolox equivalents per gram of dry weight (µmol TE/g d.w.) (Castro-Vargas H.I. *et al*, 2010). A calibration curve utilizing Trolox as standard was applied. The samples were prepared by mixing 0.10 mL of each extract with 3.90 mL of 0.1 M DPPH solution. The solutions were then left at room temperature in the dark for 30 minutes before the absorbances (Af) were measured. Instead of the extract (A0), the blank absorbance was measured at 515 nm using a 3.9 mL DPPH solution 0.1 M (in methanol) and 0.10 mL methanol. Also, the inhibition percentage was calculated. % Inhibition = (A0- Af)/A0 x 100.

Raw milk collecting, sampling and analysis.

200 L of milk were taken out of the farm's storage tank in sterile containers. It was refrigerated at 4°C for 24 hours. Milk was then completely homogenized and added to the analytical laboratory investigations.

The physicochemical parameters of milk samples (moisture content, solid non-fat content, total solid, fat content, protein content, and pH) were determined in according with methods of AOAC.

Cheddar cheese manufacturing. After being standardized to have a protein to fat ratio of 0.70:1, pasteurized at 65°C for 60 minutes, cooled to 30°C, and inoculated with lactic bacteria (*Str. Lactis, Str. Cremoris*). The raw cow's milk was then added calcium chloride solution and allowed to stand for 30 minutes. After two minutes, milk was mixed with liquid rennet (Chymax Plus).

After cutting the coagulum, the curd/whey mixture was left to heal for 10 minutes before being continually churned. In a 40-minute period, the

curd was heated from 38 to 42°C. The acidity of whey should not exceed 19-20ºT. The cedarization stage is carried out when the acidity of the whey approaches 60-70ºT with a pH 4.6-6.5. The curd was separated into three batches, each of which received ASPs and 0.15 kg dry salting. Cheeses were prepressed at 0.13 kPa for 30 minutes after 20 minutes of mellowing, then overnight at 2.5 kPa. At an interval of about 2 hours during the pressing, the cheeses were twisting. The temperature of the room where the pressing takes place must be between 25-27°C. The cheese is to be subjected to drying at temperatures of 12-14°C, for 7-10 days, humidity 85%. Ripening takes place at temperatures between 2-6°C in spaces free of foreign odors and the relative humidity of the air must reach 80-90% for 90-110 days. At intervals of 2 days each piece of cheese is turned. The cheese pieces are stored at a temperature between 2-4°C until being analyzed.

Sensory, physical and chemical analyses were performed with regard to the part of the qualitative analyses carried out to establish the qualitative parameters of the samples obtained with the addition (cheeses with annatto seeds powder (ASP) - ASP0.25– cheese with 0.25% ASP, ASP0.50– cheese with 0.5% ASP) and the control sample (ASP0).

Texture analysis. Using a texturometer with a digital dynamometer of 25N, the Mark 10 ESM 300 texturometer (Mark-10 Inc., USA) was used to analyze the texture of cheddar cheeses samples by the Texture Profile Analysis (TPA) Method. The data were recorded and processed utilizing the TexturePro CT V1.5 software. For each sample, four tests were performed.

Color analysis. The color of samples was determined using the Konica Minolta Chroma Meter CR-410 (Konica Minolta, Osaka, Japan) with a CIE Lab scale against a white standard. Color coordinates. were reported as lightness (L*, from 0 = black, to 100 = white), redness (a*, from red = +a, to green = -a) and yellowness (b*, from yellow = +b, to blue = -b).

Sensorial analysis. The seven attributes scale, which was based on unit numbers, was used to evaluate the value-added cheeses samples. Color, external appearance, scent, flavor, consistency, aftertaste, and overall acceptability are the evaluated attributes. A panel of 10 different panelists conducted a sensory assessment at 20 °C, in white light, and 45–47% relative air humidity.

Statistical Analysis. Using Minitab 17 software, the outcomes of each analysis were statistically examined in duplicate. To evaluate the variations between the samples, a one-way ANOVA and Tukey test was applied. The mean (n = 3) ±SD was used to express all experimental results.

RESULTS AND DISCUSSIONS

The phytochemical content and antioxidant activity of the ASP extract were determined and the results are displayed in *Table 1*

Table 1

Phytochemical content of the ASP extract

Parameters	***Sample ASP***
Total carotenoids (mg/100g d.w.)	14.205±0.622
Total flavonoids (mg CE/g d.w.)	2.285±0.028
Total polyphenols (mg GAE/g d.w.)	3.960±0.055
DPPH (µmol TE/g d.w.)	12.158±0.102
Inhibition (DPPH) %	79.432 ±1.015

Table 1 shows that ASP extract had a high carotenoid content of 14.205±0.622 mg/100g d.w. and antioxidant activity of 12.158±0.102 µmol TE/g d.w. Our results comply with other studies, that reported a total polyphenol content of 3.81 mg GAE/g d.w. (Quintero Quiroz *et al*. 2019) after the ultrasound assisted extraction of ASP bioactives with ethanol 96% for 20 minutes treatment time. The extracts from the seeds of annatto appear to be remarkable due to their coloring capacity and antioxidant activity.

Chemical composition of raw cow's milk.

In order to establish the quality parameters for the raw material milk, the main quality indices were determined. Results of chemical composition of cow's milk samples are in *table 2*.

Table 2

Chemical composition of raw cow's milk samples

Parameters	***Mean***
Water (%)	87.17±0.07
Total Solids (%)	12.83±0.08
Fat (%)	3.98±0.02
Protein (%)	3.25±0.04
Solid-non fat (%)	8.86±0.06
pH	6.59±0.01

Regarding the water content, it had an average value of 87.12±0.094% and that of total solids was 12.89±0.094%. Milk quality indices meet the conditions of freshness, safety, and general milk quality.

The phytochemical profile and antioxidant activity of the obtained samples are shown in *table 3*. The obtained food products were analyzed in terms of the global phytochemical profile.

Table 3

Phytochemical characterization and storage stability of added-value cheese samples

Parameters	***Storage time(days)***	***Type of cheddar cheeses***	
		ASP0.25	**ASP0.50**
Total carotenoids (mg/100g d.w.)	0	8.22±0.18[a]	10.19±0.20[b]
	110	8.62±1.11[a]	12.01±1.28[b]
Total flavonoids (mg CE/g d.w.)	0	3.28±0.20[a]	4.83±0.24[a]
	110	3.45±1.13[a]	6.63±1.14[b]
Total polyphenols (mg GAE/g d.w.)	0	5.03±0.15[a]	6.07±0.17[a]
	110	5.85±1.78[a]	9.75±1.88[b]
DPPH (µmol TE/g d.w.)	0	5.75±0.14[a]	7.22±0.19[b]
	110	5.92±1.79[a]	9.88±1.98[b]

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

As expected, the amount of powder added correlates with the differences in bioactives and antioxidant activity between the two samples. The phytochemicals content and antioxidant activity was higher in cheese with 0.50% addition. The obtained samples highlighted the satisfactory content in phytochemicals. During the storage period, the phytochemicals increased until the end of the ripening for the ASP0.50 sample while for the ASP0.25 sample the values were constant. The phytochemicals content of all cheeses increased during the ripening phase, which is consistent with the findings of Rashidinejad A. *et al* (2013), who investigated the influence of catechin addition on the phenolic content and antioxidant capabilities of low-fat cheese. Reduced analytical precision and the presence of milk-derived compounds may be responsible for the increased phenolics values.

Chemical composition of value-added cheddar cheese. The chemical composition of the prepared Cheddar cheeses is described in *table 4*.

Table 4

Chemical composition added-value cheese samples

Parameters	***Type of cheddar cheeses***	
	ASP0.25	**ASP0.50**
Water (%)	36.22±0.18[a]	36.19±0.18[a]
Dry mattter (%)	63.78±0.18[a]	63.81±0.19[a]
Fat (%)	40.03±0.03[a]	39.07±0.03[a]
Fat in dry matter (%)	62.77±0.16[a]	61.23±0.15[a]
Protein (%)	23.86±0.04[a]	25.81±0.04[b]
Ash (%)	4.24±0.08[a]	6.11±0.09[b]
Salt(%)	2.08±0.06[a]	2.13±0.03[a]

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

Table 5

Texture of value-added cheese samples

Component	***Type of cheddar cheeses***	
	ASP0.25	**ASP0.50**
Cohesiveness	0.275 ± 0.01[a]	0.655 ± 0.01[b]
Springiness, mm	0.50± 0.01[a]	1.06 ± 0.01[b]
Hardness, N	65.80 ± 0.02[a]	79.70 ± 0.04[b]
Gumminess, N	0.43 ± 0.01[a]	0.54 ± 0.02[b]
Chewiness, mJ	4.97± 0.03[a]	5.76± 0.04[b]

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

The analysis of the obtained media indicates differences between the sample with de addition of powder obtained from annatto seeds for protein and ash parameters. The other parameters that were not influenced by the addition of ASP used by us was fat, dry matter, fat in dry matter and salt.

With the addition of more annatto powder, the ash content showed a significant increase, increasing from 4.24% in the case of the sample

with an addition of 0.25% to 6.11% in the case of the sample with an addition of 0.50%.

Value-added cheddar cheeses texture analysis. In order to estimate the effect of ASP over the texture of cheddar cheeses, the hardness, cohesiveness, springiness, gumminess and chewiness were tested using the Texture Profile Analysis method. The results of these parameters are presented in *table 5*. Cheese texture is an extremely important attribute that results from a combination of physical properties, perceived with the help of the tactile senses. Hardness, described as the maximum force needed to compress the samples in the first cycle, was between 65.80 ± 0.02N for ASP0.25 and 79.70 ± 0.04N for ASP0.50. The ASP0.50 sample displayed the greatest values for cohesiveness and springiness, which are measured as the difference in deformation between the two compression cycles and measured as the ratio between the samples' resistances during the second and first compression, respectively. The energy needed to break down the food during mastication is known as chewiness, and it increased from 4.97± 0.03 mJ for ASP0.25 and 5.76± 0.04 mJ for ASP0.50.

Color evaluation of value-added cheddar cheese samples. A food's color is a crucial physical characteristic that influences the consumer's preference. The samples were analyzed in terms of CIELAB colorimetric parameters. The terms L* (brightness), a* (trend towards red or green), and b* (trend towards yellow or blue) were used to express the results. Color data of the value added cheeses where shown in *table 6*.

Table 6

Color data of ASP0.25 and ASP0.5 cheeses

Parameters	***Storage time(days)***	***Type of cheddar cheeses***	
		ASP0.25	**ASP0.50**
L*	0	71.43±0.05[a]	68.59±0.16[b]
	110	70.62±0.11[a]	67.21±0.28[b]
a*	0	11.86±0.02[a]	14.16±0.02[a]
	110	12.45±0.13[a]	15.63±0.14[b]
b*	0	47.80±0.01[a]	49.73±0.03[b]
	110	48.92±0.79[a]	50.88±0.98[b]

For each type of cheese, letters indicate a comparison across color parameters; means denoted by distinct letters in each row indicate significantly different results (ANOVA test, P 0.05).

As expected regarding the colorimetric parameters, a* and b* values suggested a red-orange to orange-yellow color, with a pleasant taste due to the presence of ASP. Both samples show high levels of lightness and yellowness, which are characteristics of carotenoid compounds, according to the L* and b* values. The significant increase of b* value with the powder concentration suggests a tendency to yellow, offered by the biologically active compounds (carotenoids) from the annatto seeds used as a functional ingredient ($p < 0.05$). All cheese samples containing spirulina showed a decrease in L* value during storage, which might be explained by the rise in acidity and proteolysis that took place during this time (Chudy *et al*. 2020) The a* and b* values, a measure of redness and yellowness, of annatto fortified samples were significantly higher at the end of storage.

Sensory evaluation of value-added cheddar cheese. To evaluate the sensory aspects of the product, we used the quantitative descriptive analysis method, which is frequently used in studies of various products, including cheese (Stone H. *et al*, 2021). *Table 7* outlines the mean intensity ratings of descriptive attributes of evaluated cheese samples.

Table 7.

Sensory attributes values given by the panelists to ASP0.25 and ASP0.5 cheeses

Sensory attributes	***Type of cheddar cheeses***	
	ASP0.25	**ASP0.50**
Color	5.71[a]	6.13[b]
External appearance	5.77[a]	4.12[b]
Scent	4.20[a]	4.60[a]
Flavor	5.22[a]	6.00[b]
Consistency	5.30[a]	6.12[b]
Aftertaste	3.93[a]	4.22[a]
Overall quality	5.81[a]	6.92[b]

Different letters within rows indicate significant differences between samples (ANOVA test, $P < 0.05$).

The addition of ASP in cheese samples increased the yellow color, so the testers noted that the cheeses varied in color, with ASP0.5 being the most yellow. The annatto seeds powder-infused

cheddar cheeses were praised for having an agreeable flavor, scent, and color. The samples' crumbly, layered, dense consistency was also appreciated. The panel of tasters gave high marks to all of the suggested samples, ASP0.50 being the most preferred cheddar cheese. Sensory analysis showed that cheese with ASP could potentially be well accepted among consumers, having quite acceptable quality attributes.

CONCLUSIONS

Research has shown that adding annatto powder to cheese, regardless of quantity, does not significantly affect the nutritional composition of the cheese, without adding significant amounts of fat or protein.

Due to the ability of the seeds to give an intense orange-yellow shade, coloring cheese with annatto is a common practice in the food industry, without affecting the taste or aroma, it is a neutral additive that does not change the organoleptic characteristics of the product and does not interfere with the natural flavor of the product.

The addition of this powder, provides an attractive visual appearance by giving the color vibrant and attractive, which can influence consumer perception and increase the product's appeal in the market. The annatto seeds powder may be explored as valuable ingredients for the development of added-value food dairy products.

These results showed that value-added cheeses obtained with annatto seeds powder could be an alternative to synthetic food coloring, whereas providing a pleasant color, with potentially beneficial effects on human health.

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