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A Parametric Study on the Bixin Oil Suspensions Produced Using Annatto Seeds (*Bixia Orella)* and Its Potential Application in Coloring Margarine Products

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Abstract

Bixin obtained from Annatto seeds (*Bixinella orella*), has been used by the food industry as a source for natural color for a long time due to its low toxicity and high colorific value. Given its potential applications in dairy and meat industry to obtain a desired reddish - orange hue, a method was sought, which would quickly extract the desired pigment from the seed coat at low cost and allow for its direct application in the desired product. The bixin mass was extracted using demineralized water (D.M.W), sunflower oil or methanol and was then re-suspended in sunflower oil to a bixin strength of 10%. A study of the extraction process, viscosity and color hue (L*a*b value) of the 10% formulated product in margarine at low doses of 0.1% was carried out. Data indicated that methanol extraction provided the desirable reddish hue in margarine and yielded a product with lowest viscosity.

Keywords: Annatto; *Bixinela orellana*; Bixin, oil – suspension; L*a*b parameter.

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1. Introduction

Annatto (*Bixinela orellana*) has been used by humans for hundreds of years as a spice in food and for cosmetic purposes owing to its deep color imparting dyestuff [5]. The major dyestuff present on the pericarp of the Annatto seeds is the apocarotene Bixin. While it is common to bring about the alkali treatment of bixin to create a water soluble analogue Norbixin; bixin is unique in its ability to provide a rich deep color ranging from deep red to orange depending on its dosage and concentration

With a growing sense of awareness in consumers about the potential ill effects of chronic consumption of synthetic colors, the trend is now shifting towards the adoption of natural colors [4, 1]. Owing to its non –toxic nature and deep color imparting properties, bixin has found ubiquitous use in the food industry and has been designed with an E number (E 160b) [3, 7].

While numerous methods and processes have been described by a number of researchers on the extraction and usage of bixin; industrial application requires due consideration to dosage, cost and usability. An oil suspension of bixin in sunflower was therefore attempted to be produced by first extracting the bixin from the annatto seed pericarp using either D.M.W (De-mineralised water), sunflower oil or methanol to produce an extract which was then suspended in sunflower oil. Tests were conducted which would help determine the best method to extract bixin from the seeds into a product which could directly be used for food applications without the need for further purification or treatment while still being cost effective and easy to perform.

2. Materials and Methods

Annatto seeds were procured from Excardx S.A., Guatemala, Peru. Bixin content of the seeds were estimated by taking 1 g of seeds and refluxing them in 100 ml of Chloroform at 60^{o}C for one hour under dark conditions. The chloroform extract was then tested for bixin content. Bixin estimation was done by dissolving samples in chloroform and analysing them 470 nm considering E1% = 2826 using chloroform as blank [6].

For each experiment, 500 g of seeds were used. Seeds were subjected to agitation in DMW, methanol or sunflower oil at 70^{o}C for 1 hour (except for methanol which was carried out at room temperature) and then passed through 60 mesh and 100 mesh sieves. The sieved extracts were then passed through 2 µ filter cloth under a vacuum of 0.132 atm. The seeds were subjected to re-extraction using the sunflower oil and methanol using the same respective methodologies. Re-extraction was not pursued for DMW extraction.

The retentates of each method were recovered and pooled, weighed and tested for bixin content and then re-suspended in sunflower oil to give a product with bixin strength of 10%

Methanol and chloroform used were purchased from Merck (A.R Grade). Refined colorless sunflower oil was purchased from the local market.

Viscosities of the 10% bixin oil – suspension samples were determined using Brookfield Viscometer (LVDV- II +Pro programmable rheometer) where measurements were made at 20^{o}C across an r.p.m. range of 10 to 100,

with a 10 rpm interval, in an ascending and descending order, keeping a 60 second gap between each reading.

The particle sizes of the 10% bixin oil- suspension samples were determined through Microscopy (Motic BA – 210 using Motic particle size detection software) at 10x magnification.

Determination of L*a*b values were done using X-rite Color i5 by mixing the samples in vegetable derived margarine at a dosage of 0.1%

All experiments were conducted in triplicates.

3. Results

The bixin content of the seeds was found to be 2.3% indicating that each 500g batch seed contained 11.5g of bixin.

Post agitation of the seeds in DMW and passing them through 60 mesh and 100 mesh sieves, the extracts took over 40 hours to complete filtration under vacuum. In comparison, the filtration of the sunflower oil extract required around 24 hours whereas, the methanol extract required only one hour.

The consolidated results are given in the tables below:

3.1 Tables

Table 1: Parametric values of the 10% bixin content sunflower oil suspension product extracted using water at 70^{o}C for 1 hour

Water Extract									
	Dry filtrate			10% Bixin suspension in sunflower oil					
Trial No.	Weight of extract (g)	Bixin content (%)	Bixin Recovery from seeds (%)	Viscosity (cP)	Average Particle size (µ)	L*a*b value			
						L	a	b	D.E
Trial 1	20.4	10.5	32.0	>300000	8.6	70.718	28.912	50.888	91.796
Trial 2	18.6	10.2	28.2	>300000	7.9	74.612	29.015	49.012	93.867
Trial 3	18.9	10.1	28.5	>300000	8.8	73.535	28.791	49.798	93.360
Average	19.3	10.3	29.6	>300000	8.4				

Table 2: Parametric values of the 10% bixin content sunflower oil suspension product extracted using methanol at room temperature for 1 hour

Methanol Extract									
	Dry filtrate			10% Bixin suspension in sunflower oil					
Trial No.	Weight of extract (g)	Bixin content (%)	Bixin Recovery from seeds (%)	Viscosity (cP)	Average Particle size (µ)	L*a*b value			
						L	a	b	D.E
Trial 1	32.8	21.6	61.8	1988	6.1	71.652	28.324	44.671	89.060
Trial 2	29.6	22.5	58.0	1392	6.8	72.158	28.178	44.583	89.378
Trial 3	32.8	22.2	63.6	1414	6.7	72.149	28.301	44.411	89.324
Average	31.7	21.2	61.1	1598	6.5				

Table 3: Parametric values of the 10% bixin content sunflower oil suspension product extracted using sunflower oil at 70^{o}C for 1 hour

Sunflower Oil Extract									
	Dry filtrate			10% Bixin suspension in sunflower oil					
Trial No.	Weight of extract (g)	Bixin content (%)	Bixin Recovery from seeds (%)	Viscosity (cP)	Average Particle size (µ)	L*a*b value			
						L	a	b	D.E
Trial 1	40.3	10.2	35.7	4793	7.6	73.206	28.947	48.527	92.477
Trial 2	40.1	13.0	45.2	4376	7.9	73.212	28.834	48.471	92.417
Trial 3	39.7	13.9	47.8	4234	7.8	73.235	28.921	48.511	92.483
Average	40.0	12.3	42.9	4467	7.8				

Re–suspension of the retentate from the water extract in sunflower oil was cumbersome. Bixin estimation of the sample was also difficult owing to poor solubility.

4. Discussion

A comparative extraction of the bixin from the seeds was performed using DMW, methanol or sunflower oil as extraction media, which was then sieved and filtered to obtain a retentate which was reconstituted with refined and de-colored sunflower oil till it reached a bixin content of 10% Adjustment of the bixin content helped eliminate variability arising from the purity of bixin obtained from the extracts. Furthermore, it helped demonstrate the properties of a possible 10% bixin oil suspension product which could directly be used for commercial applications.

By comparing Tables 1, 2 and 3, it can be observed that the methanol extracted product had the lowest viscosity with an average value of 1598 cP. This was possible due to the high bixin content of about 21.2% in the retentate which allowed for a greater quantity of sunflower oil to be added to adjust for the desired bixin content; in conjunction with a relatively low particle size value of about 6.5 μ.

DMW extracted bixin samples showed viscosities greater than the measuring range of the instrument while the direct sunflower oil extract provided a viscosity value of 4467 cP. Furthermore, as the bixin content of the retentates using DMW and sunflower oil were already close to 10%, there was little scope for addition for sunflower oil for bixin content adjustment. Also, the DMW extracted retentate may have had residual water which in conjunction to its high particle size of 8.4 μ may have contributed to its poor mixing which resulted in an increased viscosity.

The L value (indicating lightness of the sample) provides us with an idea about the depth of the color at the dosage tested, with low values being “deeper” and higher values being “lighter”.

The methanol extracted product seems to have the deepest color, followed by the sunflower oil extract. The DMW extracted product showed the lightest color.

The ‘a’ and ‘b’ values indicated the redness and yellowness respectively of the colors obtained. All the samples showed almost equal levels of redness. However, lowered ‘b’ values for the methanol extracted product resulted in a hue appearing the “reddest” amongst the three type samples. The sunflower oil and DMW extracted products showed almost comparable ‘b’ values, indicating that both products were similar in terms of overall hue.

However, in terms of cost and usability, it is important to note that while DMW may be inexpensive by itself for extraction, the sheer time required for the filtration step seems to reduce the feasibility of this process. Moreover, the residual water in the retentate makes it difficult to measure and use. A vacuum oven may be employed to dry the retentate prior to suspension in sunflower oil; however, adding a step and slowing the overall throughput of the process and increasing the energy cost of the process.

On the other hand, using the direct sunflower oil extraction process seemed to require lesser time as compared to the water extraction process with comparatively better product behaviour in terms of viscosity. However, the methanol extraction process yielded a product with the most desirable qualities of lowest viscosity, darkest and

reddest color and shortest process time.

5. Conclusion

In terms on cost and usability, the methanol extracted product seems to be a viable option given the desirable color it provides in the final application and the ease of production. However, should a solvent free method be desired, direct sunflower oil extraction may be pursued using a filter of higher pore size along with higher vacuum to help hasten filtration process.

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