artikel 8

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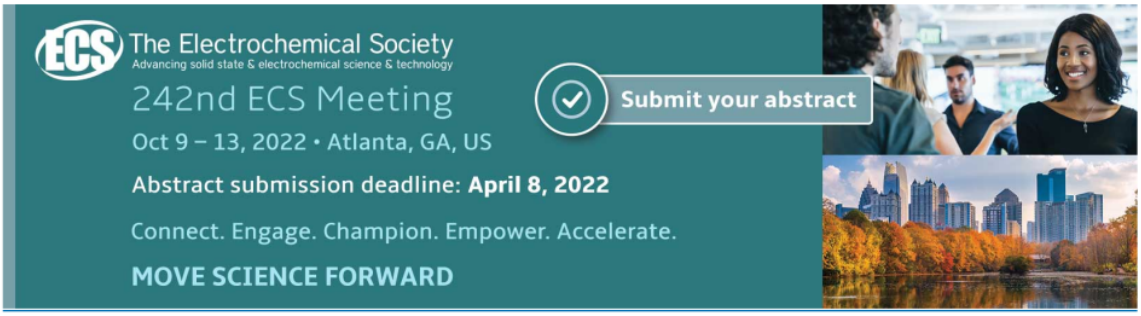

Evaluation types of solvents on the extraction of *Bixa orellana* and application of extract on a chicken sausage product as natural colour and antioxidant sources

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Abstract. *Bixa orellana* known as annatto is often used as a natural colorant in some food industries. This pigment is potential as an antioxidant due to high levels of total phenol and carotenoid in seeds. This study aims to evaluate the effect of polarity of solvents in annatto extraction on colour and antioxidant content in the extracts as well as the application of extracts of annatto on chicken sausage products. The solvent used for extraction annatto was hexane (nonpolar), chloroform (semipolar) and ethanol (polar), and concentration of extracts used in the making of chicken sausage were 1, 2 and 3% respectively. Colour observation of extract was done visually and by using a tintometer to measuring the color components while the colour observation of chicken sausage was done using Munsell chard as well as sensory evaluation by the panelist. The antioxidant content in the extracts as well as in the chicken sausages was evaluated by measuring the total phenol and carotenoid. The result of this research shows that hexane extract had a yellow colour, which composed of red and yellow colour with intensity 9 and 37. The chloroform extract had red dense colour, which composed of red, yellow and blue with intensity 12, 4 and 6.9, respectively, while the ethanol extract had orange colour, which composed of red and yellow colour with intensity of 12, and 50. The highest content of total phenol (115 mg/g) was found in ethanol extract while the highest content of carotenoid (11.77mg/g) was produced in hexane extract. Addition of 2 % hexane extract gives the best colour on the chicken sausage, and based on the measuring with the Munsell chard was produced orange colour with hue 2.5 Y; value 7; and chrome 6, with the level of joy is like (3.8). The highest total phenol content (29.95 mg/g) was found in the chicken sausage which was added of 1% extract of hexane whereas the highest carotenoid content (16.17 ug/g) found in the chicken sausage which was added of 3 % extract of hexane.

Keyword: *Annatto, extract, colour, phenol, carotenoid*

1. Introduction

Colour is the main feature of food which enhancement visual appeal to the enjoyment of eating. The synthetic colourant is commonly used nowadays but a growing trend toward natural colourant due to potential promising no or relatively lower side effects and economic viability. *Bixa orellana* (annatto) is one of the plants that have potential higher as colourant. Annatto which was obtained from *Bixa orellana* fruit is one of natural pigment used as a natural food colorant. The main pigment of annatto is carotenois

composed of bixin and nor bixin [1]. Annatto pigment have low toxicity and have hight inctorial value as well as has an outer color range comprising of red, orange and yellow hues [2]. Bixin (non polar) is more soluble in vegetable oil, on the other hand, norbixin (polar) is more soluble in aqueous solution. Furthermore [2] reported that bixin more soluble in solvent with low polarity. According to [3], [4], [5] reported that *Bixa orelana* extract has potential as a natural antioxidant. Antioxidant activity and colour properties of some extracts have been done. Flavonoids and carotenoids are regarded as natural colorant which has the activity of quenching singlet oxygen and acts as a strong antioxidant.

Some methods have been done to extract the annatto pigment as well as natural antioxidants such as soxhlet extraction, maceration, vegetable oil extraction, alkaline solution, organic solvent extraction, supercritical CO2 [6], and ball milling grind [7]. Solvents polarity produces different colors and bixin levels [2]. According to [6] who reported that organic solvent such as acetone and methanol, using for extraction, produced bixin on higher concentrations (3.5–5.2%). Furthermore, [6] statted that after extraction, the solvent was evaporated, then the powder pigment production was dissolved in vegetable oil.

As a colourant, annatto was used in cheeses, sausages, meat and candies industries [6]. The dairy industry is the biggest usage of annatto pigment [8]. In the passfew decades, there has been an increase of interest in attending on the usage of plant-derived antioxidant in reducing free radical and the current trend to substitute the synthetic with natural antioxidants [3]. Annatto seed powder could be used as a natural antioxidant in various foods especially in meat products [7]. But studied on antioxidant activity and application of annatto extract as colouring and antioxidant agents on chicken sausage products are limited.

Chicken sausage is one of the food-processed products that contain high protein. But chicken sausage without the addition of dyes has a color that is less attractive. An alternative that is often done by industries is by add dyes or to use curing. Chicken sausage also contains fat with a fairly high content of about 5% so it is easy to oxidation. Fat oxidation can produce free radicals that can cause damage to the product even triggers the onset of diseases such as coronary heart disease and cancer. A synthetic antioxidant such as BHA and BHT is commonly used to prevent food oxidation. Butylatedhidroxyanisol (BHA), butylated hydroxy toluene (BHT), tet butyl hydro quione (TBHQ) were the most frequently used as synthetic antioxidant of food due have high activity and stability. However, the use of synthetic antioxidants is potentially carcinogenic [9].

This research evaluated differences solvent polarity for extraction *Bixa orrellana* and evaluated the colour and antioxidant content of extract as well as its application as natural colourant and antioxidant on chicken sausage product. Even though many researchers were worked on extraction of annatto, however evaluation the color components of extract, application of extract in chicken sausage and evaluation of antioxidant not already done.

2. Experimental details

2.1. Preparation of annatto powder

Fresh annatto was colected from Banyumas Regency of central Java_Indonesia. Sample was sliced with size 0.5 cm, and sun dried (38–40^{o}C) until dry broken. Futhermore sample was powdered using a mechanical grinder to obtained annatto powder.

2.2. Preparation of extract

The extraction of annatto powder is carried out multilevel with soxhletasi method using 3 different solvent polarities, namely hexane (nonpolar), chloroform (semi-polar) and ethanol (polar). A total of 30 g of annatto powder was wrapped using a filter paper tied both ends, then inserted into the tube of soxhlet. The solvent is used each as much as 200 ml. Extraction was begun using hexane, was continued extraction using chloroform and the last was ethanol. Colour of extracts were analysed by visual and colour composition of extract were measured using Lovibond tintometer [10]. Furthermore, extracts were analyzed of total phenol and total carotenoid.

Before used as dyes and antioxidants in chicken sausage, the solvent in the extract was eliminated by drying using a blower oven at 40^{O}C. The condensed extract was dissolved into a food-solvent with comparison extract: solvent of 1:1 (b/v). A hexane extract was dissolved into vegetable oil, chloroform extract was dissolved into a tween-20 that had been diluted with aquadest (0.7% v/v), whereas ethanol extracts were dissolved into the aqudest.

2.3. Processing of chicken sausage

The chicken meat is ground with the addition of ice and salt. Seasoning, STPP, cassava starch, wheat flour, and egg whites are incorporated into ground meats. A mixture of ingredients that have been stirring, add with an annota extract, inserted into the sleeve, then cooked by steam blanching for 20 minutes. The resulting chicken sausage is performed colour analysis which is done in sensory and using the Munsell Chard, the total phenols and carotenoids. The content of phenolic compounds (total phenols) was determined using the Folin Tiocalteau reagent, and tannic acids used as standard [11]. The measurement of carotenoids (total carotenoids) is carried out using spectrophotometry methods [12]. Color and sensory preference on the product are done using sensory test by a semi-trained panelist of 15 people by scoring method. Color score i.e white (1); white brownish (2); yellow (3); reddish yellow (4); yellowish red (5). Color measurement using Munsell Colour Chart is done against hue, value and chroma. Hue states the name of the color, the value represents the level of brightness or color brilliance while chroma declares color intensity, strength or purity. Preference scores include; nor (1) so so (2), quite like (3), like (4) and really like (5).

2.4. Analysis of variables

The variables tested was analyzed using analysis of variance (ANOVA). If the analysis showed a significant influence, it was followed by the DMRT (Duncan multiple-range test) with a 95% confidence interval.

3. Result and discussion

3.1. The colour of the extract

The mayor pigments of annatto are bixin and non-bixin [7]. Bixin has two different stereoisomer configuration: cis-bixin and trans-bixin. The cis configuration is soluble in polar organic solvent that produces orange colour and insoluble in vegetable oil. The configuration of cis-bixin was instability properties in solution and it was readily converted to trans-bixin. Trans-bixin which has red colour is an isomer that more stable [13]. The colour analysis of extract was shown in table 1.

Table 1. Colour analysis of extract.

Extracts	Visual colour	Color composition	
		Colour	Intensity
Hexane	Yellow	(Red; Yellow)	(9; 37)
Chloroform	Red dense	(Red; Yellow; Blue)	(12; 4; 6.9)
Ethanol	Orange	(Red; Yellow)	(12; 50)

The result showed that polarity of solvents had different visually colour and its intensity. The colour of extracts produced of multilevel extraction using hexane, chloroform and ethanol ranged from yellow to red. The yellow colour of hexane extract was produced, due to hexane extract was the first extract in multilevel extraction. Bixin is the most component of carotenoids of annatto and it dissolved into solvent during extraction. Hexane (non-polar) resulted in yellow colour extract visually while ethanol (polar) gave orange visually. The colour of the pigment is affected by the concentration of the colour compound [7]. The red color of the extract is thought to be caused by some of the carotenoids extracted in solvents, and the use of high temperatures during extraction using hexane and chloroform causes cis-bixin to

convert to trans-bixin which produces a red colour. The bixin content decreased when exposed to a process temperature above 60^{o}C [14].

3.2. Total phenol of extract

The content of the total phenolic was carried out using Folin-Ciocalteau reagent with the standard solution of tannic. Phenolic compounds have a particularly strong antioxidant effect [15]. Extract ethanol gives the highest total phenol than hexane and chloroform extract (figure 1).

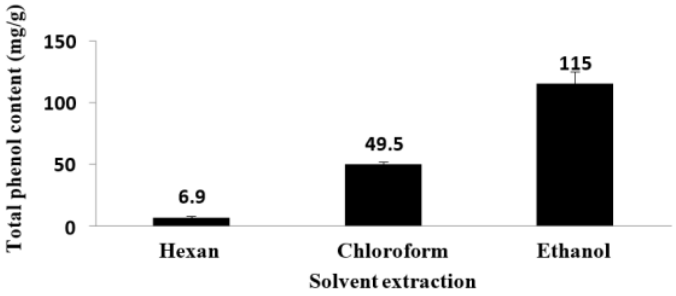

Figure 1. Total phenol of extract produced from different solvent.

Ethanol was more efficient to extract total phenol due to ethanol is polar solvent. Ethanol has an ability to extract glicosidic and non-glicosidic compounds of phenol than a nonpolar solvent such as hexane and chloroform. This result in agreement with [13], who reported the highest total phenol of *Merremia borneensis* was produced in ethanol extract than in extract of hexane and chloroform [15]. According to [15] also reported that the highest total phenol of pears extracts was produced in ethanol extract. Furthermore [15] reported that the selection of the solvent and the conditions of extraction is an important analytical step in the development of the technique for the qualitative and quantitative analysis of the biologically active compounds in raw plant material. The solvent of the extraction is one of the main factors in the prognosis of the qualitative and quantitative composition of the isolated phenolic compounds.

3.3. Total carotenoid of extract

The main carotenoid composition of annatto is bixin and nor bixin the carboxilic acid mono methyl ester constitute 90% of total pigment [16]. Figure 2 shows carotenoid content of hexane, chloroform and ethanol extracts obtained using multilevel extraction. Carotenoid content of extracts was shown in figure 2.

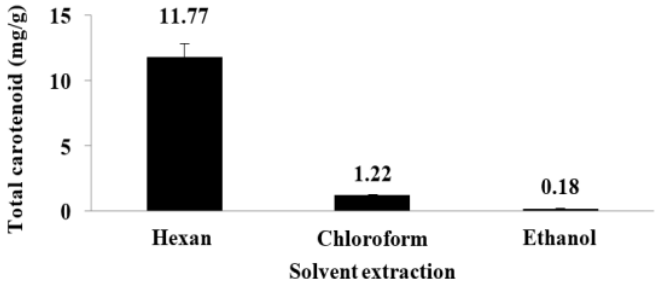

Figure 2. Carotenoid content of hexane, chloroform and ethanol extracts obtained using multilevel extraction.

The highest carotenoids total were produced in hexane extract and the lowest in ethanol extract. Hexane which was first used in the first time in multilevel extraction is non polar solvent, so it can dissolve bixin more effectively than chloroform and ethanol which has a lower polarity level. The main carotenoid in annatto is bixin. Bixin is non polar compound, therefore the use of hexane to extract of annatto produced more high levels of carotenoids than chloroform and ethanol. Hexane is a non-polar solvent, making it more effective to dissolve non-polar bixin. Bixin is the most component of carotenoids of annatto and it dissolved into solvent during extraction. According to [6] who reported that organic solvent that used for the extract of annatto, produces bixin on higher concentrations.

3.4. Colour of chicken sausage measured using a chart Munsel

The results of the color readings of chicken sausages using Munsell chard show the use of different polarity of the solvent, as well as different of extract concentration, produces different chicken sausage colors. Color of chicken sausage on the addition of annatto extract shown in table 2.

Table 2. Colors of chicken sausage based on measurement using Munsel Chart.

Treatment	Hue	Value	Chroma	Visual
Hexane 1%	2.5 Y	7	6	yellow
Hexane 2%	2.5 Y	8	6	yellow
Hexane 3%	7.5 YR	7	8	reddish Yellow
Chloroform 1%	2.5 Y	7	4	pale yellow
Chloroform 2%	2.5 Y	7	6	yellow
Chloroform 3%	2.5 Y	7	6	yellow
Ethanol 1%	2.5 Y	6	2	grey
Ethanol 2%	2.5 Y	7	4	pale yellow
Ethanol 3%	5 Y	8	4	pale yellow

The highest color of chicken sausage is a reddish yellow produced by a 3% hexane extract, gave chicken sausage with a hue value of 7.5 YR, the value of 7 and chroma of 8. Hexane that is a nonpolar solvent can extract bixin more efficiently than chloroform and ethanol. The dissolving of hexane extract using vegetable oil also gives an increase in the color of the chicken sausage. Vegetable oil contains carotenoids to give increasing the color intensity of the yellow in chicken sausage. The higher extract concentrations also give an increase to color intensity because it is suspected to contain higher bixin.

3.5. The color of the chicken sausage measured with sensory

The addition of annatto extract on chicken sausage produces different colors. The colour of chicken sausage with the addition of annatto extract was shown in figure 3.

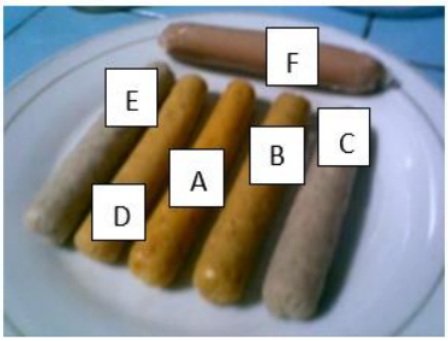

Figure 3. Chicken sausage with the addition of annatto extract (A: hexane extract; B: chloroform extract, C: ethanol extract, D: vegetable extract (data not shown, E: control, without extract, F: sample).

The addition of a 3% extract of hexane gives a reddish yellow color with a score of 3.9. The color of chicken sausage with such treatment does not differ real with chicken sausage with the addition of 2% extract of hexane, as well as 2% and 3% extracts of chloroform. The result of the color measurement chicken sausage with sensory test shown in figure 4.

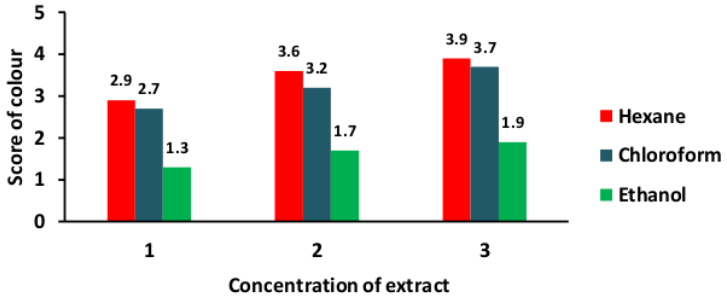

Figure 4. Colour of chicken sausage based on measurement using the sensory test.

Chicken sausage with the addition extract of ethanol resulted in the lowest color score between 1.3 and 1.9 (yellowish-white color). Extraction using ethanol which is the final stage extraction suspected produces a low bixin concentration. The dissolving of ethanol extracts using aquadest also leads to lower bixin concentration so that the resulting yellow color intensity is also lower.

3.6. Panelist preference on chicken sausage

The result of the preference test on chicken sausage with the addition of extract annatto is shown in figure 5

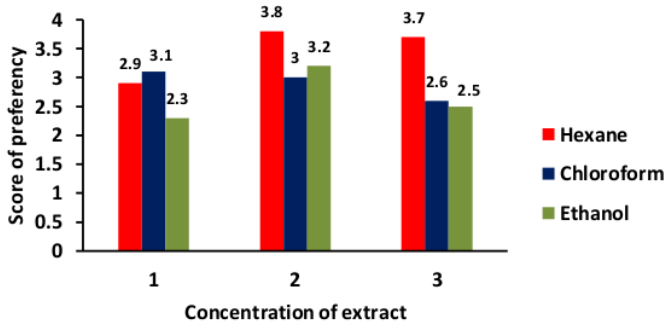

Figure 5. Panelist preference on chicken sausage based on measurement using the sensory test.

Figure 5 shows the preference level on chicken sausage influenced by the type and concentration extract of annatto. Chicken sausage with the addition of a 2% extract of hexane is the most favored product of panelists with a preference score of 3.8 that does not differ with the addition of a 3% hexane extract (3.7) which shows like on chicken sausage product. Chicken sausage with the addition of a 1% extract of ethanol is the most disliked product of panelists with a preference value of 2.3 (so so-quite like). This is due to chicken sausage with the addition of ethanol extract produces the lowest color ie yellowish white. These results show chicken sausage with orange-yellow color is more liked by the panelist.

3.7. Total phenol of chicken sausage

Total phenols contained in an ingredient relates to the antioxidant activity [17]. The content of the total phenol of chicken sausage which added with extract of annatto is shown in figure 6.

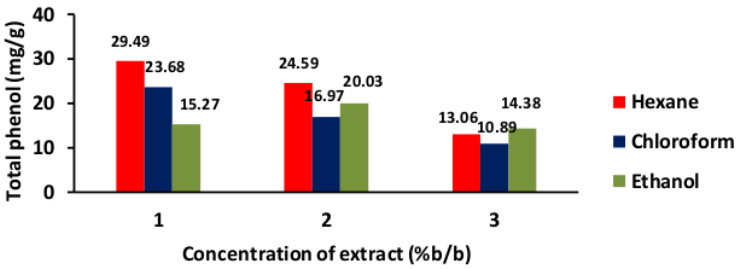

Figure 6. Total phenolic of chicken sausage with the addition of annatto extract.

The results of the study showed the addition of a 1% extract of hexane produce the highest total phenol (29.94 mg/g) while the lowest total phenol produced in chicken sausages with the addition of a 2% extract of chloroform (10.30 mg/g). The higher phenol levels in the extract of hexane which was dissolved in vegetable oil are caused by the addition of a phenol compound derived from the vegetable oil. The study by [3] which stated that annatto contains saponin, tannins, and terpenoids. Furthermore [7] stated that content total phenol of annatto seed powder 62.08 ± 2.21 mg of GAE/g). The presence of phenol compounds in chicken sausage which added extract of annatto is expected to contribute to increased antioxidant activity. Antioxidants will react with oxygen thereby reducing oxidation that causes damage.

The results of this study also showed that an increase in the concentrated extract of hexane, chloroform, and ethanol provides different results, depending on the type of solvent used. The increase concentration extract of hexane and chloroform from 1 to 3% cause decreases total phenol levels while this phenomenon does not occur in ethanol extracts. According to [18], the content of total phenol in extract depending on the solvent used for extraction. The total decrease of phenol with increased annatto extract was allegedly due to reaction interactions between phenols and proteins. According to [19] the phenol compounds can interact to form a complex with proteins. The parameters that are affected protein-phenolic interaction are temperature, pH, type and protein concentration and type and structure phenolic compound.

3.8. *The total carotenoid content of the chicken sausage*

Carotenoids are one of the sources of natural dyes which can serve as an antioxidant [20]. According to [21], carotenoid compounds have the ability as an oxygen singlet quenchers and potentially as other reactive oxygen scavengers. The carotenoid content of chicken sausage adding with extract of annatto is shown in figure 7.

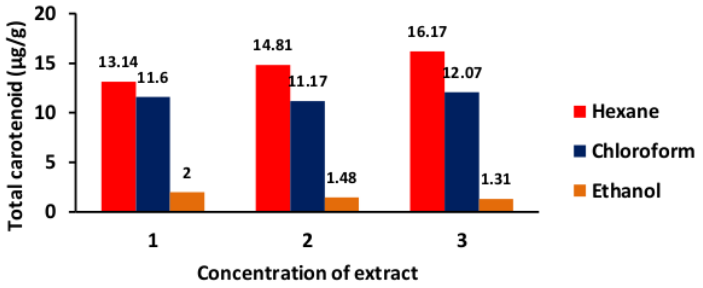

Figure 7. Total carotenoid of chicken sausage with the addition of annatto extract.

Based on figure 7, the highest carotenoid levels are produced in chicken sausages with the addition of 3% hexane extract (16.17 µg/g) while the lowest carotenoid levels (1.31 ug/g) is produced in chicken sausages with the addition of 3% ethanol extract. High carotenoid levels in chicken sausage with the addition of hexane extract caused by hexane is a nonpolar solvent, so it can extract bixin which is the main carotenoid in annatto. According to [22], the main pigment of annatto is bixin that was a nonpolar compound. Annatto also contains norbixin which was a polar compound. The content of bixin in the annatto reaches 80% [23]. The content of bixin in annatto seed powder amounting to 40.33 ± 0.32 mg/g while the content of norbixin 31.61 ± 3.88mg/g [7]. The results of the study also showed an increase in the concentration of extracts which was added to chicken sausage has no significant effect on carotenoids. It is suspected that the combined concentration range is not significantly different resulting in no different carotenoid levels.

4. Conclusion

The colour of extracts produced of multilevel extraction using hexane, chloroform and ethanol ranged from yellow to red. Addition of 2 % hexane extract gives the best colour on the chicken sausage, and based on the measuring with the Munsell chard was produced orange colour with hue 2.5 Y; value 7; and chrome 6, with the level of joy is like (3.8). The highest total phenol content (29.95 mg/g) was found in the chicken sausage which was added of 1% extract of hexane whereas the highest carotenoid content (16.17 ug/g) found in the chicken sausage which was added of 3 % extract of hexane.

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