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Formaldehyde Adulteration in Meatball is Still Prevalent and it is Hard to Identify Only from its Physical Properties

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Abstract

Human carcinogenic compound formaldehyde (alternative name, formalin) adulteration in food is inappropriate and illegal. However, its simple and cheap procedure in giving desired food preservation effect becomes the factor of its prevalence in Indonesian community. Our current study focuses on the usage of formaldehyde in meatball products which are sold in bulk without refrigeration in traditional market set up. Eighty different packages of meatballs were collected following accidental sampling method from small groceries in Tembalang, Semarang, Central Java in 2019. Samples were characterized for their formaldehyde content (AOAC 931.08), total plate count, moisture and organoleptic properties. Formaldehyde was found in 1 sample at the level of 176.2 ppm. Whereby, it was among only 4 % of samples that met the maximum microbial limit of 5 log CFU/g. The particular sample was characterized by pale color, rather slimy surface, chewy and weak meatball aroma. Note, this characteristic is not unique and is shared by several other samples. Therefore, this study brings an update that formaldehyde adulteration is still prevalent. The adulterated product has hardly distinctive sensorial properties which make self-evaluation difficult, thus exposing consumers to potential food safety risks.

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Introduction

Meatball is popular in Indonesia. Many food workers offer the meatball as a soup-dish or mixed in noddle-based dishes. Not only it is available as ready-to-eat, restaurant level, products, meatball is also widely sold as unprepared/uncooked version for use at household level. In this study, the term ‘meatball’ refers to the second type of product described above. According to Indonesian Standard SNI 3818:2014, meatball is characterized with ≥11.0% protein content, ≤10% fat content, ≤70% moisture, and ≤3% ash. Its high moisture content allows microbial growth, hence meatball is categorized as perishable product that must be stored in cold/frozen temperature. However, in traditional market set up in Indonesia, many vendors sell meatball without chilling storage. The product is often sold as ‘bulk’ where buyers can buy in variable quantity or packed in unlabelled packaging with inadequate sealing (Hermanianto & Andayani, 2002). This poor hygienic practice poses risk to accelerate meatball microbial spoilage (Nasution *et al.*, 2018).

There are at least two alternative methods to extend the shelf life of meatball, first, imposing a strict cold-chain process combined with good packaging, or second, by adding food preservative. The first method is the most popular and considered as the best practice in meatball industry. However, the lack of sufficient cold-chain infrastructure makes this method to be practical only for modern market set up. The bigger percentage of Indonesian population still rely on traditional market and small vendors which often closer to their neighbourhood. Here, the poor hygienic practice is common. Therefore, it is not surprising to have meatball manufacturers to opt for the second method, food preservative, to extend the shelf-life of their product. The prevalence of cheap prohibited chemicals being added into meatball is also evident. One study in 2015 reported about 15 out of 98 meatball samples (15.3%) were formaldehyde positive (Pratmanitya & Aprilia, 2016). Whereas, the annual report of Indonesian Food and Drug Agency (BPOM) in 2017 showed that 537 out of 8950 food samples were adulterated by borax, formalin, yellow methanyl, and rhodamine B. Formalin is a known adulterant that is added to obtain preservation effect in perishable food products, especially for those distributed in warm Indonesian climate without chilling. It is added to meatball due to its potent antimicrobial effect (Nikolic *et al.*, 2019).

Formaldehyde is classified as human carcinogen in 2004. Several cancers such as lung cancer, nasopharyngeal cancer, leukemia have been associated with the prolonged exposure of this substance (Protano *et al.*, 2022). Due to its health risk, the exposure of formaldehyde in daily life, including from food access route, must be kept up to date. The current study aims to provide update on the prevalence of formaldehyde adulteration in meatball product in Tembalang, Central Java, Indonesia. We also evaluate the physical and microbiological characteristics of samples in an attempt to formulate visual identification method of formaldehyde adulteration in meatball for consumers.

Materials and Methods

Materials

Beef meatball samples were obtained from small groceries and food vendors in Tembalang, Central Java, Indonesia. Typical presentation of meatball at small vendor facility is shown in **Figure 1**. Chemicals for characterization included saturated 1,8-dihydroxynaphtalene-3,6-disulphonic acid, distilled water, physiological NaCl solution, Plate Count Agar medium, standard formaldehyde (37%), and sulfuric acid.

Figure 1. Typical presentation of meatball at small vendor facility.

Methods

Sampling

This is a descriptive study (qualitative and quantitative) using survey and accidental sampling technique. There are 12 sub-districts in Tembalang. We obtained 5-7 packs of bulk meatball for every sub-district, amounting to 80 packs in total. Each pack contained 10 meatballs. Procurement of samples were conducted in the morning and followed immediately by visual and physical characterization. Subsequent evaluations (moisture, microbial and formaldehyde content) were performed on the same day after the characterization.

Visual and Physical Characterization

Meatball samples were evaluated for its color, aroma (typical meaty meatball aroma), texture and the amount of slime on surface. Assessment was performed by single trained panelist using scoring sheet 1-5 (low to high). The single panelist procedure was aimed to keep the scoring consistency. Details on scoring can be seen at **Table 1**.

Tabel 1. Scoring scale of several bulk meatball quality attributes

Color	Aroma	Slime	Texture
1: Pale	1: Weak	1: Dry	1: Brittle
2: Rather pale	2: Rather weak	2: Rather dry	2: Rather brittle
3: Normal	3: Normal	3: Normal	3: Chewy
4: Grey	4: Rather strong	4: Rather slimy	4: Rather hard
5: Dark grey	5: Strong	5: Slimy/ sticky	5: Hard

Microbiological analysis

The number of microbes was determined using Total Plate Count (TPC) (SNI 2897: 2008). Five grams of macerated meatball samples were diluted with 45 ml physiological NaCl (0.85%) solution. One ml of suspension is further diluted with 9 ml of the same solution. This procedure was repeated until 10^{-6} dilution were obtained. Plate Count Agar (PCA) was sterilized and cooled down to 45 ± 1 °C. One ml of 10^{-4}, 10^{-5}, 10^{-6} dilution were inoculated into 15 ml PCA in sterile petri dish (duplicate). Firm agar was then incubated at 34 °C for 24 hours. Total microbe is calculated by following formula

TPC (log CFU/ml) = Log_{10} (Colony count x (1/dilution factor))

Moisture analysis

Moisture content was determined using thermogravimetric method. About 2 grams of sample were oven dried at 105 °C until constant weight. The moisture can then be calculated from the initial and final sample weight according to Indonesian Standard SNI 3818:2014.

Formaldehyde content analysis

The determination of formaldehyde content followed chromotropic acid method (AOAC 931.08, 2003) which consists of two steps, a qualitative assay whereby the positive test is followed by a quantitative measurement using a UV-Vis Spectrophotometer. Briefly, 5 g of macerated meatball was added with 50 ml distilled water. The mixture was heated at 95±2 °C for 1 h, while shaken periodically every 5 min. Upon cooling, the filtrate was removed and transferred into a volumetric flask and diluted up to 100 ml mark. Chromotropic acid standard solution was prepared by diluting 1,8-dihydroxynaphthalene-3,6-disulfonic acid (ca 500 mg/100 mL) in ca 72% H_2SO_4. Solution is light straw-colored. The qualitative assay was conducted by mixing 1 ml of sample mixture with 5 ml of chromotropic acid, followed by heating at 95±2 °C for 15 min. The appearance of violet or red-violet color indicates a positive test.

Quantitative determination was applied to positive sample. A spectrophotometer standard curve was produced using formaldehyde standard solution in the concentration of 0, 2, 4, 6, 8 and 10 ppm. Similar chromotropic acid procedure was applied to the standard stock solution. The absorbance of the resulting product, including the sample, was measured in a spectrophotometer (BK-UV 1900, Biobase, China) at 480 nm wavelength (blue). Standard curve was produced from the absorbance and the known concentration. The linearity equation $y = 0.0454x + 0.59$ with correlation coefficient (R^2) 0.9962 were then obtained.

Results and Discussion

Formaldehyde Analysis

Qualitative assay on 80 different bulk meatball samples resulted in one positive test. Meatball sample with MS23A code produced violet color solution on addition of chromotropic acid. The acid reacts with the formaldehyde in the sample producing complex molecule in violet color (Farid *et al.*, 2015). The color difference between negative and positive tests can be seen in **Figure 2**. The positive test was further quantified using spectrophotometer which showed that MS32A sample contained formaldehyde at the level of 176.2 ppm.

(a) (b) (c)

Figure 2. The color appearance of a blank (a), positive test (b) and negative test (c) in chromotropic acid measurement of formaldehyde.

Formaldehyde in any concentration is prohibited to be use in food as stipulated in the decree of Indonesian Health Minister No 033-2012 about food additives. According to (Pontel *et al.*, 2015), accumulation of formaldehyde in living cells causes the degradation in cell function and triggers unwanted cell growth such as cancer. Exposure of formaldehyde can be acute or chronic. In chronic exposure, formaldehyde inhalation causes asthma symptoms and irritation to mucous membrane of eyes, nose, mouth and respiratory tract (Abdollahi & Hosseini, 2014). Whilst, in an acute exposure of 523 ppm may cause death or other symptoms such as burning sensation at mouth and throat, digestion tract ulceration, nausea, diarrhea, gastrointestinal bleeding and kidney failure (Abdollahi & Hosseini, 2014).

The current study result with one positive test shows that the adulteration of formaldehyde in food products is still prevalent. Although the regulations are in place, both manufacturer and consumer must have adequate knowledge on the danger of formaldehyde and on how to avoid product that may have been adulterated with.

Visual and Physical Characteristic of Meatball

Observation to the quality attributes of meatball has been summarized in **Table 2**. Samples with pale color, weak aroma, dry surface and brittle texture were 5%, 30%, 48% and 3%, respectively. The lowest score in each respective attribute indicates a non-compliant to Indonesian Standard SNI 3818:2014 for meatball.

Table 2. Summary of quality attribute evaluation of meatball samples

Score*	Quality Attribute			
	Color	Aroma	Slime	Texture
1	5%	30%	48%	3%
2	25%	34%	35%	43%
3	59%	30%	13%	34%
4	8%	4%	4%	14%
5	4%	0%	0%	6%

*The definition of scoring level for each attribute refers to Table 1.

The sample of interest, MS23A, which contains formaldehyde showed characteristic of pale color, weak meatball aroma, rather slimy surface and chewy texture (**Figure 3**). This observation is in accordance with (Faradila *et al.*, 2014), which states that formaldehyde adulterated meatball is characterized by paler color, weaker aroma and chewy texture.

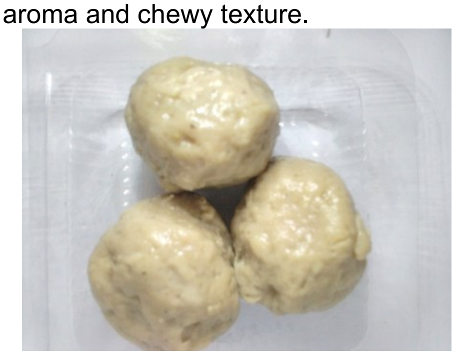

Figure 3. The visual appearance of MS23A sample

However, our observation revealed that this characteristic is not unique only to the sample of interest, but also shared by other samples that are formaldehyde negative. Therefore, the sole usage of visual and physical observation is not accurate, and thus less useful for the identification of formaldehyde adulteration.

Moisture and Microbiological Analysis

Table 3 shows the total of microbes and moisture content of meatball samples. The minimum microbe level was found to be 4.98 log CFU/g, whereas the maximum was 7.87 log CFU/g, averaging at 7.1 log CFU/g. According to SNI 3818-2914, the maximum total microbe in meatball is 5 log CFU/g. Hence, there are 96% of the samples which do not meet this standard. It is not surprising to find that the adulterated meatball sample was among the 4% of compliant samples. The MS23A is the one sample with the lowest microbe level. Here, we can see that formaldehyde effect to retard microbial growth is apparent.

The moisture analysis is Table 3 shows that the mean moisture level is 65.9%, whereas the maximum and minimum are 79.2% and 52.1%. The maximum allowance is 70% (SNI 3818-2914) moisture, hence, about 16% of the samples exceed the limit. The presence of water is essential to microbial growth. The high moisture content combined with poor hygienic practice can be responsible for the high microbial count in the sample.

Table 3. Summary of microbial count and moisture analysis on meatball samples

Parameters	Total Microbe	Moisture
Sample amount	80	80
Minimum	4.98 log CFU/g	52.1%
Maximum	7.87 log CFU/g	79.2%
Mean	7.1 log CFU/g	65.9%
Maximum allowed level*	5 log CFU/g	70%
Compliant	4%	84%
Non-compliant	96%	16%

* SNI 3818-2914

Conclusion

This study has provided an update on the prevalence level of formaldehyde adulteration in meatball product in Tembalang, Central Java, Indonesia. One out of 80 samples was found to be positive in formaldehyde at the level of 176.2 ppm. The correlation between formaldehyde content with other quality attribute has also been investigated. It is revealed that adulterated sample does not have a unique visual and physical trait. Thus, identification at the consumer level is deemed to be difficult. Enforcement of the existing regulation to punish malicious meatball producer should be combined with education to consumers about the danger of formaldehyde. This could help to mitigate the health risk posed by formaldehyde adulteration in food products.

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