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Investigation of Hazardous Food Additives in Indian Packaged Pickles through Chemical and Spectrophotometric Analyses

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KEYWORDS

Packaged pickles, Hazardous additives, Preservatives, Unpermitted colors, Synthetic antioxidants

ABSTRACT:

Pickles are the most popular accompaniment with a meal in almost every Indian household due to their rich flavor, taste enhancing properties and long shelf life. Packaged pickles are even more appealing due to their convenient usage. However, similar to any other packaged foods, pickles are also not out of the grip of adulteration. Multiple recent incidents, spreading across India, indicate indiscriminate use of synthetic preservatives, unpermitted colors and antioxidants in packaged pickles. With the growing popularity of "organic" food among the consumers, more and more pickles are being marketed as "ORGANIC" which claims to be free of any hazardous additives. Unfortunately, many of these pickles are without any authorized certification for the same. Therefore, it is highly important to analyze these pickles for detection and quantification of any possible hazardous additives in those.

In the current study, 15 commercially available Indian packaged pickles were investigated for the presence of preservatives like Benzoic acid (BA), antioxidants, such as, Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) and unpermitted colors such as Sudan IV and Metanil Yellow. Out of 15, eight pickles are of conventional/regular type, five are from uncertified organic type and two are from certified organic category. BA and BHA were analyzed quantitatively by titrimetric and spectrophotometric methods, respectively, whereas BHT and Sudan IV were analyzed qualitatively by thin layer chromatography and spectrophotometry, respectively. All 15 samples tested negative for BHT, Sudan IV and Metanil yellow, implying that the usage of these three additives are satisfactorily regulated in the pickle manufacturing sector. Use of BA and BHA in conventional pickle was also found to be satisfactory, as only one of those products exceeded the permissible limit. However, the situation was disappointing for "certified organic" and "marketed as organic" products. Those were found to contain BHA and BA which are completely non-permitted in organic products.

Findings from this project are expected to help in implementing better quality control regulations, besides creating awareness among customers about hazardous food additives in packaged pickles.

INTRODUCTION

Pickles started its journey as a highly efficient ancient form of preserving fruits and vegetables but soon turned into one of the most relished accompaniments of Indian foods due to its rich sensory attributes. As the process of pickling is time-consuming, pre-packed pickles have gained popularity and have become one of the most frequently used convenience foods in India. With the increasing consumption of processed, pre-packed convenience foods, the usage and intake of food additives has also increased. Food additives are compounds that are purposefully added to food to lengthen its shelf life or alter its sensory attributes, such as flavour, appearance, and texture (FSSAI, 2016). Based on their function, food additives are classified into several categories. It includes preservatives, antioxidants, colorants, emulsifying agents, etc. Excess usage of these additives can pose several health impacts such as cancer, neurological disorders, asthma, skin rashes, allergies, etc. (Tuormaa, 1994; Ghosh et al., 2017; Zanfirescu et al., 2019). Therefore, it is necessary to regulate their usage. In India, the usage of food additives is regulated by the Food Safety and Standards Authority of India (FSSAI) along with other organizations like the Bureau of Indian Standards (BIS) and the Codex Alimentarius Commission (CAC).

The symptoms shown after ingestion of the commonly used additives are mostly not instantaneous, but rather

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chronic (Kumar et al., 2019; Tuormaa, 1994). Therefore, often it may happen that certain health conditions cannot be traced back to the consumption of those additives through a pickle. Being rich in flavour and aroma, the off-flavours generated in the pickles by the excess additives get easily concealed, making their detection highly difficult by common consumers. Hence, it is highly important to evaluate the extent and severity of the danger of hazardous additives in pickles by systematic analyses of the packaged pickles.

With the recent trend of switching to organic foods, organic pickles are being preferred over their conventional counterpart. Unfortunately, cases of mislabeling of organic products are not uncommon (Moreira et al., 2021). There are many products available in the market that are advertised or marketed as organic but do not contain any certification or logos from organic product regulatory agencies, such as NPOP, PGS or Jaivik Bharat (Directorate of Marketing Inspection, 2005). Therefore, it is important to test organic pickles, with or without certification, besides regular conventional pickles. The objective of this study is to investigate the presence of hazardous food additives such as Benzoic acid (BA), Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Sudan IV and Metanil yellow (Fig. 1) in packaged pickles. The Food and Agricultural Organization (FAO) of the United Nations has established daily acceptable intakes (ADI) for additives like BA, BHA and BHT at 5 mg/kg, 0.5mg/kg and 0.05mg/ kg body weight/day, respectively (Carocho et al., 2014) while Sudan IV and Metanil yellow are not permitted at all.

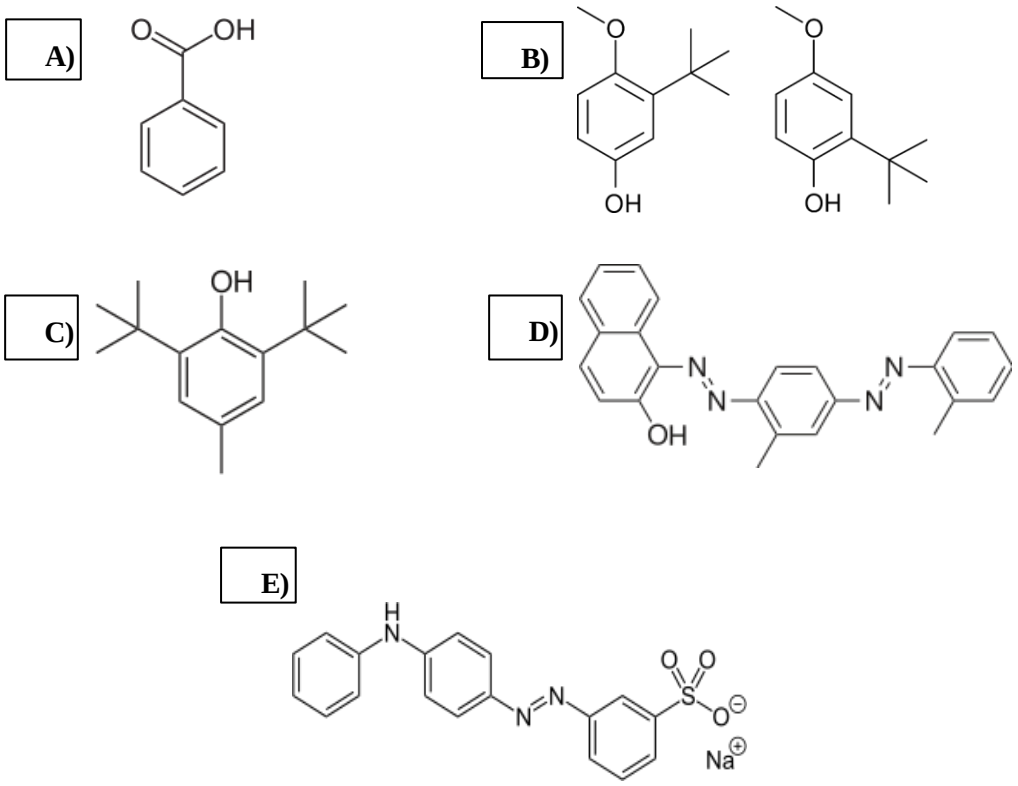

Figure 1: Chemical structure of A) Benzoic acid (Neurotiker, 2007), B) Butylated hydroxy anisole (Drevor, 2012), C) Butylated Hydroxytoluene (Derksen, 2007) and D) Sudan IV (Harbin, 2009) and E) Metanil yellow (Ju, 2015)

MATERIALS AND METHODS

Selection and collection of samples and reagents

Prior to our experiments, an online market survey was carried out to collect information on various pickles available in the market. A total of 15 pickle samples were selected based on availability, of which eight conventional or regular pickles were procured from local retail stores. The remaining five "marketed as organic" and two "certified organic" pickles were procured from e-commerce sites. The samples were coded with alphabets A to O.

The selected samples were tested qualitatively and quantitatively using chemicals that were received from the chemical manufacturer without any processing or purification. Ammonia, Hydrochloric acid, Sodium chloride, Chloroform, Borax, Methanol, Petroleum Ether, Gibb's Reagent, BHA Standard, and Ethyl acetate were procured from S.D. Fine Chemical Limited; Sodium hydroxide, BHT standard and Sudan IV from SRL (Sisco Research Laboratories Pvt. Ltd.); Acetonitrile was purchased from Qualigens (Thermo

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Fisher Scientific India Pvt. Ltd.); Benzoic acid standard was obtained from LobaChemie Pvt. Ltd. Mumbai, India. TLC Silica gel 60 F254 from Merck. Analytical tests were carried out using spectrophotometric, titrimetric and chromatographic methods.

EXPERIMENTAL PROTOCOLS

Quantitative estimation of Benzoic Acid (BA)

BA was extracted from the pickles samples using the extraction protocol described in FSSAI, 2016 with slight modifications**.** The pulverized pickle sample (15 g) was mixed with 1 ml of 10% NaOH solution which was then made up to the volume of 50 mL with the addition of saturated NaCl solution. After 2 hours of occasional shaking, contents were filtered and filtrate was collected. The filtrate (10 mL) was transferred to the separatory funnel and was acidified with 2-3 mL HCl (1:3, v/v) solution. The acidified filtrate was extracted with 15 mL portions of chloroform. The collected chloroform extract was washed with distilled water to make it free from mineral acid and dried overnight. The residues were dissolved in neutralized methanol and titrated against standard NaOH (0.005N) using a phenolphthalein indicator. The amount of benzoic acid present was calculated using the formula mentioned below:

$$\text{Benzoic acid (ppm)} = \frac{\text{Mol. wt of benzoic acid} \times \text{Vol.of NaOH used(mL)} \times \text{Normality of NaOH} \times \text{Volume made up(mL)} \times 1000}{\text{Weight of sample taken(g)} \times \text{Aliquot of filtrate taken for estimation(mL)}}$$

…. Equation (1)

The estimation efficiency of the protocol was determined by incorporating a known amount of standard BA (1000 ppm) in the pickle matrix which was devoid of any BA. Extraction and analysis were carried out following the exact procedure mentioned above. The result obtained was compared with the actual amount of BA incorporated into the pickle and estimation efficiency was calculated using the following formula.

$$\text{Correction factor} = \text{BA incorporated (ppm)}/\text{BA obtained (ppm)}$$

…. Equation (2)

The values obtained from Equation 1 were corrected using the correction factor calculated by Equation 2 as follows.

$$\text{Actual amount of BA (ppm)} = \text{Equation 1} \times \text{Correction factor}$$

Quantitative estimation of Butylated Hydroxyanisole (BHA)

BHA was extracted from the pickles samples using the extraction protocol described in FSSAI, 2016 with slight modifications**.** The multiple standard addition method was used in the present study for the colorimetric determination of BHA content. The standard BHA in the concentration range of 0.01 mg/mL to 0.08 mg/mL was used to construct the linear calibration curve. Regression of the standard calibration graph ($R^2 = 0.9918$) showed a strong positive relationship between absorbance and concentration. The amount of BHA in sample extracts was calculated using the linear equation (y=4.7262x - 0.0189).The standard graph is attached in Appendix-1. Pulverized pickle sample (5 g) was taken and 12.5 mL of 95% methanol was added. The contents were shaken for some time and heated around 40-50^0 C for about 15 minutes. The contents were further centrifuged at 3000 rpm for 5 minutes. The supernatant was collected and the same extraction process was repeated with 10 mL of 95% methanol. Before filtration, the volume was made up to 25mL with 95% methanol. The solution was mixed with calcium carbonate (1 g) and filtered using Whatman filter paper. To the aliquot (2 mL) of filtrate, 2 mL of methanol (95%, v/v), 8 mL of borax solution (0.5%, w/v), and 2 mL of Gibb's reagent (0.01%, w/v) were added and contents were incubated for 15 min. Contents were diluted precisely to 20 mL with N- butanol and absorbance was recorded at 610 nm in a colorimeter. BHA concentrations in the samples were calculated using the BHA standard curve.

To ensure that any of the dyes present in the pickle does not interfere with the colour generated by BHA-Gibbs-Borax at 610 nm, the final extract in methanol was scanned in a UV visible double beam spectrophotometer at wavelengths between 300 and 700 nm. No peak was

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found beyond 480 nm, which ruled out any interference from the pickle dyes in the absorbance at 610 nm.
The estimation efficiency of the protocol was determined by incorporating a known amount of standard BHA (1000 ppm) in the pickle matrix which was devoid of any BHA. Extraction and analysis were carried out following the exact procedure mentioned above. The result obtained was compared with the actual amount of BHA incorporated into the pickle and estimation efficiency was calculated using the following formula.

Correction factor = BHA incorporated (ppm)/BHA obtained (ppm)

…. Equation (3) The values obtained from the graph were corrected using the correction factor calculated by Equation 3 as follows:

Actual amount of BHA (ppm) = BHA concentration from the graph × Correction

Qualitative estimation of Butylated Hydroxytoluene (BHT)

BHT was extracted from the pickles samples using the extraction protocol described in FSSAI, 2016 with slight modifications**.** The pulverized pickle sample (10 g) was mixed with petroleum ether (100 mL) and was shaken for 3 min in a rotary shaker. The contents were centrifuged at 2000 rpm for 5 min and the supernatant was transferred to a separatory funnel and extracted with 45 mL portions of acetonitrile. The acetonitrile extracts were evaporated to dryness under a vacuum using a rotary evaporator at a temperature not exceeding 40°C. The residue was dissolved in 2 mL methanol and used for thin layer chromatography (TLC). After multiple trials of different solvent mixtures, the mixture of petroleum ether and benzene (1:1, v/v) was selected as eluent for the development of the TLC plate. The obtained extract (15 µL) was spotted on the TLC plate along with the BHT standard (4 µL) and a TLC plate was developed. Gibb's reagent was sprayed on the developed TLC plate and dried at 103 ± 2 °C for 15 min to visualize the clear spot. The Retention factor (Rf) of sample extracts was compared with the BHT standard.

R_f= Distance traveled by solute from the baseline / Distance traveled by the solvent from baseline

The limit of detection (LOD) for the above protocol was determined by spiking pulverized pickle samples with various quantities (1000 ppm, 500 ppm, 100 ppm and 50 ppm) of commercially purchased BHT crystals. LOD of the protocol was found to be 1000 ppm, below which spots were not visible.

Qualitative estimation of Sudan IV dye

Sudan IV dye was extracted from the pickles samples using the extraction protocol given by Singh et al., 2017 with slight modifications**.** The pickle sample (5 g) was weighed and homogenized by adding ethyl acetate (20 mL). The mixture was centrifuged at 3000 rpm for 5 min. The supernatant was filtered through a cotton plug and was diluted 10 times before scanning in a UV double-beam spectrophotometer. The limit of detection was determined for the above protocol by spiking pickle samples devoid of Sudan IV with various quantities (5 ppm, 10 ppm, 25 ppm, 50 ppm and 75 ppm) of commercially purchased Sudan IV dye. Extraction and spectrophotometric analyses were carried out by following the exact above-mentioned procedure. The survey scan of all standards and ethyl acetate extracts of the pickles were carried out between 300 - 700 nm wavelength at a normal speed and step of 1 nm. Prior to the measurement of absorbance, baseline correction was done using ethyl acetate. The spectra shown by standard Sudan IV solution was used to compare the presence of Sudan IV dye in other pickle extracts.

Qualitative analysis of Metanil Yellow (MY)

Metanil yellow dye was extracted from the pickles samples by following the extraction protocol given by Kourani et al., 2020 with slight modifications.The pickle sample (1 g) was weighed and homogenized by adding an acetic acid solution. The mixture was shaken for 6 hours in a mechanical shaker and centrifuged at 3000 rpm for 5 min. The supernatant was collected, diluted with an equal volume of acetic acid and used for UV-scanning. In a separate test tube, 0.5 mL of the acetic acid extract was diluted with propanol (2.5 mL) mixed with 50 µL of 1N HCl. In the presence of MY, the HCl-acidified extracts are supposed to show a redshift of the absorption peaks. The limit of detection was determined for the above protocol by spiking pickle samples devoid of MY with various quantities (5 ppm, 10 ppm, 25 ppm, 50 ppm and 75 ppm) of commercially purchased MY dye. Extraction and spectrophotometric analyses were carried out by following the exact above-mentioned procedure. The survey scan of all standards, acetic acid extracts and acetic acid-HCl extracts was carried out between 300 - 700 nm wavelength at a normal speed and step of 1 nm. Prior to the measurement of absorbance,

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baseline correction was done using ethyl acetate. The spectra shown by standard MY solution was used to compare the presence of MY dye in other pickle extracts.

RESULTS AND DISCUSSION

The results from quantitative estimation of BA showed its presence in all 15 samples (Fig.2). Among the conventional pickles, one out of eight exceeded the permitted level of 250 ppm. It showed that the number of cases of indiscriminate use of chemical preservatives in pickles is not very common. However, such a violation can pose an immense threat as the detected level exceeded the permissible limit by over 3 times. On the other hand, all "marketed as organic" and certified organic pickles showed the presence of BA. According to NPOP, organic products should not have any BA (Directorate of Marketing and Inspection, 2005) This demonstrates a sheer violation of rules and regulations set up by the food safety regulatory bodies (Directorate of Marketing and Inspection, 2005; FSSAI, 2018) and also exhibits mis-labeling of the products.

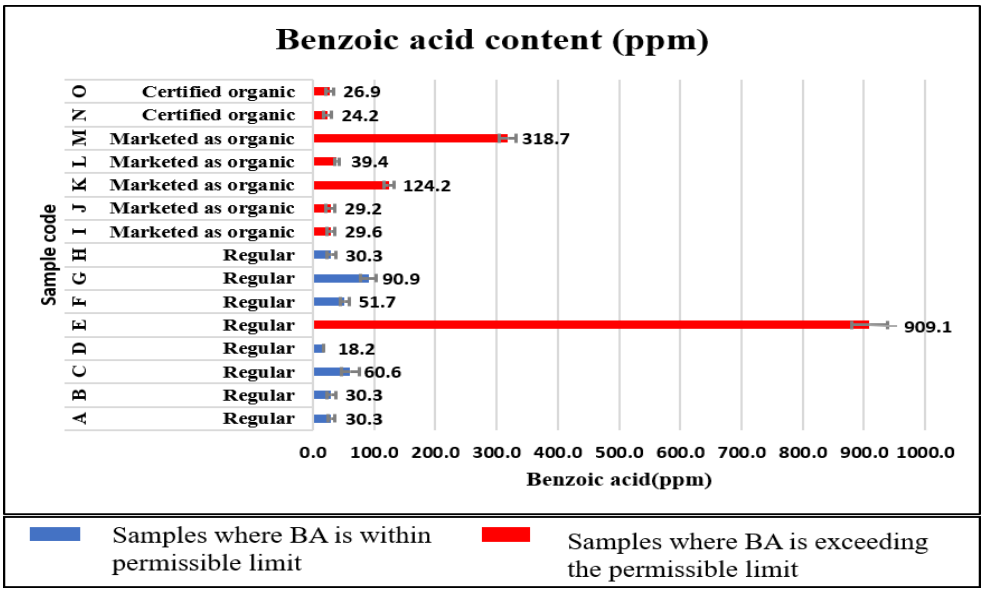

Figure 2: Benzoic acid content in pickle samples

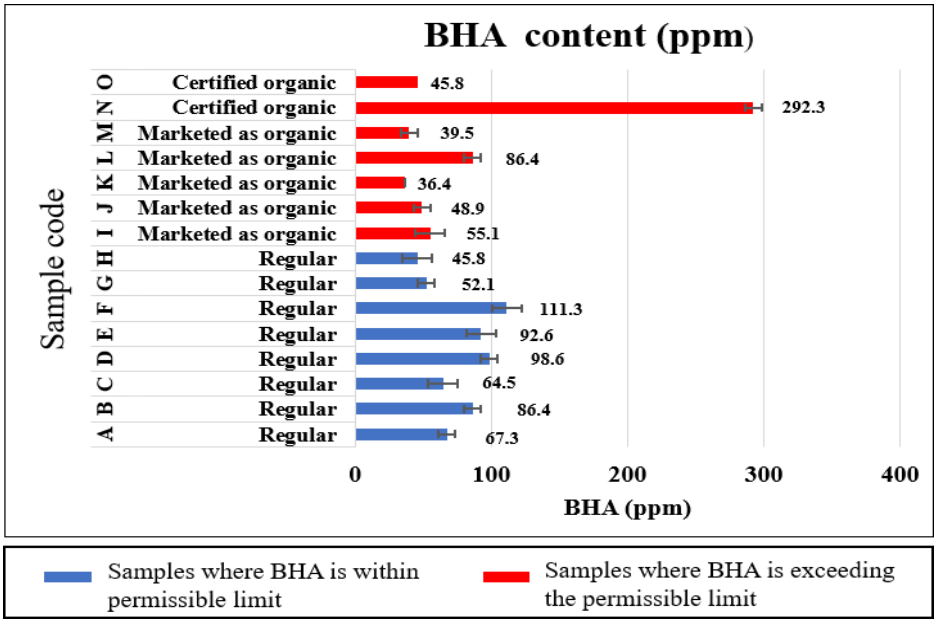

Figure 3: BHA content in pickle samples

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The results from quantitative estimation of BHA showed its presence in all 15 samples (Fig. 3). All the conventional pickles were found to contain BHA within the permitted level of 200 ppm. It clearly indicates that the manufacture of conventional pickles is well supervised in terms of BHA. On the contrary, the results with the remaining seven "marketed as organic" and "certified organic" pickles are quite disappointing. All seven samples showed the presence of BHA though NPOP certification of organic products specifically forbids its presence in processed fruit and vegetable products. The result clearly shows the lack of regulation in the usage of additives for producing "organic". One of the certified organic samples (Sample N, turmeric pickle) had BHA in superfluous amounts suggesting indiscriminate use of BHA. However, another plausible reason for this observation might be the interference from curcumin, a natural antioxidant present in abundance in turmeric. Being structurally very similar in terms of phenolic groups, curcumin may react with the testing reagent (Gibb's reagent) and show up as BHA in the analysis.

Qualitative estimation of BHT by TLC indicated that BHT is absent in all 15 samples as none of the spots in any sample extract showed the same Rf as the standard (0.94) (Table1). The outcomes from the qualitative analysis of BHT indicated that all 15 samples were devoid of BHT or may contain in quantities lower than the LOD of the protocol (1000 ppm).

Qualitative testing of Sudan IV was done by UV-Vis spectrophotometry. None of the samples showed the characteristic peak of Sudan IV at 350 nm and 520 nm (Fig.4). The results indicated that all the 15 samples either did not contain Sudan IV or contained below the LOD of the protocol (25 ppm).

Qualitative testing of MY was done by UV-Vis spectrophotometry. The findings (Fig. 5) from the qualitative estimation of Metanil yellow also showed that none of the 15 samples contained Metanil yellow or may contain below LOD (25 ppm).

Table 1: Comparison of R_f values of the pickle extracts with that of BHT standard

Sample	Category	Rf value	Presence of BHT
Standard BHT	Analytical grade	0.94	-
A	Regular	0.00	Absent
B	Regular	0.08	Absent
C	Regular	0.00	Absent
D	Regular	0.26	Absent
E	Regular	0.59	Absent
F	Regular	0.36	Absent
G	Regular	0.00	Absent
H	Regular	0.15	Absent
I	Marketed as organic	0.28	Absent
J	Marketed as organic	0.00	Absent
K	Marketed as organic	0.62	Absent
L	Marketed as organic	0.38	Absent
M	Marketed as organic	0.63	Absent
N	Certified organic	0.83	Absent
O	Certified organic	0.00	Absent

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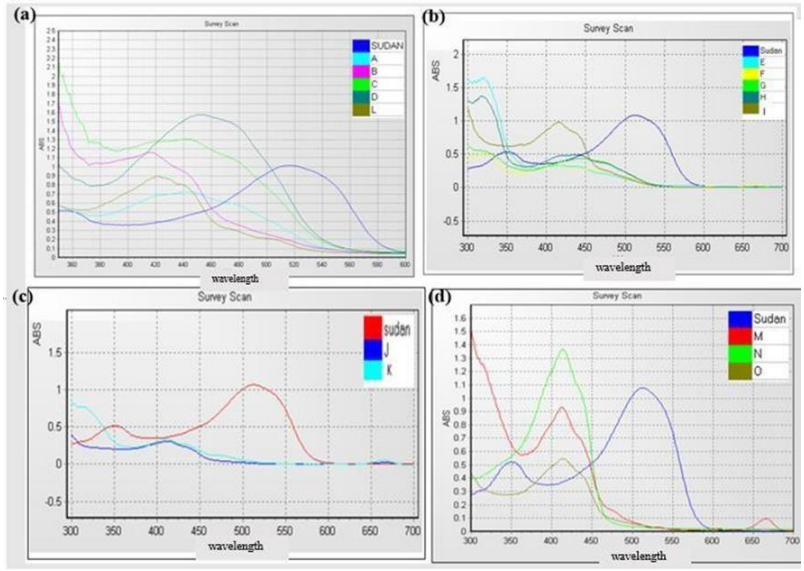

Figure 4: UV-Vis absorption spectra for Sudan IV standard and sample A-D and L (a), E-I (b), J-K (c) and M-O (d)

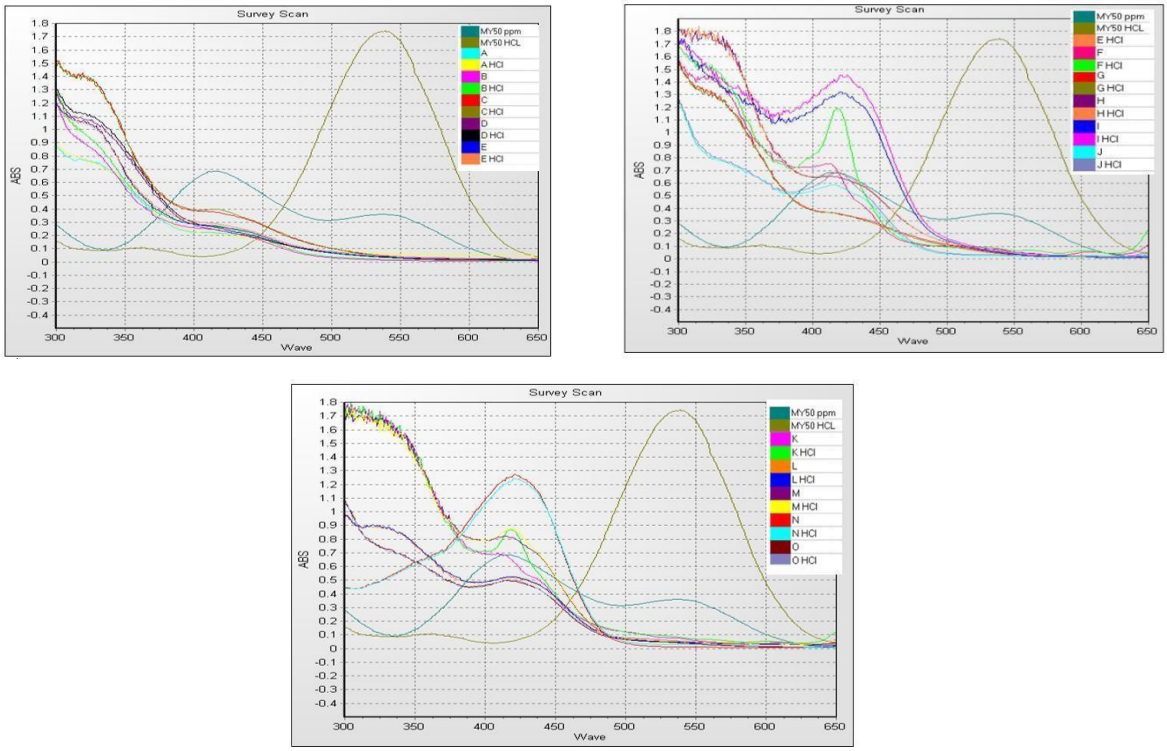

Figure 5: UV-Vis absorption spectra for Metanil Yellow standard and sample A-D and L (a), E-I (b), J-K (c) and M-O (d)

CONCLUSION

According to this study, it can be concluded that the usage of food colors such as Sudan-IV and Metanil yellow is satisfactorily regulated in the pickle manufacturing sector in India. BA and BHA are regulated satisfactorily in conventional pickles. However, the situation is disappointing in both "certified organic" and "marketed as organic" products. The usage of BHT was found to be well-regulated in all three categories. The findings of this project are extremely important, as these help to evaluate the real scenario of the usage of additives in Indian branded pickles. However, this study is not devoid of limitations. The number of pickles tested from each category could be

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more and uniform. Limited resources and logistics restricted the sample number chosen for the analysis.

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CONFLICTS OF INTEREST

The authors report that there are no competing interests to declare.

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APPENDIX-1: STANDARD CALIBRATION GRAPH OF STANDARD BHA