AMERICAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY (AJFST)

ISSN: 2834-0086 (ONLINE)

VOLUME 3 ISSUE 2 (2024)

PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

American Journal of Food Science and Technology (AJFST)

Volume 3 Issue 2, Year 2024
ISSN: 2834-0086 (Online)
DOI: https://doi.org/10.54536/ajfst.v3i2.3786
https://journals.e-palli.com/home/index.php/ajfst

Detection of Adulteration in Besan (*Cicer Arietinum*) Using Microscopic Technique

Padmapriya Rajan[1], Kyatanahalli S. Nagabhushana[1*]

Article Information

Received: September 11, 2024

Accepted: October 14, 2024

Published: November 30, 2024

Keywords

Birefringence, Besan, Cicer Arietinum, Food Adulteration, Polarized Light Microscope

ABSTRACT

Detection of food adulteration in an unambiguous way requires a methodology that provides distinct differentiators between the authentic and adulterated samples. We present a simple method to detect the adulteration of Besan (chickpea flour), an extensively used food ingredient for different food products in the Indian culinary industry. As many as 10 different samples, both branded and non-branded samples, were procured from the market and were evaluated for their purity. Polarized light Microscope was used as a simple evaluation tool to assess the purity of the sample. The quality of the image in light, polarized light, polarized light with analyzer, and dark mode bring about a clear distinction between pure besan and adulterated sample. Sample analyses were done in 50 X to 1000 X magnification. This non-destructive analysis of the sample brings about even a very small degree of adulteration in the sample. From the analysis, it was clear that out of 10 samples tested, 5 samples were seen as adulterated. The chief adulterants were yellow pea flour and corn flour. This simple method provides easy detection of adulterants and adulteration in food samples.

INTRODUCTION

Food adulteration is one of the major pervasive businesses which stimulate a lot of health issues among consumers and is a very crucial thing to be considered from an economic point as well. This intentional addition of foreign substances to food products tends to benefit the business but affects the product's nutritional value. Though it has been a historical problem for centuries finding the simplest and authentic technique that could reveal the mixing of foreign substances in food samples is still a challenging problem. Because the common adulterants are harmless cheap food products (or) dangerous chemicals which has the same physical nature as the original food sample. It also affects the quality of food products, and the awareness about food safety procedures is yet to be explored by vendors (Oseyemi, 2023). On the other hand, ensuring the quality of food products also involves extensive experimental procedures (Júnior *et al.*, 2023). So, finding the simplest non-targeted method to identify adulterants would also act as a preliminary test to ensure the quality of the food products. Many analytical techniques like HPLC, TLC, GC-MS, LC-MS, UV-Vis, NMR, and FTIR are available to detect adulteration but sample preparation and statistical procedures to analyze the spectral data are yet time-consuming. Microscopy techniques are one of the easiest ways to find adulterated substances but they are still to be explored as authenticated techniques for adulteration in the food industry. Chickpea (*Cicer arietinum*) flour or Besan is a rich source of proteins and an indispensable flour used in most Indian traditional sweets and snacks. Because of its high demand in India, it is rather being adulterated with low-cost foreign substances like corn flour and other legume flours. Besan is the safe choice for people suffering from diabetes as it contains high fiber and protein and less starch content. Rice and corn flour are not a good choice because of its high starch content. The exact difference in the nutrients of besan and corn flour has been well discussed in the literature (Vinod *et al.*, 2023). The protein content of besan is 22% and for corn flour, it is around 6.9%, mixing of these two flours causes severe protein deficiency. The total carbohydrate content of besan is 58% and for corn flour, it is 76%, and another important factor is the nature of the starch. Besan has resistant starch (RS) which gets digested slowly, so it causes a lower spike in blood glucose levels. Corn flour has native starch which increases the spike in blood glucose levels. Mixing these two flours creates a severe nutritional imbalance in diabetic patients as well as others. Yellow peas (*Pisum sativum*) are another common foreign material that could be easily mixed with besan since it has the same physical nature as the besan. *Pisum sativum* has 70% carbohydrates, particularly starch content varies from 39% to 46% and it is also rich in proteins which is about 25% (Wu *et al.*, 2023). From an economic point of view, consumers are supposed to pay more money than the actual money for the besan when they mix these adulterants with the besan. Finding these substances in besan is yet a difficult task because of its similar physical nature as other flours chemometric methods have become prominent in the food industry in analyzing data through spectroscopic methods. On the other hand, non-targeted method is a recent trend in identifying unknown components in the actual food sample (Gao *et al.*, 2019). In the case of besan, adulteration of grass pea flour and pea flour in chickpea flour has been found by using Near-infrared reflectance spectroscopy along with chemometrics (Bala *et al.*, 2022). Similarly, the addition

[1] Prayoga Institute of Education Research, SY No. 133, Ravugodlu, Bolare Post, Off Kanakapura Road, Bangalore, India
[*] Corresponding author's e-mail: ksn@prayoga.org.in

of maize flour to chickpea flour has also been predicted through near-infrared spectroscopy followed by a partial least square regression method (Bala *et al.*, 2022a). It was also mentioned that there are no chemical methods to identify the adulteration of corn flour in besan, so the urgency to find the technique to differentiate besan from corn flour is increasing among researchers. Microscopy technique has been used to find the adulteration of pea flour in chickpea flour based on seed testa macrosclereids (Dattatreya *et al.*, 2010). Polarized light Microscopy is one of the newly emerging authentic techniques to identify the foreign substances in food samples. Recently, it has been used to identify the percentage of "wheat flour in Galician bread" (Fernández-Canto *et al.*, 2022). In this work, we propose the microscopic technique to find the adulteration of maize flour and yellow pea flour in chickpea flour based on the physical difference between the particles through stereo microscopy and the physical difference between the 'Maltese cross' pattern of both the particles under polarized light microscopy.

MATERIALS AND METHODS

Preparation of Samples

To prepare the pure besan Bengal gram dal was grained and sieved with the appropriate consistency of besan which is available in the market and corn flour has been purchased from the local market in Bangalore, Karnataka. A small amount of besan which ranges from 0.005g to 0.009g was placed on the microscopic slide and mixed with two to three drops of water then the slide was allowed to dry at room temperature. Once dried, a small drop of glycerin is added and it is spread over the surface of the slide for better visualization. The same procedure has been followed for the corn flour, yellow pea flour, and all branded samples as well as with the mixture of besan with adulterants.

Methods

Polarizing Microscope "Leica DM750M" has been used to take the images of the sample. All the images have been taken under four modes. This microscope is equipped with one polarizer, and one analyzer with the non-rotating mechanical stage to place the sample.

First Mode

Once the Polarizer and Analyzer are removed it acts as a normal stereo microscope. So, the first image has been taken under normal conditions.

Second Mode

In this mode, the polarizer has been inserted before the sample stage and the images have been taken under polarized light.

Third Mode

The analyzer has been inserted above the sample stage and the image has been taken with both polarizer and analyzer.

Fourth Mode

In this mode, the polarizer has been rotated to get the dark field mode where the direction of the polarizer is exactly perpendicular to the direction of the analyzer. This has been verified by using cotton. Since cotton has the birefringence property by nature it reveals the colors when it is placed under exactly crossed polars. By doing this experiment the dark mode of the microscope has been confirmed.

Birefringence Property of Starch

Starch has a semi-crystalline nature which owns the property called birefringence by nature and can produce 'Maltese cross' patterns under crossed polars. Many microscopy techniques have been utilized in the food industry so far, among them polarizing light microscopy has been specifically used to characterize the food products that have optical anisotropy (or) birefringence and the 'Maltese cross' pattern of starch granules before and after heat treatment had been recorded earlier (Corradini & McClements, 2017). The clear-cut image of this pattern under 700X has also been reported in the literature (Sivak & Preiss, 1998). Along with that, it is also evident that the morphology, crystallinity, and thermal properties of different types of starch granules from wheat have been well discussed through light microscopy (Kumar *et al.*, 2016). This property had been used to identify the presence of starch in food components

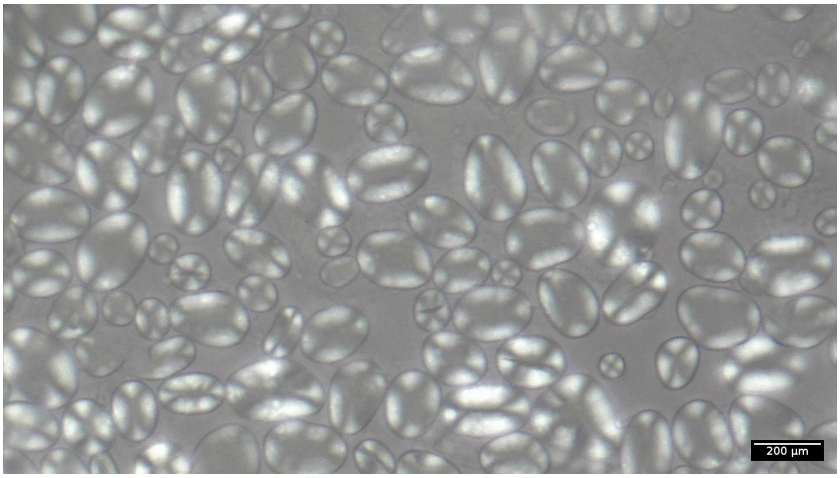

Figure 1: Besan under dark mode (500x)

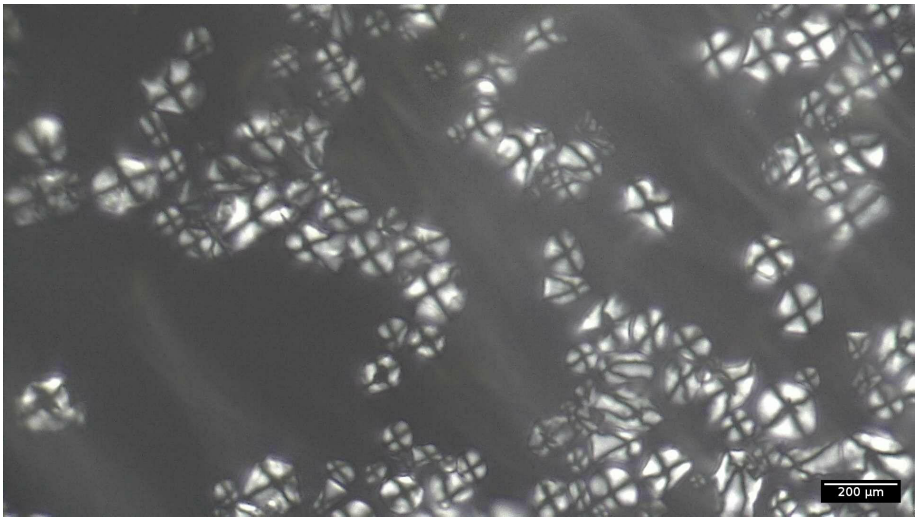

Figure 2: Corn Flour under dark mode (500x)

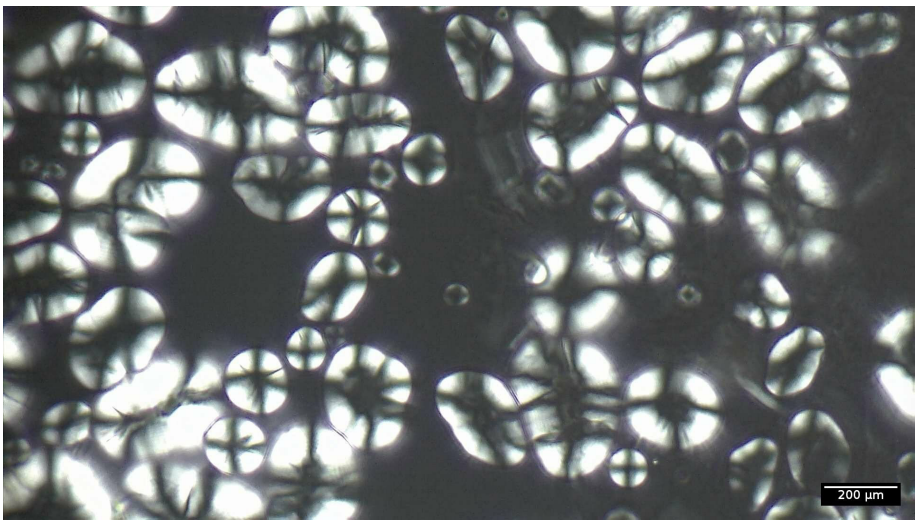

Figure 3: Yellow pea flour under dark mode (500x)

and particularly it was observed that corn starch had a polygon shape (McMahon, 2004). In this study, all three flours have starch but the nature of the starch differs. While besan and yellow pea flour have resistant starch, corn flour possesses native starch. Accordingly, all three flours produce different interference patterns under crossed polars and this pattern also varies between the size, shape and nature of the particles. Figure 1, Figure 2, and Figure 3 have been taken under the dark mode of the polarization microscope which provides clear-cut difference between the interference pattern of all three samples. For besan, it is oval and corn flour has an exact 'Maltese cross' pattern. Yellow pea flour has a slightly differentiated pattern with a dark patch in the center along with cracked lines in its sides.

RESULTS AND DISCUSSION

The microscopic images of besan, corn flour, and yellow pea flour under all four modes provide unique features of each particle along with its size and shape. We intentionally mixed besan with corn flour and yellow pea flour in equal ratio and observation was made under all the modes, which helps us to differentiate all three samples through the naked eye easily. These images provide the clear-cut difference between the particle shape and size.

Besan

Besan particles are represented in Figure 4 and they are elliptical in shape, Figures 4a, 4b, 4c, and 4d represent besan particles in all four modes, Figure 4a is brighter as we pass light in all directions whereas Figure 4b and Figure 4c are slightly dark as we pass polarized light. Particularly, Figure 4c provides a slight structural difference because of the difference in the direction of the polarizer and analyzer. Finally, dark mode provides the interference pattern of besan particles which is an oval shape. It could be noted that a few besan particles have a thin line at their center but the size and shape of the particles are different from yellow pea flour and corn flour.

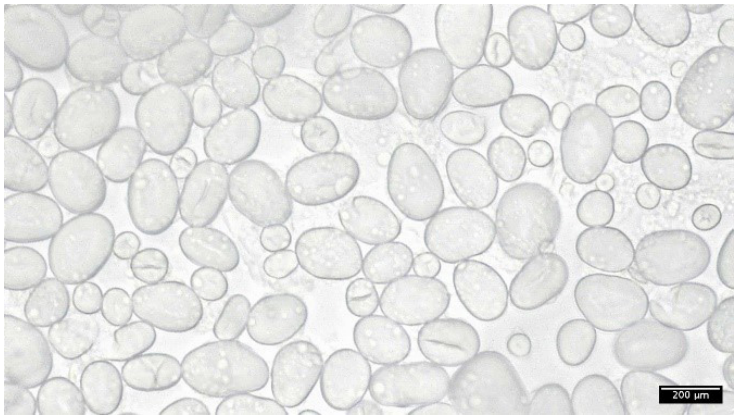

Figure 4a: First Mode

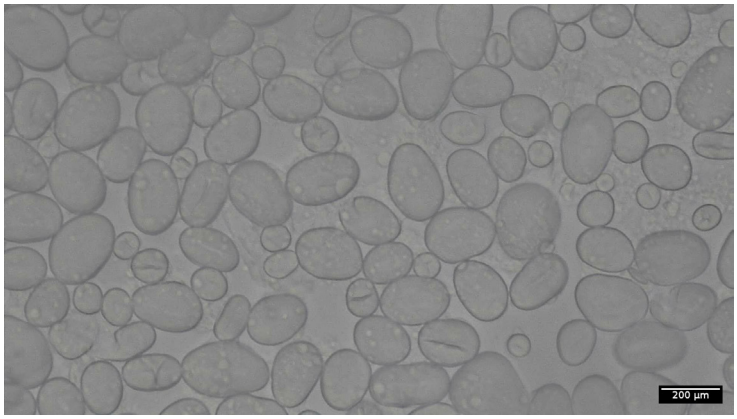

Figure 4b: Second Mode

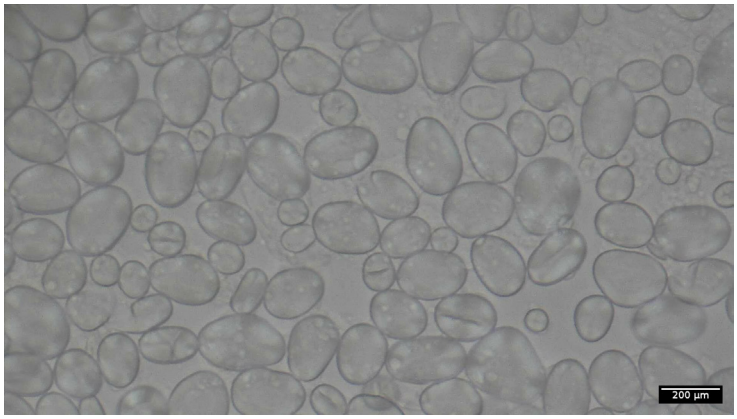

Figure 4c: Third Mode

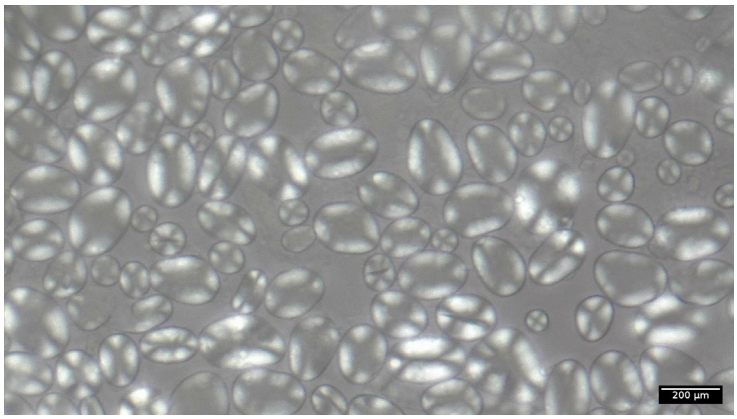

Figure 4d: Fourth Mode
Figure 4: Besan (500x)

Corn Flour

Corn flour particles are represented by Figure 5 and they have irregular polygon shapes with a central black dot in it. As we mentioned, the second mode and third mode don't provide much structural difference except for the variation in brightness. The fourth mode Figure 5d provides the exact 'Maltese cross pattern' under crossed polars because of the nature of the particles.

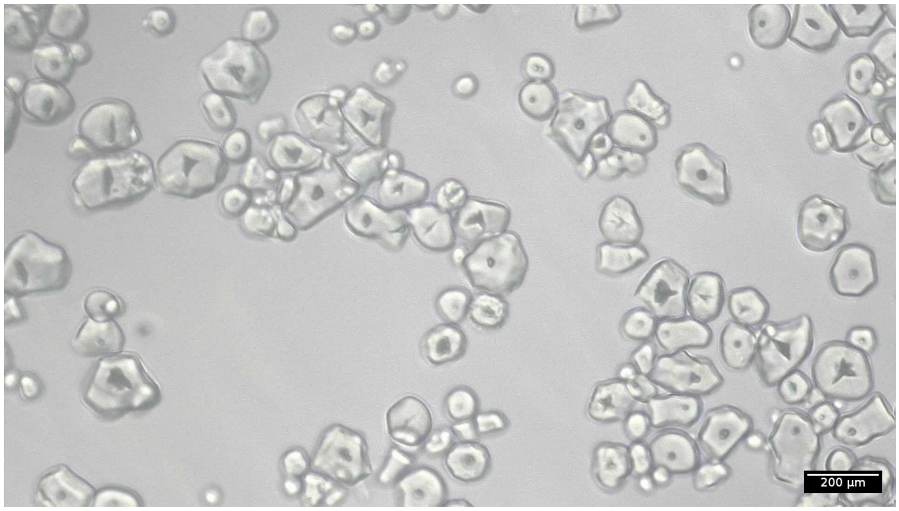

Figure 5a: First Mode

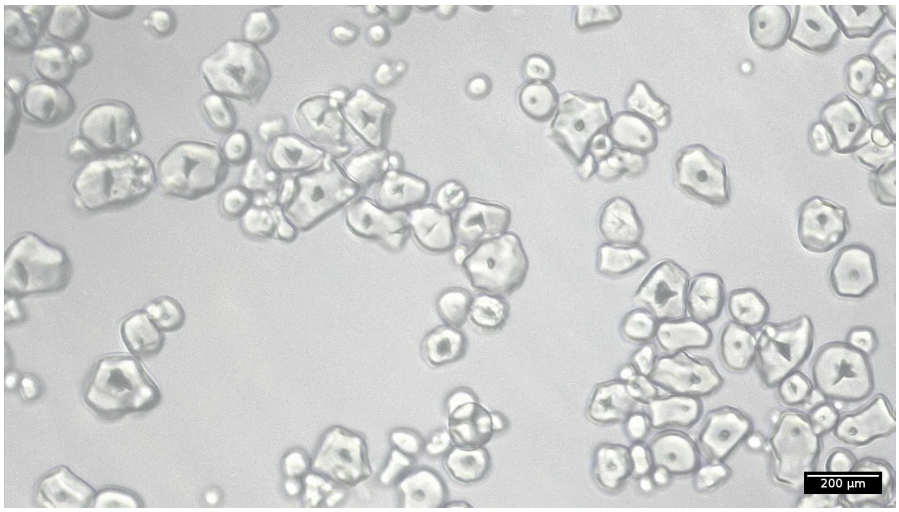

Figure 5b: Second Mode

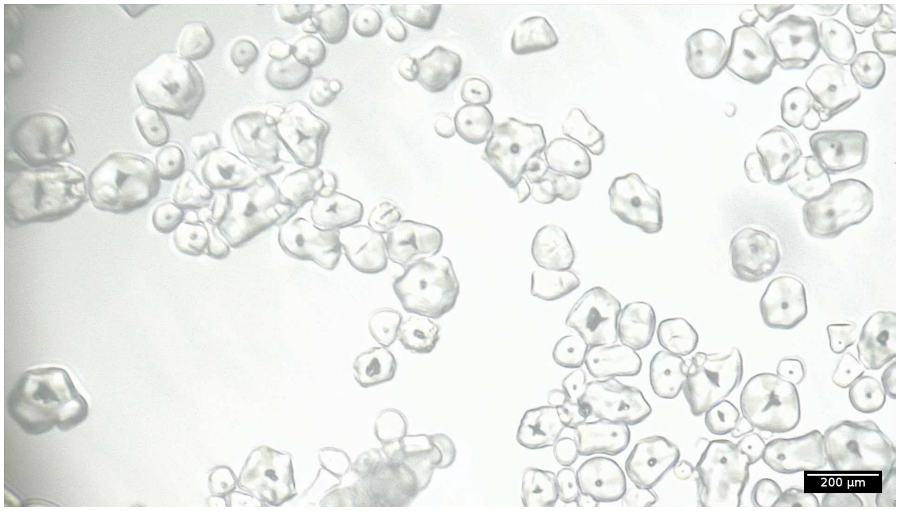

Figure 5c: Third Mode

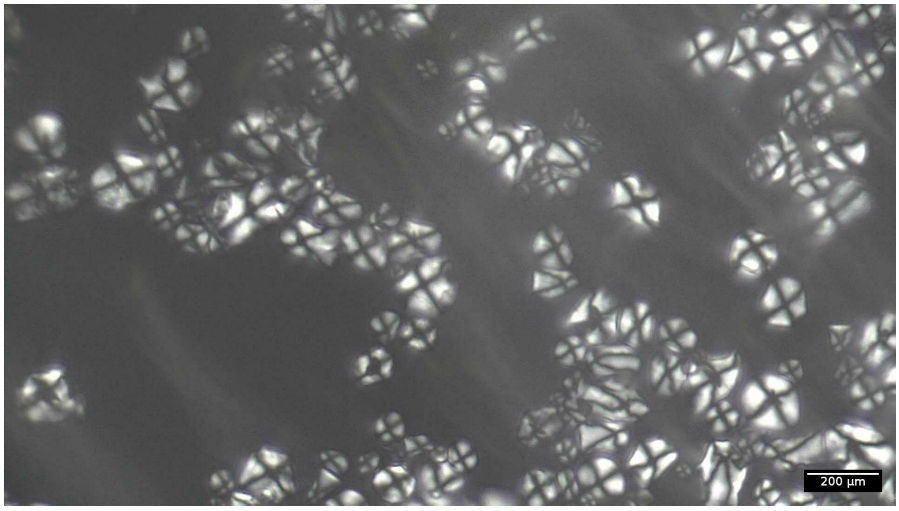

Figure 5d: Fourth Mode
Figure 5: Corn Flour (500x)

Yellow Pea Flour

Yellow pea flour particles are bigger in size and the internal structure of the particles varies based on their size. Also, some particles have a light crack inside them. These particles are represented by Figure 6, particularly, Figure 6c reveals the internal structure of the particle which is different from besan.

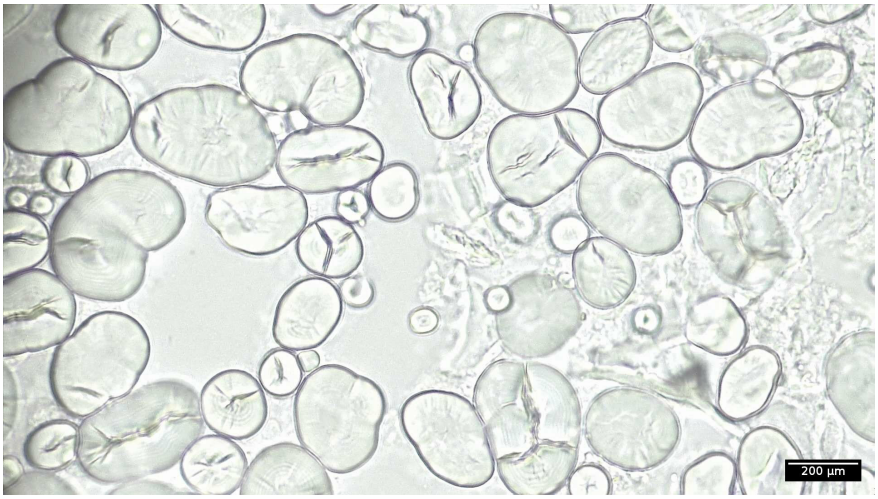

Figure 6a: First Mode

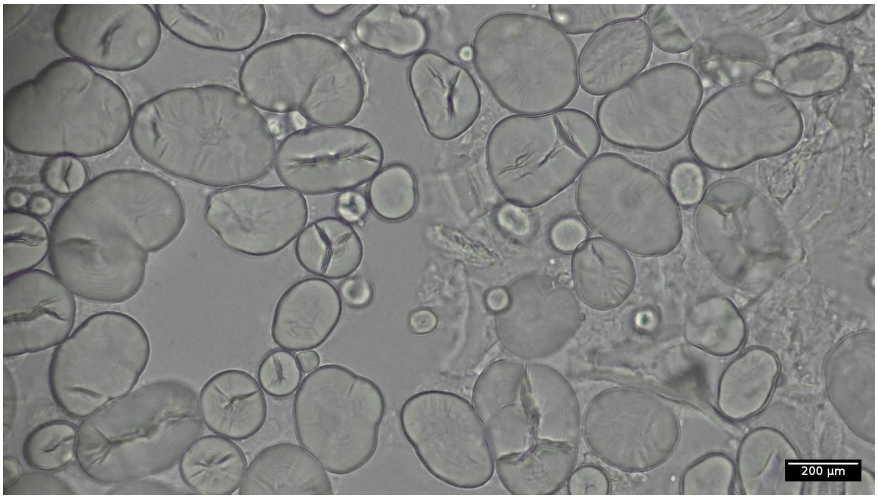

Figure 6b: Second Mode

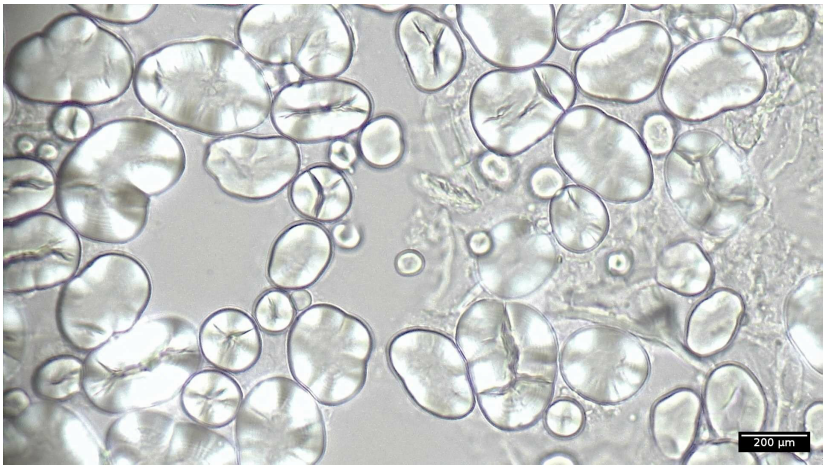

Figure 6c: Third Mode

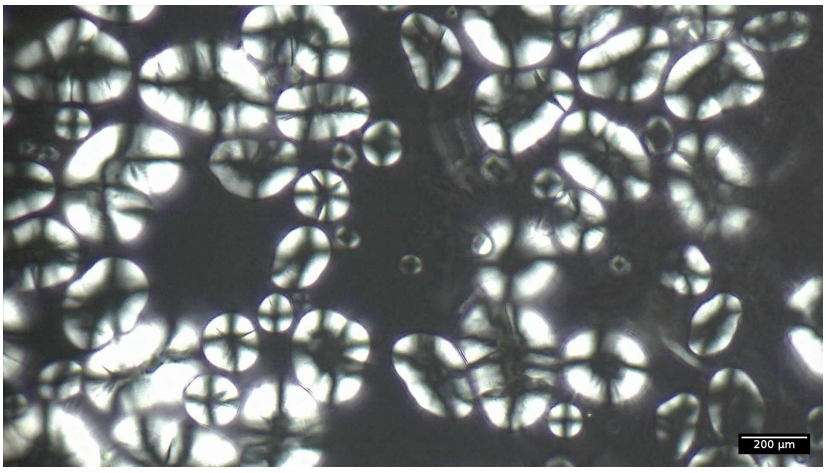

Figure 6d: Fourth Mode
Figure 6: Yellow Pea Flour(500x)

Besan with Adulterants

To see the possibilities of identification of adulterants in besan we mixed besan with corn flour and yellow pea flour with equal ratio and the samples were examined through the microscope under all four modes. Figure 7 and Figure 8 represent the mixture of besan with corn flour and the mixture of besan with yellow pea flour respectively. Adulterant particles could be easily identified by seeing the images through the naked eye Figures 7a and 7d provide the unambiguous difference between besan and corn flour, similarly, Figures 8a,8c, and 8d provide the unambiguous difference between the besan and yellow pea flour.

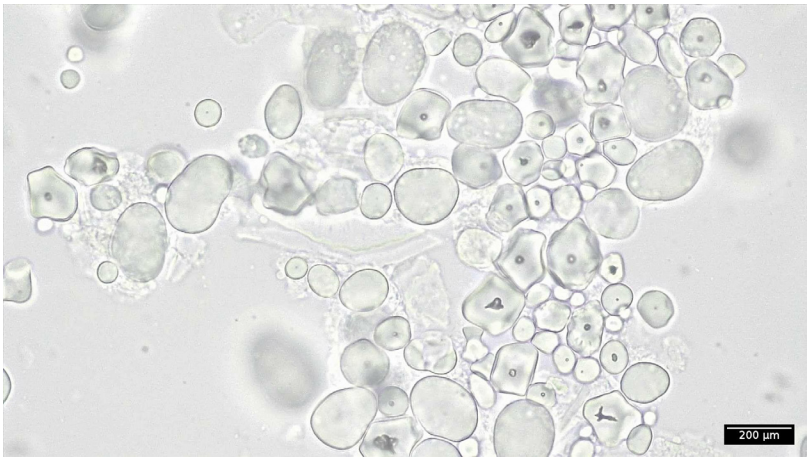

Figure 7a: First Mode

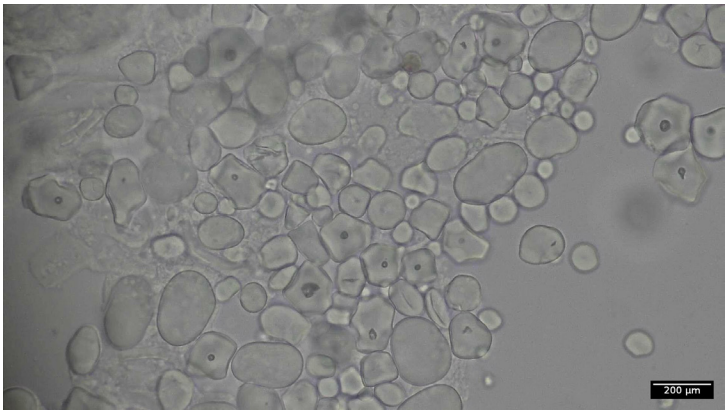

Figure 7b: Second Mode

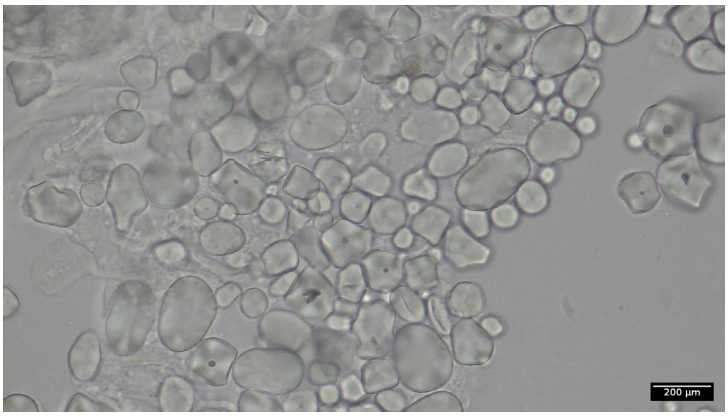

Figure 7c: Third Mode

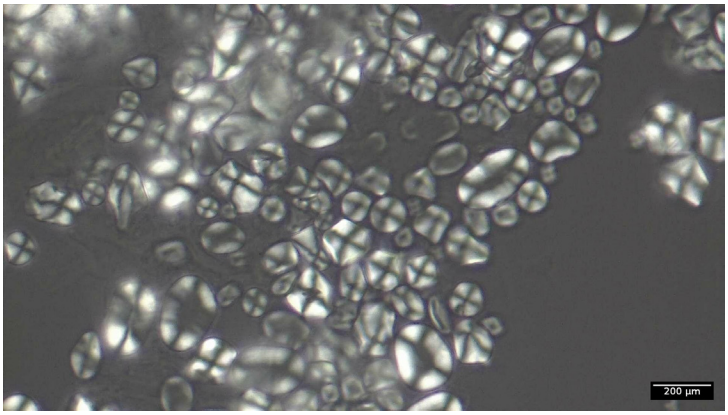

Figure 7d: Fourth Mode
Figure 7: Besan and Corn Flour (500x)

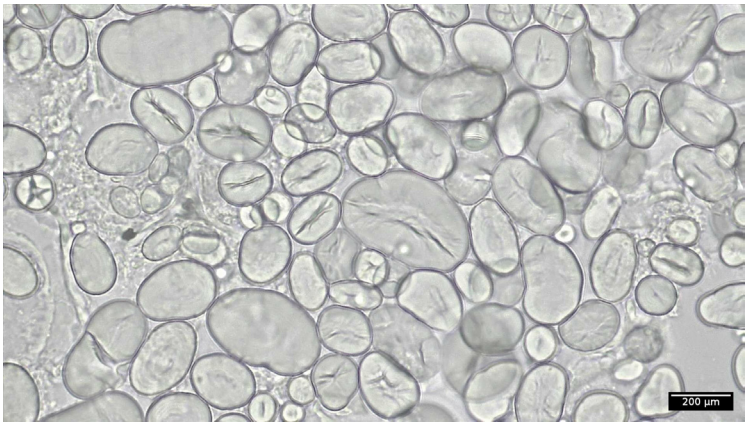

Figure 8a: First Mode

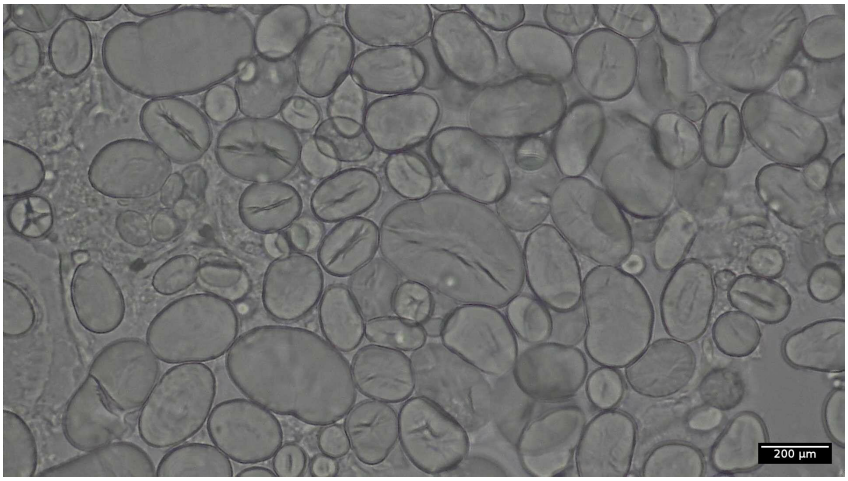

Figure 8b: Second Mode

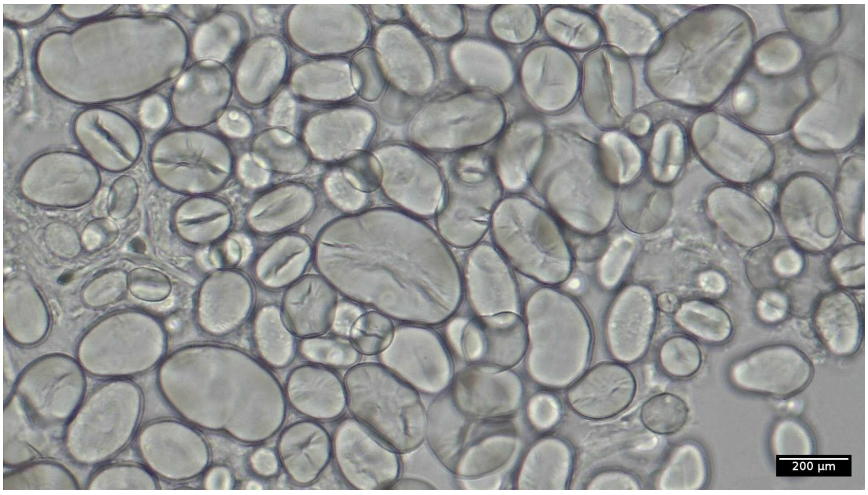

Figure 8c: Third Mode

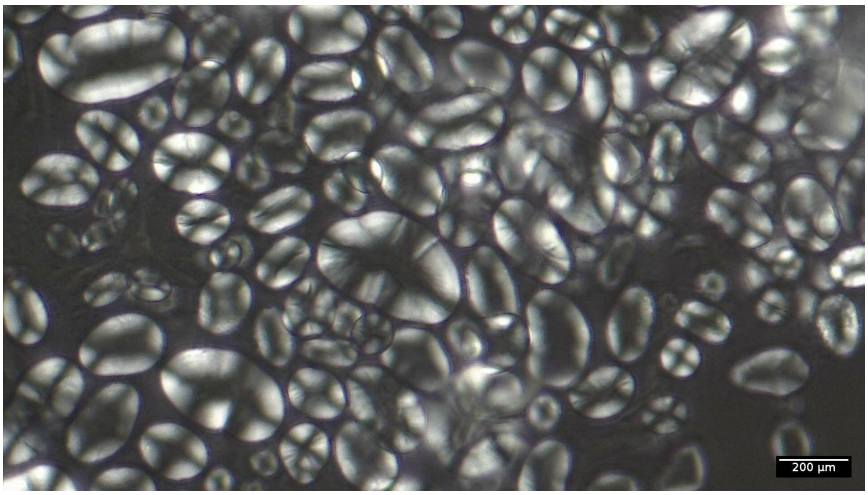

Figure 8d: Fourth Mode
Figure 8: Besan and Yellow Pea Flour(500x)

Market Samples

To see the practical applicability of this technique ten market samples have been purchased from the supermarkets in Bangalore and they were examined under a microscope under all four modes. Among them, five samples were adulterated with corn flour, yellow pea flour, and unknown adulterants. Around 0.005g of the samples were used from each pack and to prepare slides we followed the same procedure which we used for pure besan and adulterants.

Brand 1

This brand got adulterated with corn flour, Figure 9 represents this brand and it could be easily noted in 9a that two to three particles have irregular polygon shapes with a central dot and those particles reveal a 'Maltese cross pattern' in a dark mode which could be seen in 9d. This confirms the adulteration of corn flour in the sample.

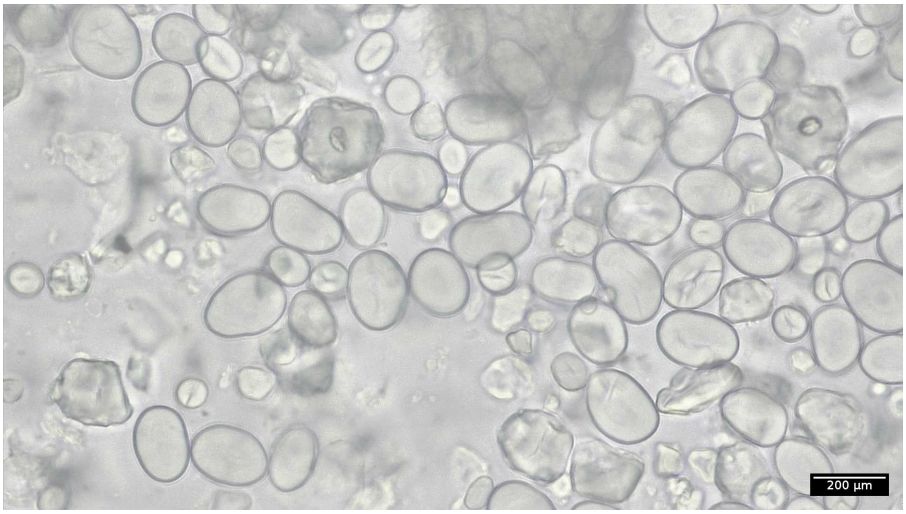

Figure 9a: First Mode

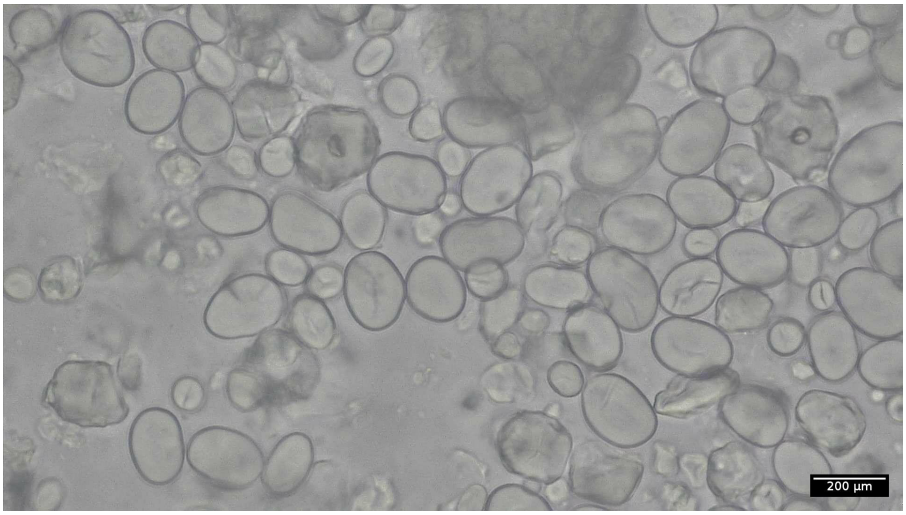

Figure 9b: Second Mode

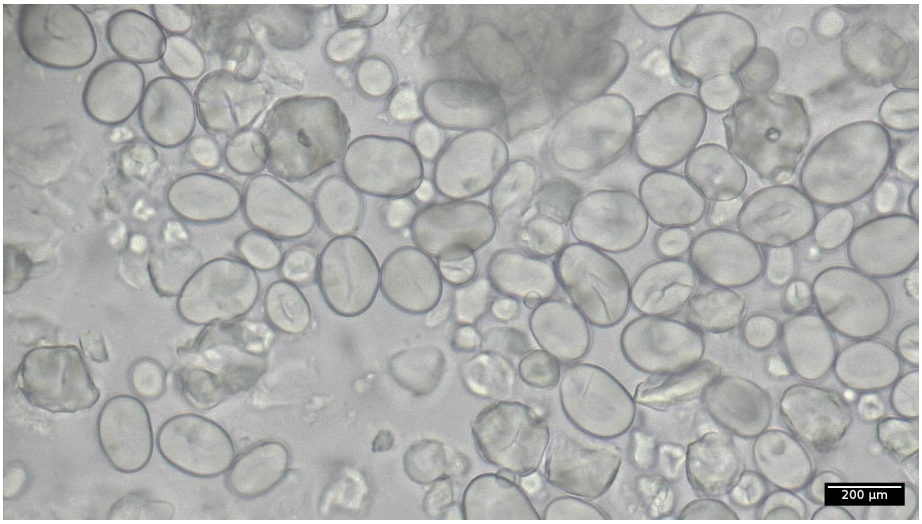

Figure 9c: Third Mode

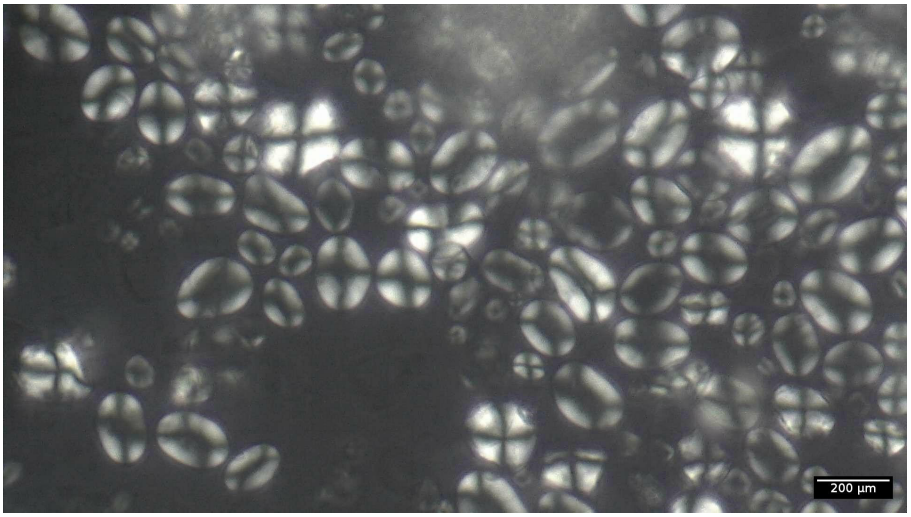

Figure 9d: Fourth Mode
Figure 9: Brand 1 (500x)

Brand 2

This brand is represented by Figure 10, it can be noted that a few particles are bigger, and from Figures 10c and 10d it is clear that the internal structure and interference pattern of these particles matches with yellow pea flour. So, it is confirmed that it is being adulterated with yellow pea flour.

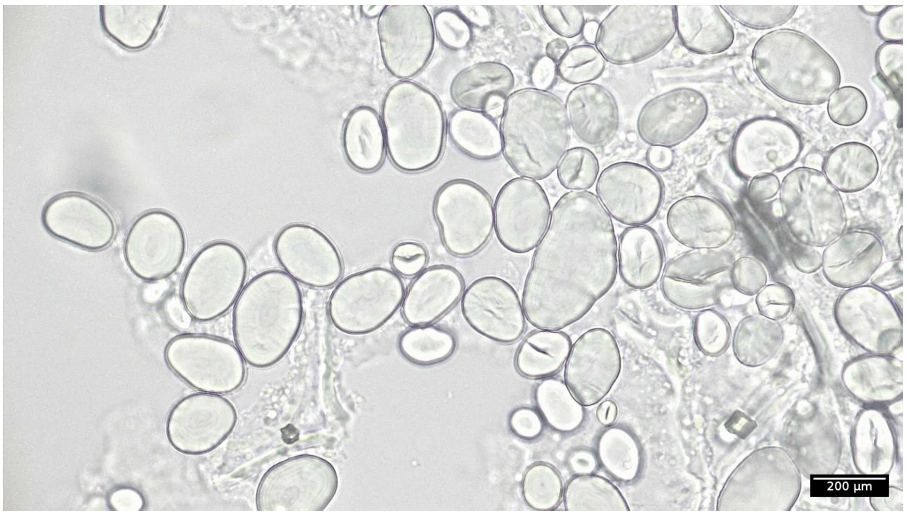

Figure 10a: First Mode

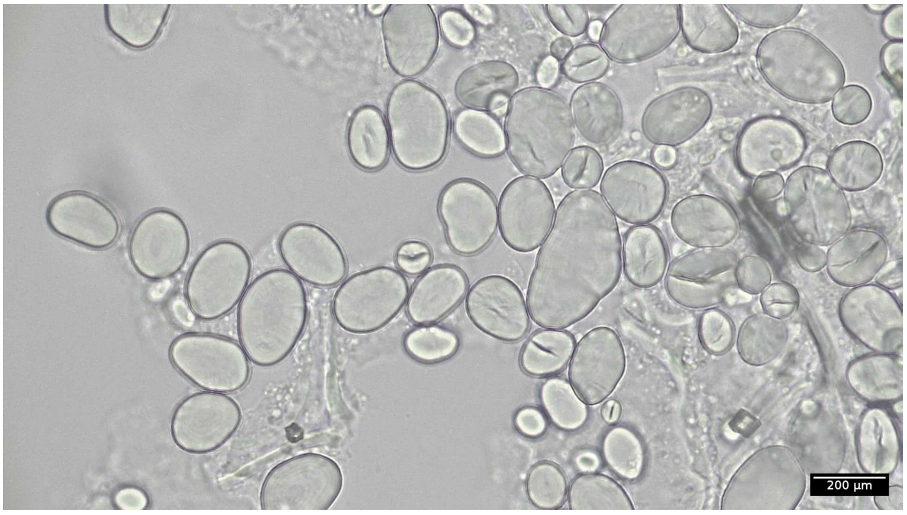

Figure 10b: Second Mode

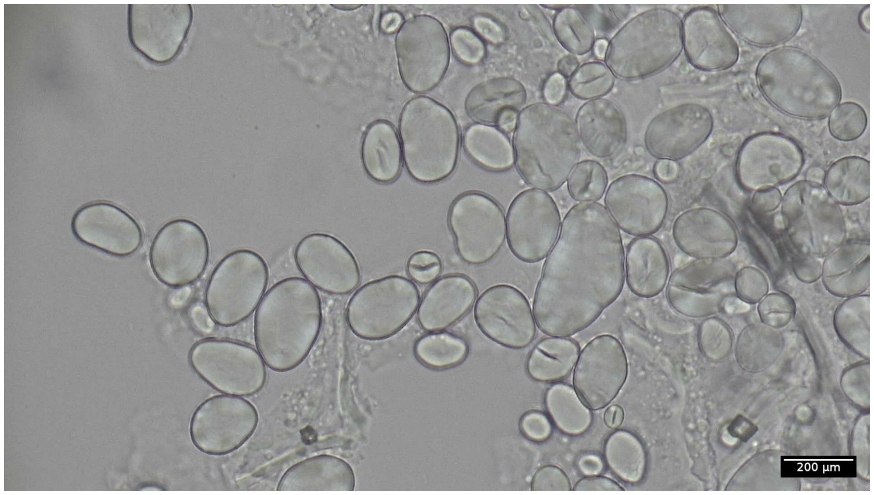

Figure 10c: Third Mode

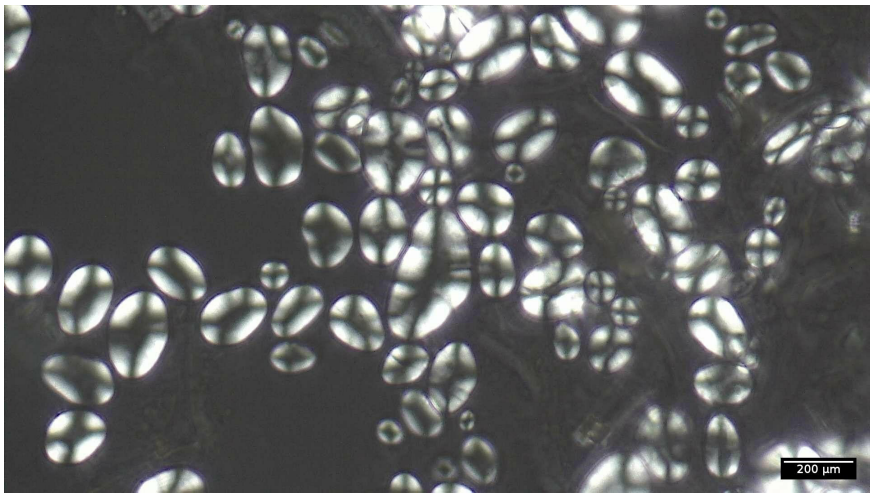

Figure 10d: Fourth Mode
Figure 10: Brand 2 (500x)

Brand 3

This brand is being adulterated with both corn flour and yellow pea flour, Figure 11 represents these particles and it could be noted in Figure 11a that a patch of particles and a few lone particles are in irregular polygon shape, and around two particles are bigger in size and shape which is similar to yellow peas. In dark mode those irregular polygon-shaped particles reveal a 'Maltese cross pattern' and the interference pattern of those bigger particles exactly matches with yellow pea particles. Hence, it could be confirmed that these particles are adulterated with both corn flour and yellow pea particles.

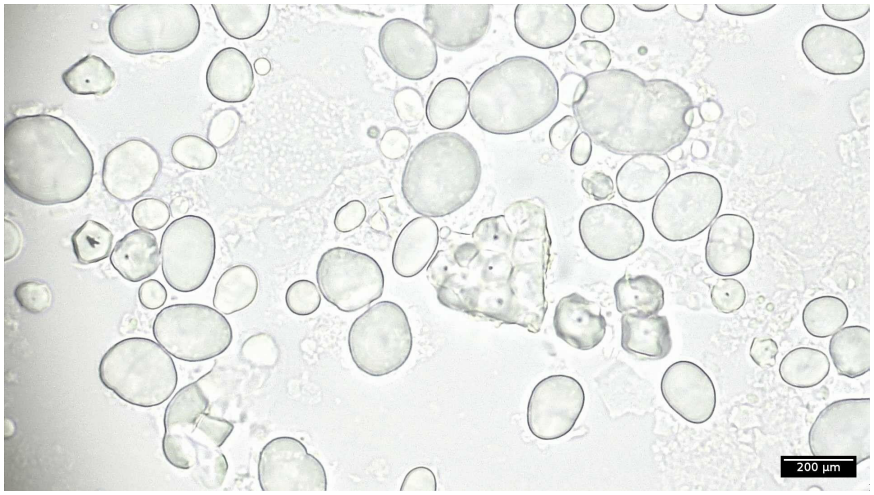

Figure 11a: First Mode

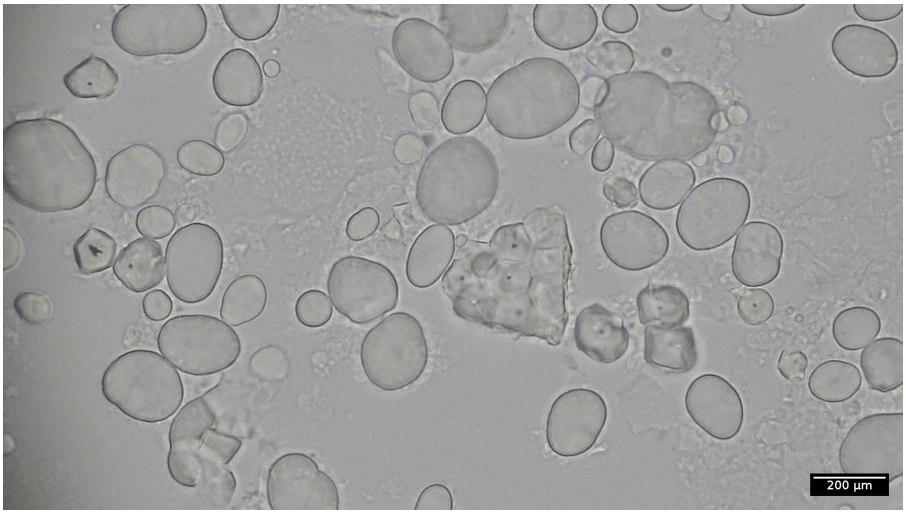

Figure 11b: Second Mode

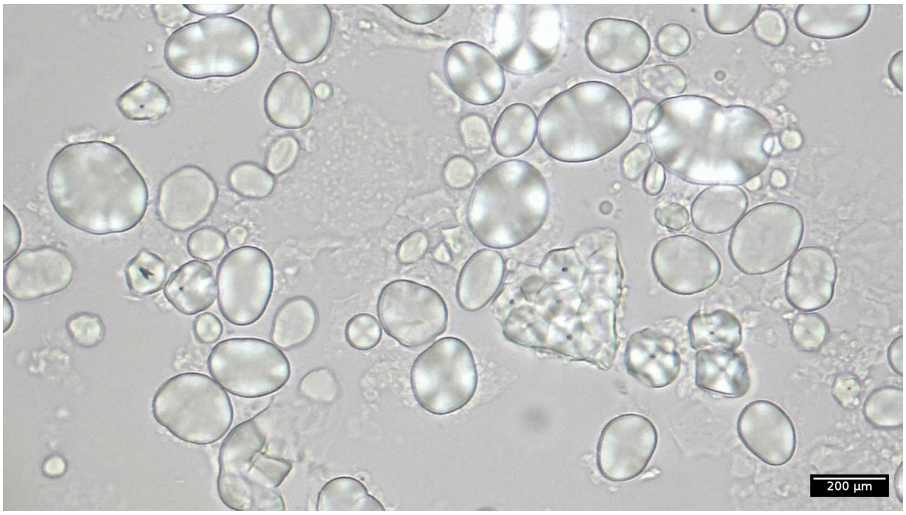

Figure 11c: Third Mode

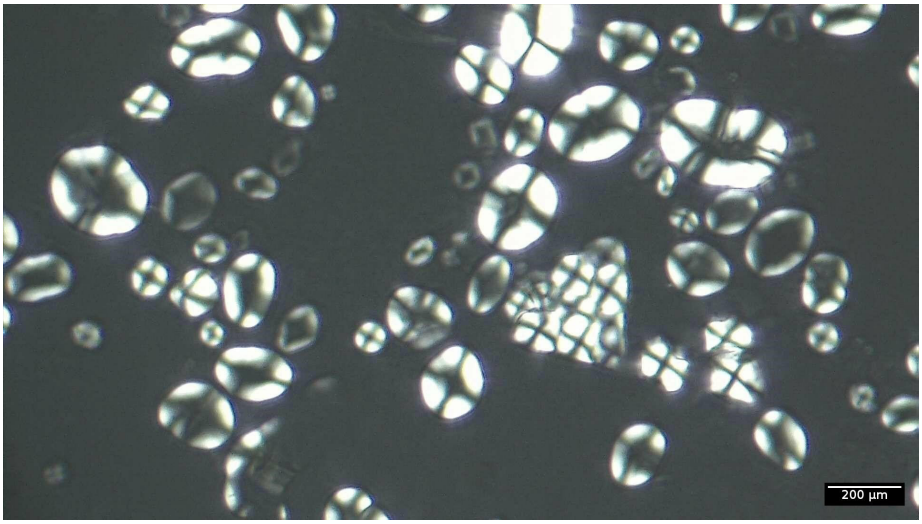

Figure 11d: Fourth Mode
Figure 11: Brand 3 (500x)

Brand 4

This brand is represented by Figure 12 around six particles having irregular polygon shape with a central black dot at the center and it also reveals the 'Maltese cross pattern' in dark mode. So, it could be confirmed that it is being adulterated with corn flour.

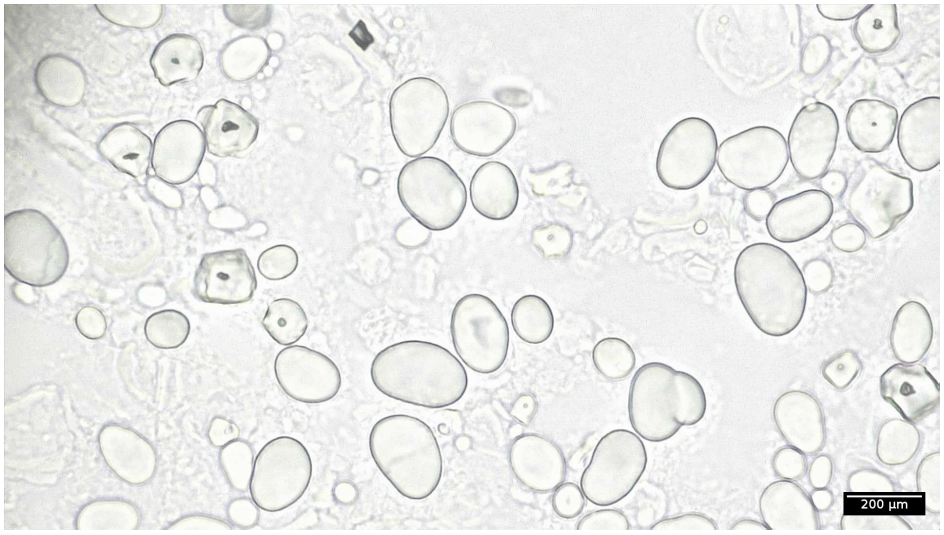

Figure 12a: First Mode

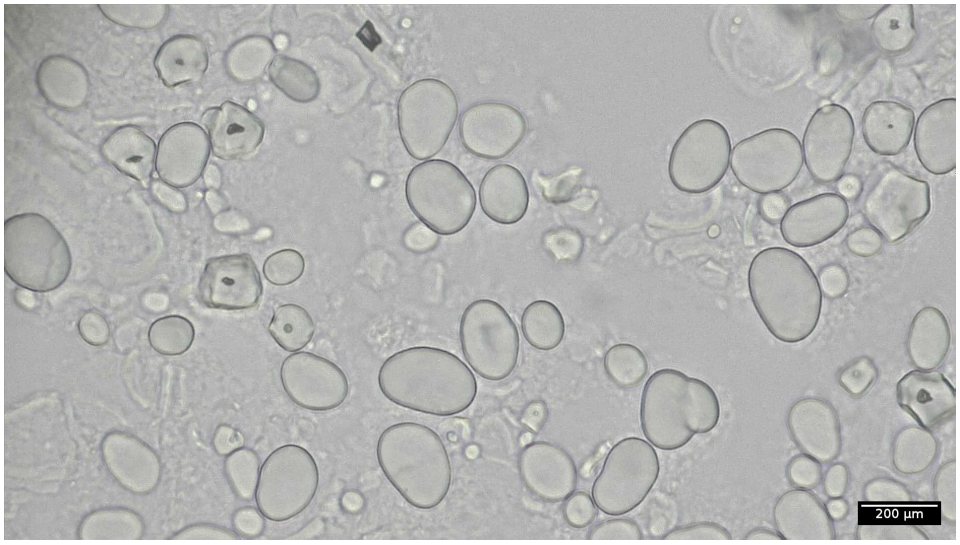

Figure 12b: Second Mode

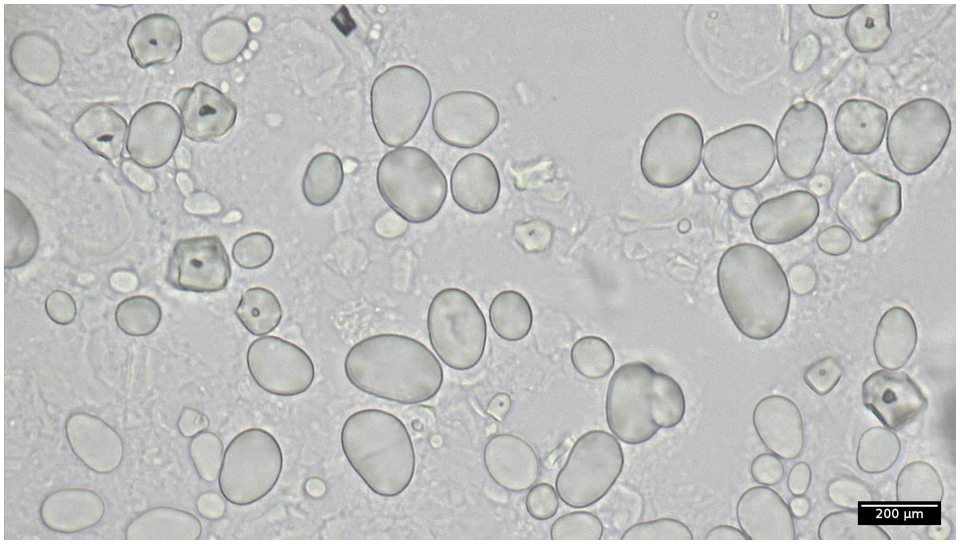

Figure 12c: Third Mode

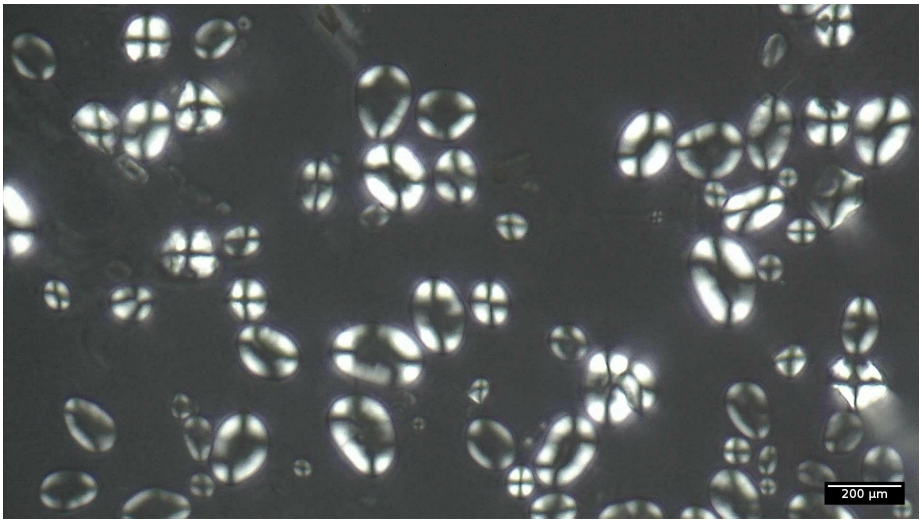

Figure 12d: Fourth Mode
Figure 12: Brand 4 (500x)

Brand 5

This brand is represented by Figure 13, almost half of the particles have some weird black patches inside them which cannot be seen in the pure besan particles. In Figure 13d also it could be noted that those particles reveal different interference patterns because of those dark patches which are not matching with the pure besan. Another important factor is these particles are not matching with corn flour particles and yellow pea flour particles also but it could be confirmed that it is being adulterated with some unknown foreign substances. So here this technique by using a polarization microscope acts as a non-targeted method to identify the adulteration hence it provides a clear-cut image of pure besan particles.

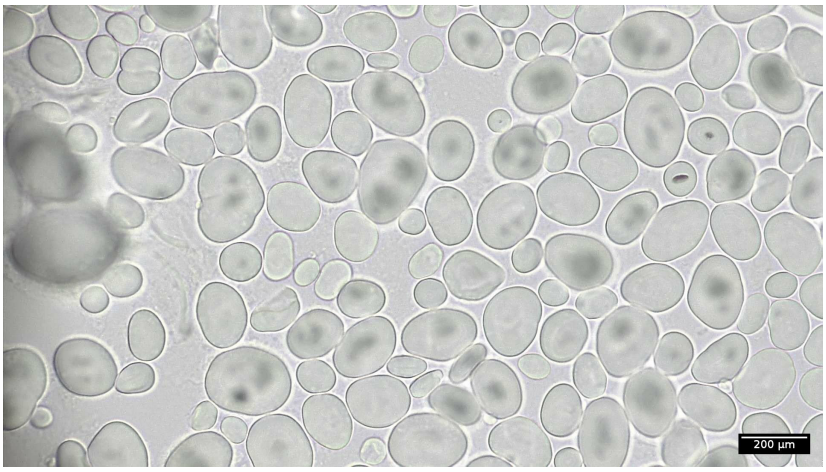

Figure 13a: First Mode

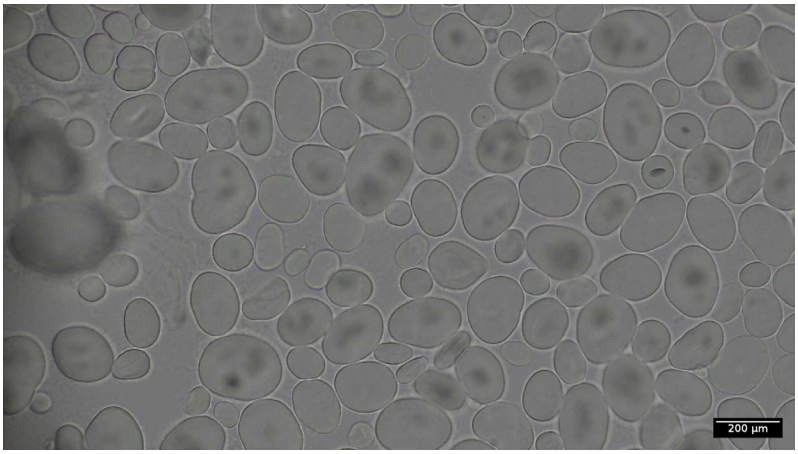

Figure 13b: Second Mode

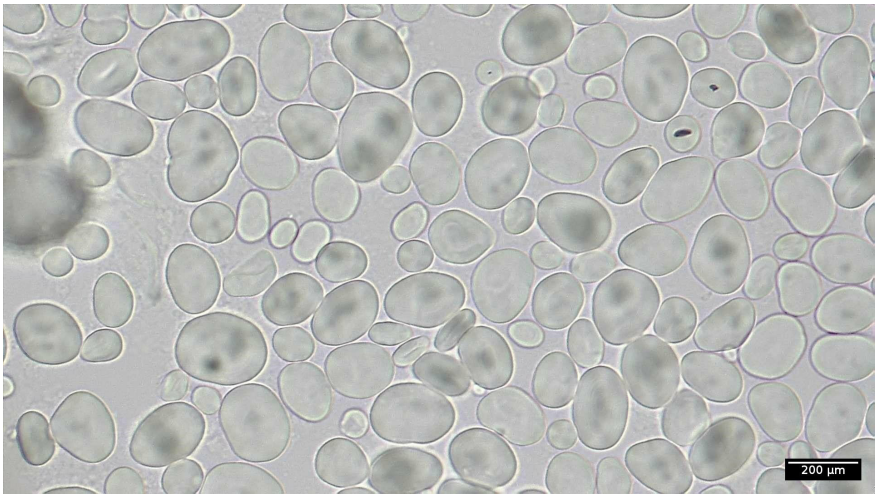

Figure 13c: Third Mode

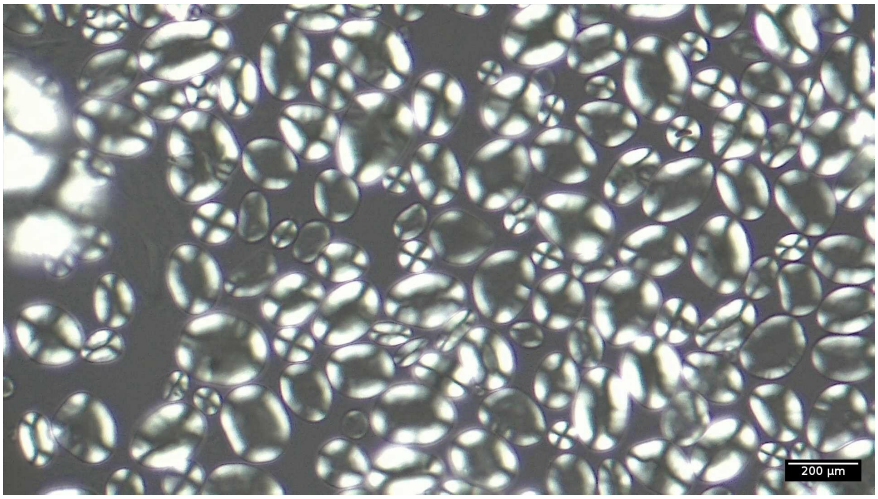

Figure 13d: Fourth Mode
Figure 13: Brand 5 (500x)

Brand 6

This brand has been represented by Figure 14 and it is pure besan, there is no shade of corn flour particles and yellow pea flour in the image. Also, the particle shapes exactly match the pure besan.

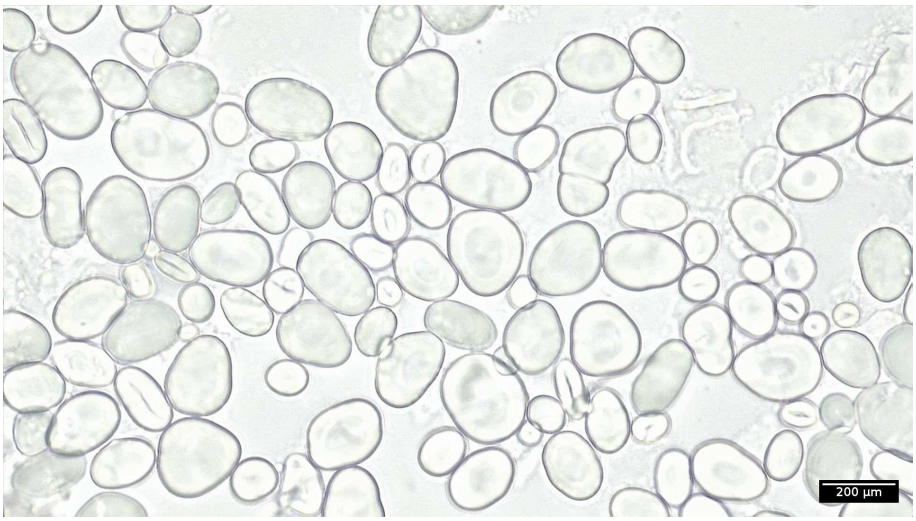

Figure 14a: First Mode

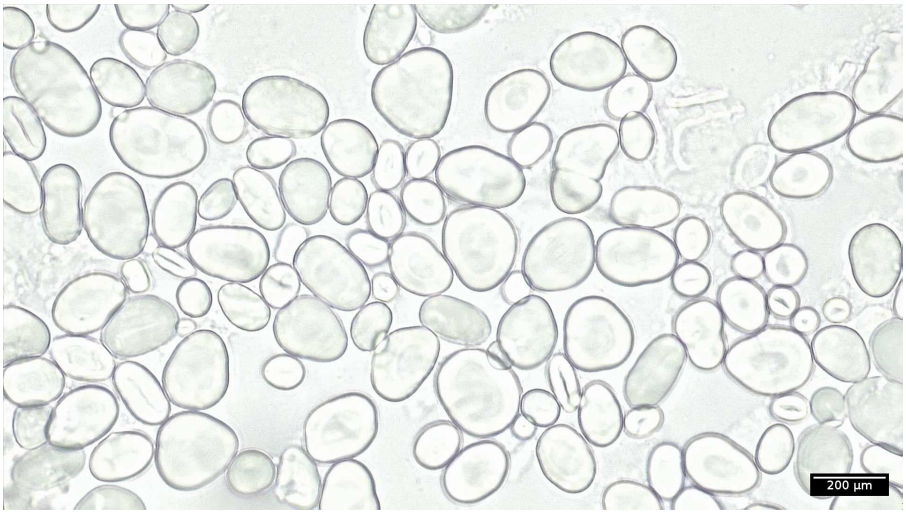

Figure 14b: Second Mode

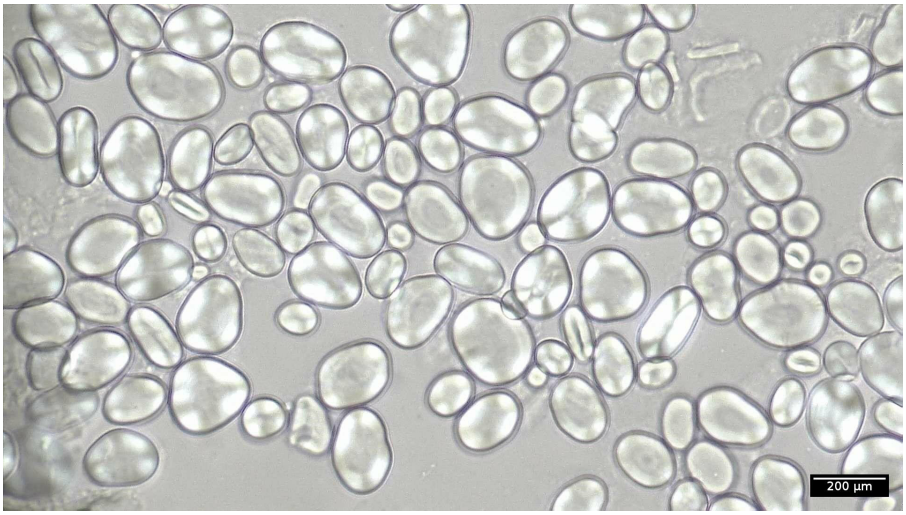

Figure 14c: Third Mode

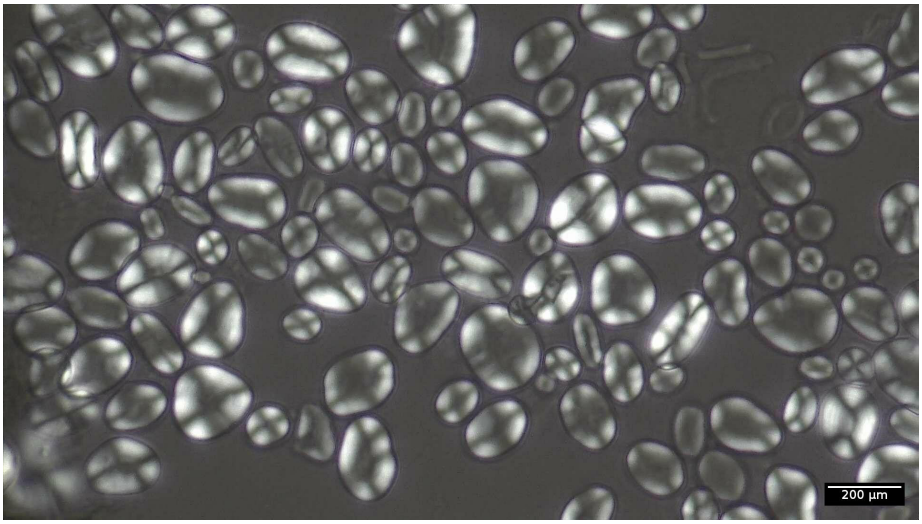

Figure 14d: Fourth Mode
Figure 14: Brand 6 (500x)

Brand 7

This brand is also pure besan which doesn't have the shade of corn flour and yellow pea flour but it could be noted in Figure 15d that a few particles have light cracks in the sides as yellow peas. But it is visible from 15a and 15c that those particles match with pure besan particles.

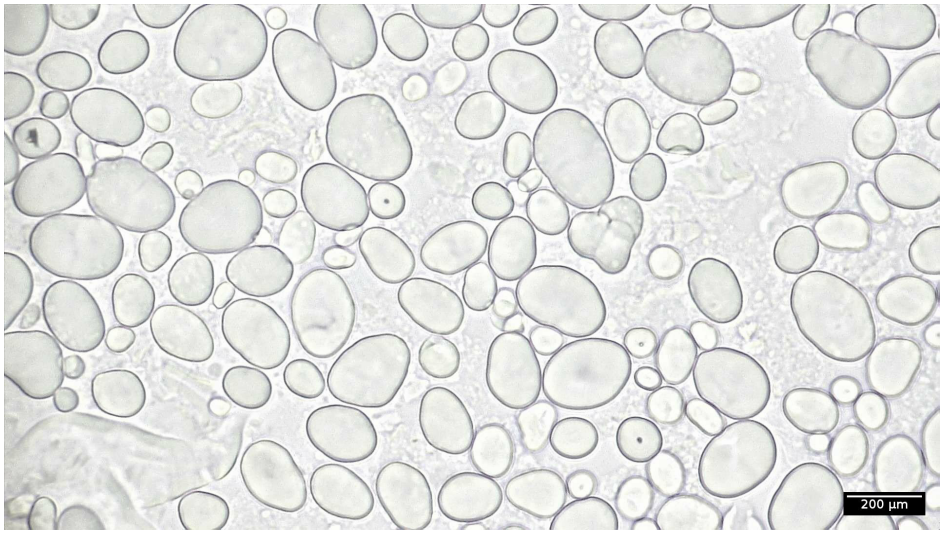

Figure 15a: First Mode

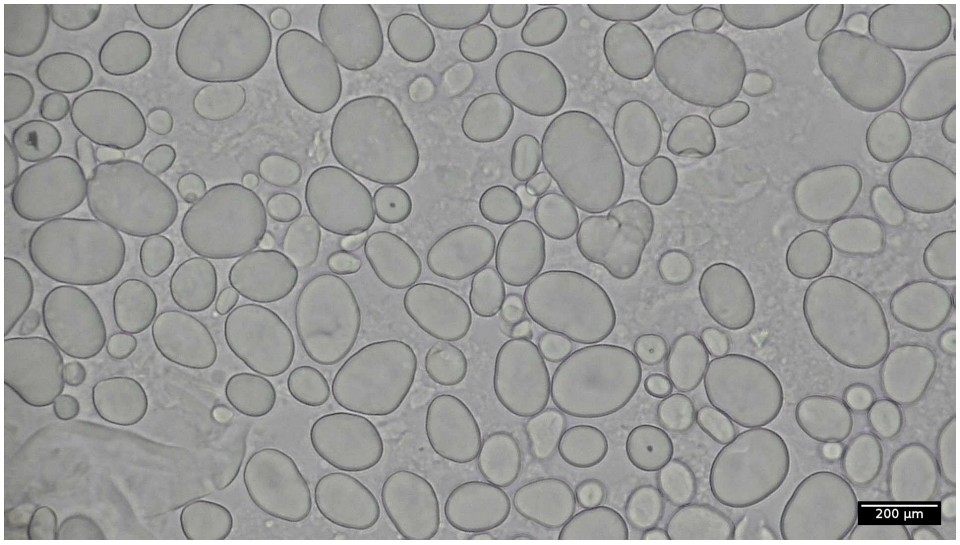

Figure 15b: Second Mode

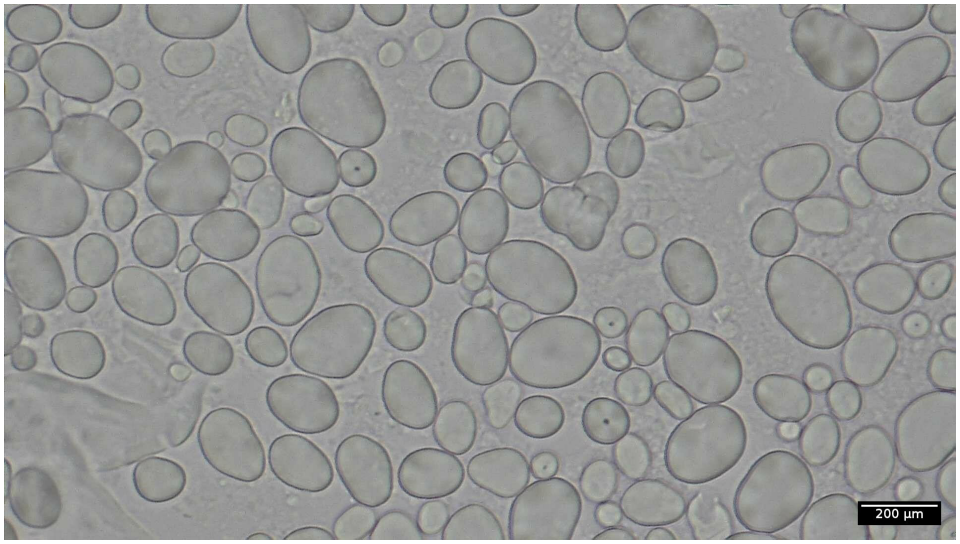

Figure 15c: Third Mode

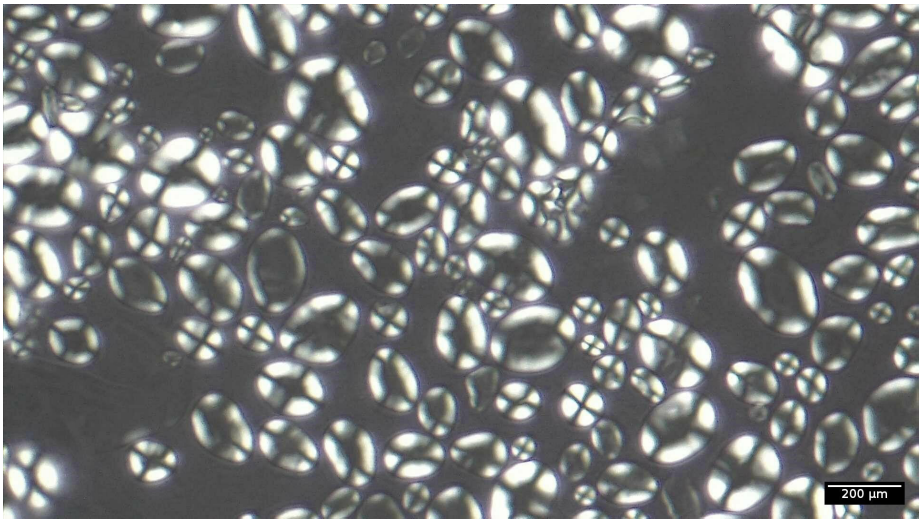

Figure 15d: Fourth Mode
Figure 15: Brand 7 (500x)

Brands 8, 9 and 10

Figure 16, Figure 17, and Figure 18 represent brands 8, 9 and 10 respectively. It could be seen there is no shade of corn flour and yellow pea flour. These particles match exactly with besan particles. So, it can be confirmed that it is being non-adulterated.

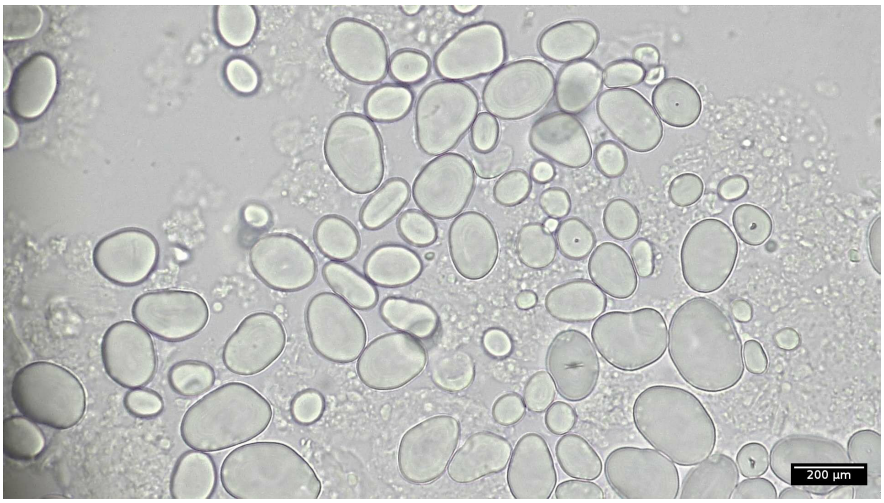

Figure 16a: First Mode

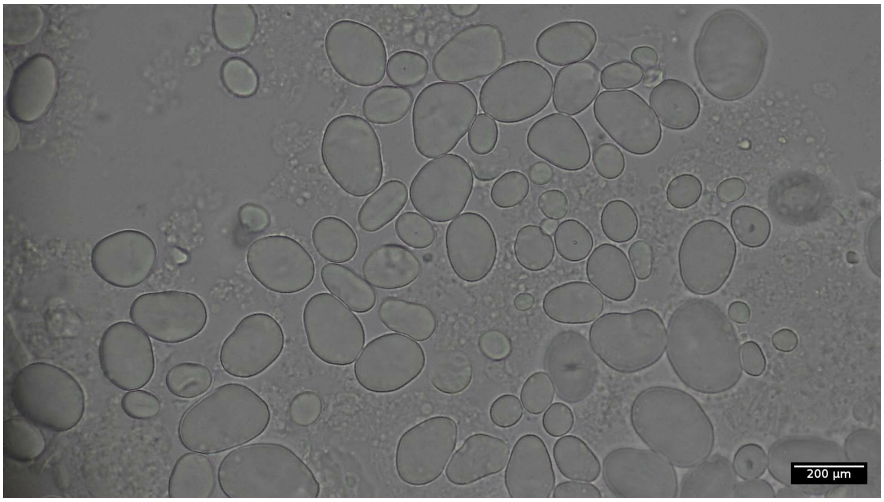

Figure 16b: Second Mode

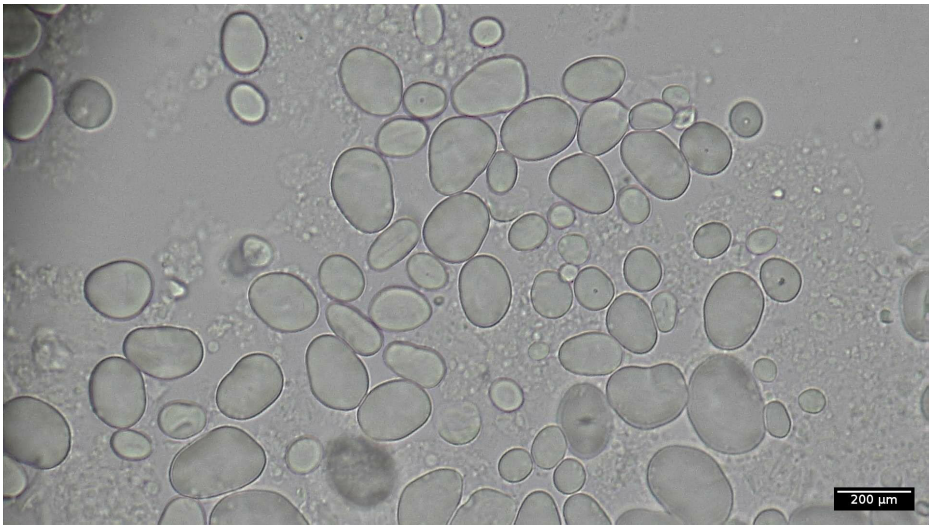

Figure 16c: Third Mode

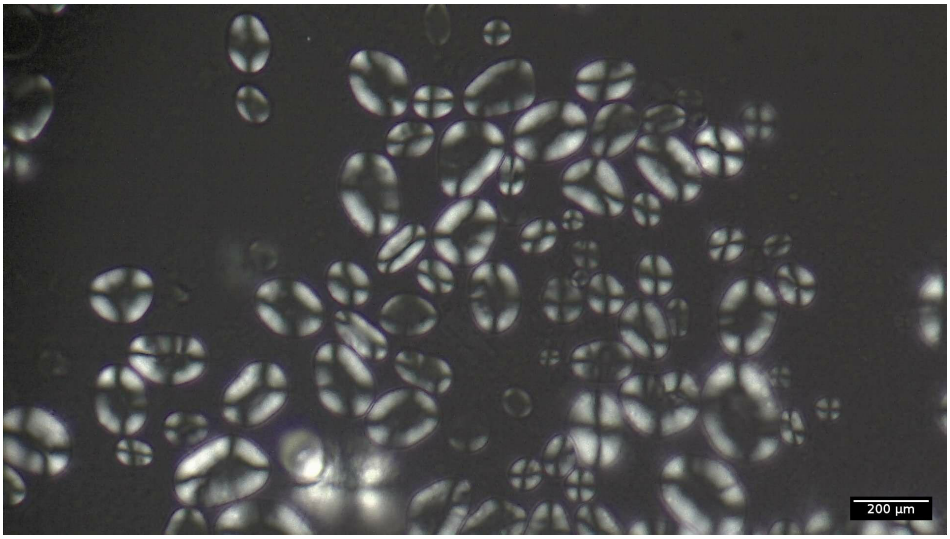

Figure 16d: Fourth Mode
Figure 16: Brand 8 (500x)

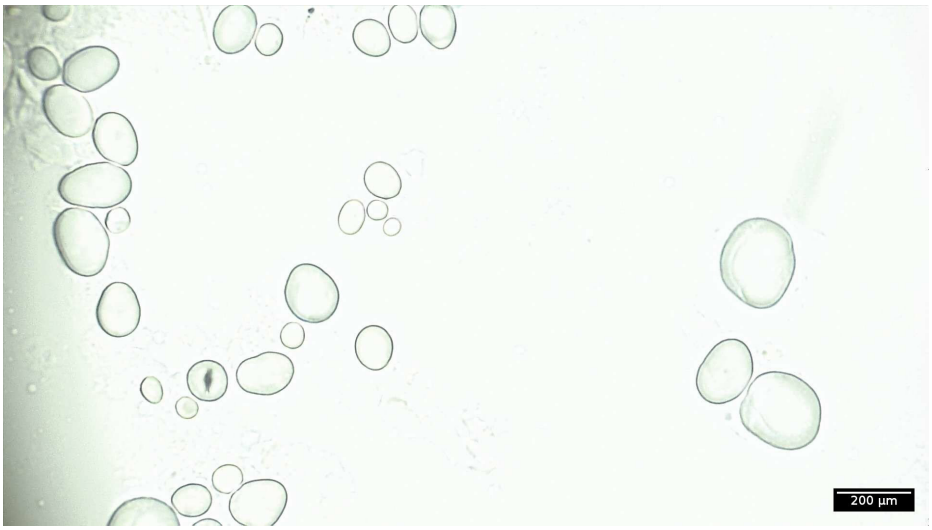

Figure 17a: First Mode

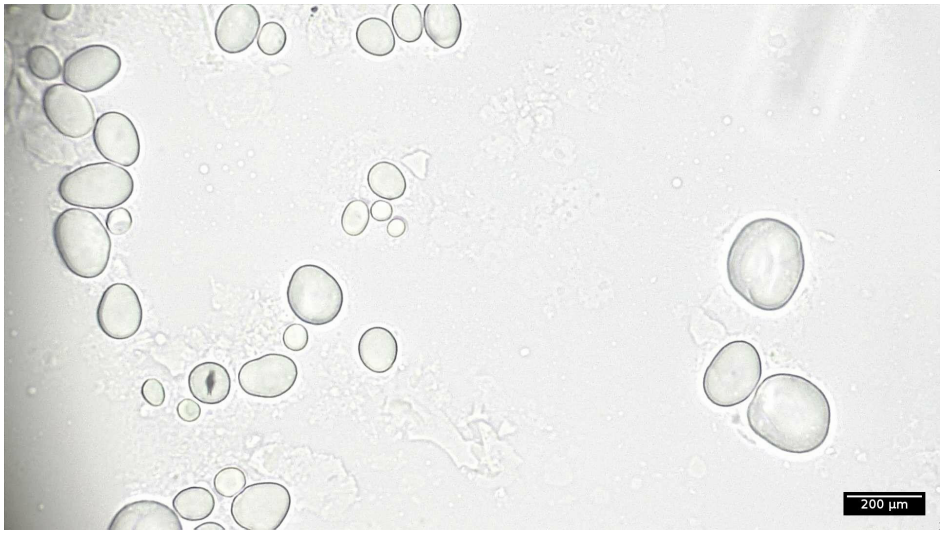

Figure 17b: Second Mode

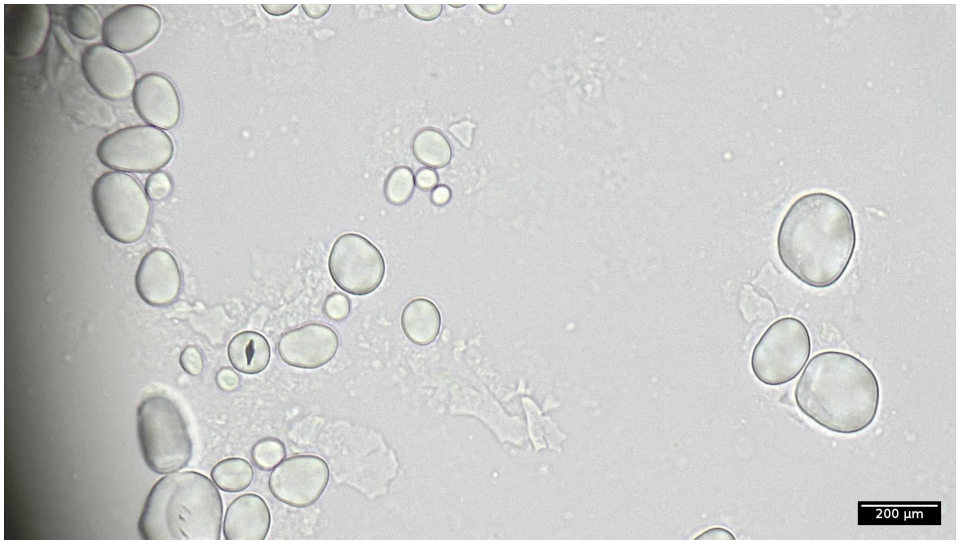

Figure 17c: Third Mode

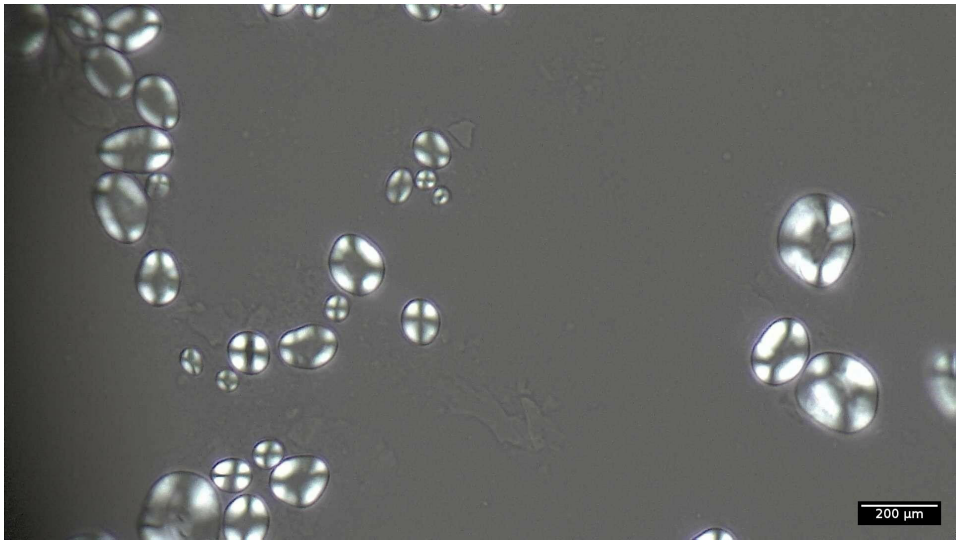

Figure 17d: Fourth Mode
Figure 17: Brand 9 (500x)

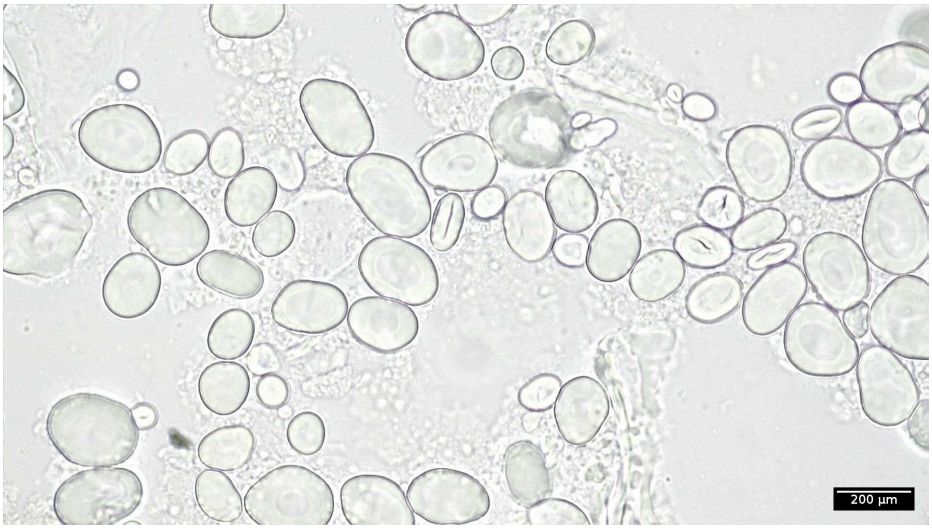

Figure 18a: First Mode

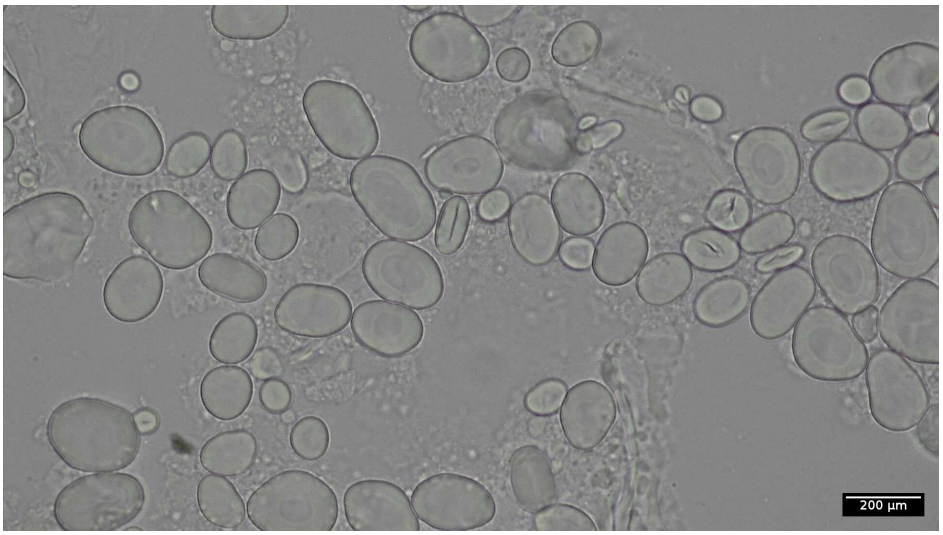

Figure 18b: Second Mode

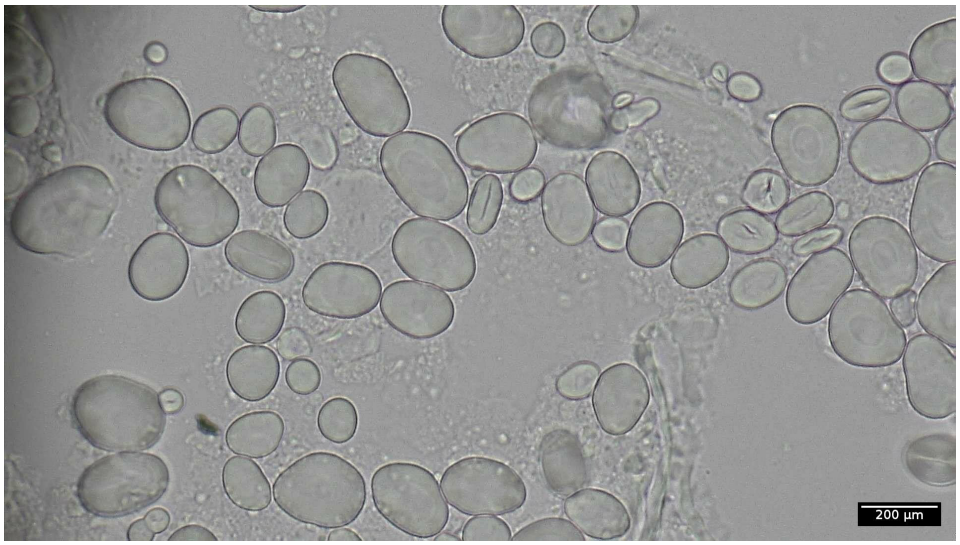

Figure 18c: Third Mode

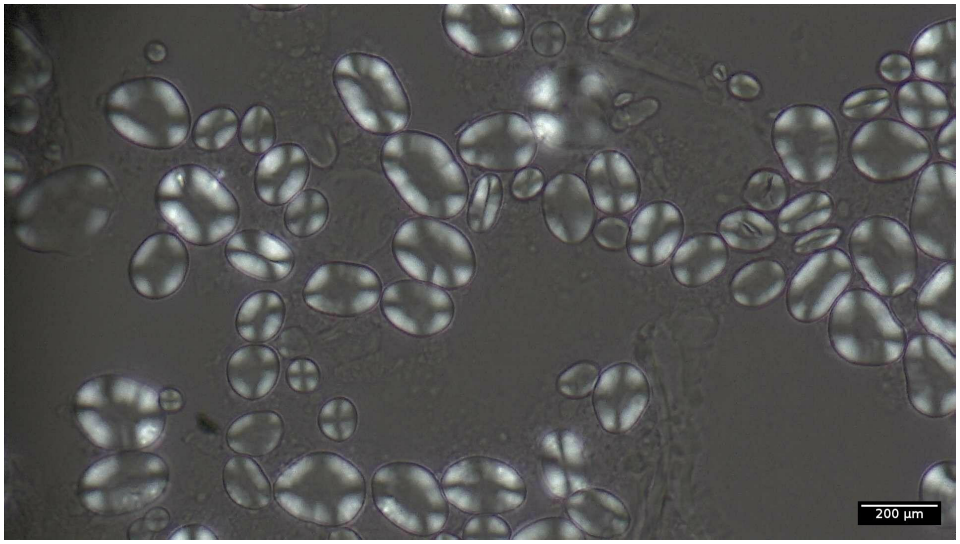

Figure 18d: Fourth Mode
Figure 18: Brand 10 (500x)

CONCLUSION

Food adulteration affects most of the human beings without our knowledge and it is the main cause for most of the health issues. So still the world needs to find authenticated and easy techniques from both targeted and non-targeted aspects. We provided the first clear-cut image of besan, corn flour, and yellow peas particles under a microscope as well as under crossed polars using a polarization microscope. These images help us to differentiate adulterant particles without any chemical methods. One more advantage is a pinch of sample is enough for this technique. Ten samples have been purchased from the market around Bangalore and tested for adulteration through this technique. It has been very successful in finding foreign substances in the sample.

Abbreviations Used

HPLC High-Performing Liquid Chromatography
TLC Thin Film Liquid Chromatography
GC-MS Gas Chromatography-Mass Spectrometry
LC-MS Liquid Chromatography – Mass Spectrometry
UV-Vis Ultraviolet-visible Spectroscopy
NMR – Nuclear Magnetic Resonance Spectroscopy
FTIR Fourier Infrared Transform Spectroscopy
PLM Polarized Light Microscope
RS Resistant Starch.

Acknowledgments

We thank Dr. HS Nagaraja, Chief Mentor of Prayoga for the useful discussions during the project period.

Supporting Information description

This supporting information contains all the images taken during the experiment.

REFERENCES

Bala, M., Sethi, S., Sharma, S., Mridula, D., & Kaur, G. (2022). Non-destructive determination of grass pea and pea flour adulteration in chickpea flour using near-infrared reflectance spectroscopy and chemometrics. *Journal of the Science of Food and Agriculture, 103*(3), 1294–1302. https://doi.org/10.1002/jsfa.12223

Bala, M., Sethi, S., Sharma, S., Mridula, D., & Kaur, G. (2022a). Prediction of maize flour adulteration in chickpea flour (besan) using near infrared spectroscopy. *Journal of Food Science and Technology, 59*(8), 3130–3138. https://doi.org/10.1007/s13197-022-05456-7

Corradini, M. G., & McClements, D. J. (2017). Food ☆. In *Elsevier eBooks*. https://doi.org/10.1016/b978-0-12-409547-2.14314-8

Dattatreya, A. M., Nanjegowda, D. K., & Viswanath, P. (2010). Microscopic detection of adulteration of Bengal gram (*Cicer arietinum*) flour with other legume flour based on the seed testa macrosclereids. *Journal of Food Science and Technology, 48*(1), 114–119. https://doi.org/10.1007/s13197-010-0168-0

Fernández-Canto, N., Romero-Rodríguez, M. Á., Ramos-Cabrer, A. M., Pereira-Lorenzo, S., & Lombardero-Fernández, M. (2022b). Polarized light microscopy guarantees the use of autochthonous wheat in the production of flour for the Protected Geographical Indication 'Galician Bread.' *Food Control, 147,* 109597. https://doi.org/10.1016/j.foodcont.2022.109597

Gao, B., Holroyd, S. E., Moore, J. C., Laurvick, K., Gendel, S. M., & Xie, Z. (2019). Opportunities and challenges using non-targeted methods for food fraud detection. *Journal of Agricultural and Food Chemistry, 67*(31), 8425–8430. https://doi.org/10.1021/acs.jafc.9b03085

Júnior, A. A. M., Massie, B. B., Sigauque, F. J. L., Dimande, A. A., & Fernandes, G. D. (2023). Quality of Fortified Zea Mays L (Maize) and Triticum Durum (Wheat) Flours. *American Journal of Food Science and Technology, 2*(2), 46-53. https://doi.org/10.54536/ajfst.v2i2.1997

Kumar, R., Kumar, A., Sharma, N. K., Kaur, N., Chunduri, V., Chawla, M., Sharma, S., Singh, K., & Garg, M.

(2016). Soft and hard textured wheat differ in starch properties as indicated by trimodal distribution, morphology, thermal and crystalline properties. *PLoS ONE, 11*(1), e0147622. https://doi.org/10.1371/journal.pone.0147622

McMahon, K. A. (2004). Practical botany-the Maltese cross. *Tested Studies for Laboratory Teaching, 25,* 352-357.

Oseyemi, F. H. (2023). Appraising the Knowledge and the Practice of Food Safety Procedure and Hygiene among Food Vendors in Akure South Local Government Area of Ondo State, Nigeria. *American Journal of Physical Education and Health Science, 1*(1), 31-38. https://doi.org/10.54536/ajpehs.v1i1.1614

Sivak, M. N., & Preiss, J. (1998). Physicochemical structure of the starch granule. *Advance Food Nutrition Research, 41,* 13-32.

Vinod, B. R., Asrey, R., Rudra, S. G., Urhe, S. B., & Mishra, S. (2023). Chickpea as a promising ingredient substitute in gluten-free bread making: An overview of technological and nutritional benefits. *Food Chemistry Advances, 3,* 100473. https://doi.org/10.1016/j.focha.2023.100473

Wu, D., Li, W., Wan, J., Hu, Y., Gan, R., & Zou, L. (2023). A Comprehensive Review of Pea (*Pisum sativum* L.): Chemical Composition, Processing, Health Benefits, and Food Applications. *Foods, 12*(13), 2527. https://doi.org/10.3390/foods12132527