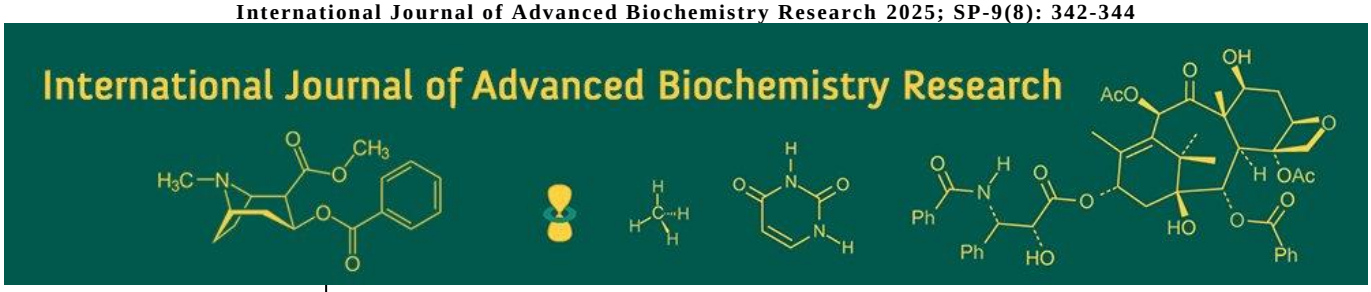

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Boric acid detection in wheat flour using "Tears of the Wine" phenomenon

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Abstract
Adulteration in food commodities is problematic issue as it compromises consumer confidence. Wheat flour is added with boric acid because boric acid acts as antimicrobial agent and insecticide. Boric acid is toxic particularly in long run as it damages vital organs and thus not approved as preservative in food products in many countries including India. Thus fast, easy and cheap methods are required for boric acid adulteration. No method is reported using "Tears of the Wine" phenomenon in literature and this study is first to report use of this physical phenomenon for easy detection of boric acid. Using "Tears of Wine" phenomenon based method where visible cottony growth can be observed on conical flask ream we were able to detect minimum level of 0.4% w/w boric acid adulteration in wheat flour.

Keywords: Adulteration detection, boric acid, wheat flour, tears of wine

Introduction
Adulteration in food commodities is on-going problem [1]. Wheat flour is added with boric acid because boric acid is insecticide and thus prolongs shelf life of wheat flour. Boric acid acts as antimicrobial agent and insecticide [2-4]. Boric acid is toxic particularly in long run it damages vital organs [5-7] and thus not approved as preservative in food products in many countries including India [5, 8]. Thus fast, easy and cheap methods are required for boric acid adulteration. No method is reported using "Tears of the Wine" phenomenon in literature and study is first to report use of this physical phenomenon for easy detection of boric acid.

Materials and Methods
Wheat grains were purchased from local market and was of "MP Tukadi Sharbati" variety. Pure wheat flour was prepared by milling wheat grains in flour mill. AR grade boric acid H_3BO_3 having 99.5% purity was procured from Molychem, Mumbai, India. Boric acid was in dusty fine powdery form. Spiked samples were prepared by taking pure wheat flour by weight and then adding boric acid by weight. Control samples were prepared either form pure wheat flour or from pure boric acid.
Boric acid adulteration was checked using conical flask, beaker and volumetric flask geometries. Room temperature, 30 °C, 50 °C and 70 °C temperature effect was checked. Effect of time was also studied for 72 hour at 12 hour time period. Adulteration detection was tired at 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1% w/w levels of boric acid in wheat flour. For all trials methanolic extract of samples was used. It was prepared by taking 10 g of sample in 100 ml breaker and extracting it using 50 ml methanol as solvent. For extraction slurry of sample was prepared with ethanol by through mixing and then filtrate is collected using Whatman 41 filter. Filtrate was used for detection.

Results and Discussion
Extraction with methanol vs with water
Boric acid has 17% solubility in methanol compared to 5% in water. Methanol does not make viscous paste when mixed with wheat flour while water is imbibed by flour proteins making paste. On this two accounts methanol is better solvent than water for boric acid extraction. Further it was experimentally observed that water extract leaves more resides after completer evaporation than methanol extract i.e. nonspecific extraction is more.

Effect of vessel type

Table 1: Effect of vessel type

Level of spiking	Vessel	Temp °C	Results
1%	Beaker	30	+
1%	Conical Flask	30	+++
1%	Volumetric Flask	30	++

Presence of visible white fur/precipitate at the rim of vessel indicated adulteration with boric acid. Earliest detection observed was after 24 h of incubation. Afterward detection does not improve. Thus 24 h incubation was considered satisfactory for detection.

Conical flask was most suitable closely followed by volumetric flak to visualize tear of wine phenomenon based cottony growth for detection of boric acid in wheat flour. Beaker was found unsuitable.

Vessels with wide openings like beaker facilitate faster evaporation of solvent rising on sides of vessel due to "tears of wine" phenomenon thus give faster detection in theory but practically it is observed that this results in spread of boric acid on whole of beaker surface and thus dilutes its concentration. Narrow opening vessels have problem of slow evaporation but advantage of concentrating effect. In our study it was found that 100 ml conical flak was good. As conical flak is easy to handle too, use of conical flak is recommended.

Table 2: Effect of temperature

Level of spiking (%)	Vessel	Temperature (°C)	Observation (after 24 h)
1%	Beaker	70	-
		50	-
		30	+
1%	Conical Flask	70	-
		50	-
		30	+++
1	Volumetric Flask	70	-
		50	-
		30	++

Visual “tear of wine" phenomenon based cottony growth of boric acid on top rim of vessel was observed best at 30 °C temperature. Among selected geometries conical flaks performed most satisfactorily.

It was theorized in the beginning of study that temperature is the big factor which can make evaporation of solvent rising on the vessel walls faster. Higher temperature was thus supposed to make detection both rapid and more sensitive due to overall more boric acid accumulation on top of vessel. In practice this never happens. It is interesting to think why temperature does not help. It can be deducted that heating of vessel walls reduces surface tension between methanol and glass but this effect alone can only reduce but cannot stop rising of methanol on glass walls as still there is positive attraction between methanol and glass. Another factor play role here i.e. heating causes high degree of direct evaporation. This results in effective stopping of evaporation of ethanol from walls. Further this directly evaporated pure ethanol can condense on vessel walls starting reflux washing down any boric acid on walls back in vessel. If directly evaporating vapor can reach to top of vessel it can wash down boric acid cottony growth form there back in flak. This thinking was confirmed practically i.e. heating even removes already happened cottony growth. Note that direct methanol evaporation is not responsible of cottony growth. This is supported by the fact that when water evaporates from salty water it leaves most of salt behind and as boric acid is solid like salt it behaves similarly. It should be noted hare that boric acid has 171 °C melting point and 300 °C boiling point and is not logical that it evaporates with heating at 70 °C or lower which is boiling point of alcohol. Conclusion of this trials was that 30 °C is suitable temperate for detection compared to 50 or 70 °C.

Table 3: Effect of concentration

Level of spiking (%)	Vessel	Temperature (°C)	Observation (after 24 h)
0.8	Conical Flask	30	+++
0.6	Conical Flask	30	++
0.4	Conical Flask	30	+
0.3	Conical Flask	30	-
0.2	Conical Flask	30	-
0.1	Conical Flask	30	-

Visual “tear of wine" phenomenon based detection of boric acid in wheat flour was observed up to 0.4% level of spiking of boric acid in wheat flour and this confirmed the LOD for given method as 0.4% boric acid in wheat flour w/w.

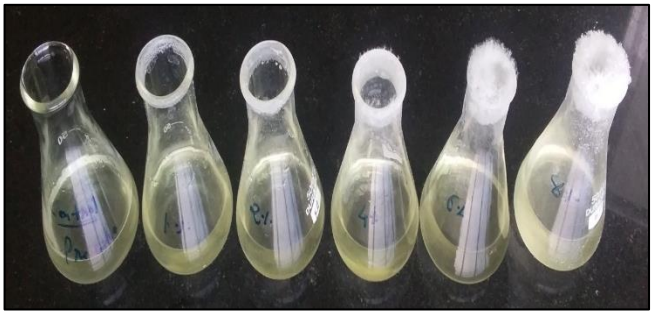

Fig 1: Control and 1% to 8% spiked samples detection

Fig 2: 1% Detection comparison with control

Fig 3: 0.8% Detection comparison with control

Fig 4: 0.6% Detection comparison with control

Fig 5: 0.4% Detection comparison with control

This effect was straight forward i.e. more concentration more was white particulate matter on top of vessel.

Reason for Tears of wine phenomenon and cottony growth of boric acid

Methanol has low surface tension i.e. self-attraction. Methanol has more attraction for glass and water than for itself. Thus methanol follows "love not thyself but thy neighbor" philosophy with glass and water. Also note that methanol does not show azeotropic behavior with water. When boric acid methanol solution is left in glass vessel boric acid rises on side of glass vessel with methanol. Evaporation of methanol concentrates water it in which forces more methanol to rise. Eventually extra water falls off the walls as tear drops [9]. As internal of vessel become saturated with vapor, no more evaporation occur internally at given level and solvent front moves up in vessel. Eventually when boric acid methanol mixture reaches vessel top total evaporation of solvent causes formation of cottony appearance. The cotton like appearance can be explained by the fact that boric acid itself act as scaffold for rise of methanol and its evaporation. Directionality of boric acid crystals finally give directional growth. Even if there is no water in methanol still we find cottony growth as it appears that cottony growth is independent of water reflux i.e. presence or absence of water in methanol is not making or breaking change.

Conclusions

Boric acid adulteration in wheat flour can be detected using “Tears of Wine” phenomenon based method where visible cottony growth can be observed on conical flask ream. This method can detect minimum level of 0.4% boric acid adulteration in wheat flour.

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