

# **FOOD QUALITY (ADULTERATION OF HONEY)**

## **(Project – Report)**

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Science degree in Microbiology

By

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### **BONAFIDE CERTIFICATE**

This is to certify that this Project Report is the bonafide work of Anitha.S (38300003) And Ramya.B (38300014) who carried out the project entitled “**FOOD QUALITY (ADULTERATION OF HONEY)**” under our supervision from October 2020 to March 2021.

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## DECLARATION

I Anitha.s. (38300003) and Ramya.B. (38300014) hereby declare that the Project Report entitled “ **FOOD QUALITY (ADULTERATION OF HONEY)**” done by me under the guidance of **Dr.M.Masilamani selvam** at **Sathyabama Institute of Science and Technology, Chennai** is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Microbiology.

**DATE:**

**PLACE:**

**SIGNATURE OF THE CANDIDATE**

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**Abstract:**

Honey is characterized as a natural and raw foods that can be consumed not only as a sweetener but also as medicine due to its therapeutic impact on human health. It is prone to adulterants caused by humans that manipulate the quality of honey. Although honey consumption has remarkably increased in the last few years all around the world, the safety of honey is not assessed and monitored regularly. Since the number of consumers of honey adulteration have increased in recent years, their trust and interest in this valuable product has decreased. Honey adulterants are any substances that are added to the pure honey. In this regard, this paper provides a comprehensive and critical review of the different types of adulteration, common sugar adulterants and detection methods, and draws a clear perspective toward the impact of honey adulteration on human health. Adulteration increases the consumer's blood sugar, which can cause diabetes, abdominal weight gain, and obesity, raise the level of blood lipids and can cause high blood pressure. The most common organ affected by honey adulterants is the liver followed by the kidney, heart, and brain, as shown in several in vivo research designs.

**Keywords:** honey; adulteration; sugar adulterants; toxicity

| <b>CHAPTER<br/>No.</b> | <b>TABLE OF CONTENTS</b>                                                         | <b>PAGE<br/>No.</b> |
|------------------------|----------------------------------------------------------------------------------|---------------------|
|                        | <b>TITLE</b>                                                                     |                     |
|                        | <b>ABSTRACT<br/>LIST OF ABBREVIATIONS<br/>LIST OF TABLES<br/>LIST OF FIGURES</b> |                     |
| <b>1</b>               | <b>INTRODUCTION</b>                                                              | <b>4</b>            |
| <b>1.1</b>             | <b>WHAT ADULTERATION?</b>                                                        | <b>4</b>            |
| <b>1.2</b>             | <b>INDIRECT ADULTERATION OF<br/>HONEY</b>                                        | <b>4</b>            |
| <b>1.3</b>             | <b>METHODS DETECTING INDIRECT<br/>HONEY</b>                                      | <b>4</b>            |
| <b>1.4</b>             | <b>DIRECT ADULTERATION OF HONEY</b>                                              | <b>5</b>            |
| <b>2</b>               | <b>REVIEW OF LITERATURE</b>                                                      | <b>7</b>            |
| <b>3</b>               | <b>AIM AND SCOPE</b>                                                             | <b>10</b>           |
| <b>3.1</b>             | <b>AIM</b>                                                                       |                     |
| <b>3.2</b>             | <b>OBJECTIVES</b>                                                                |                     |
| <b>4</b>               | <b>METERIALS AND MEHODOLOGY</b>                                                  | <b>11</b>           |
| <b>5</b>               | <b>RESULTS</b>                                                                   | <b>16</b>           |
| <b>6</b>               | <b>SUMMARY AND CONCLUSION</b>                                                    | <b>18</b>           |
|                        | <b>REFERENCES</b>                                                                | <b>19</b>           |

#### **LIST OF ABBREVIATIONS AND SYMBOLS:**

|      |   |                           |
|------|---|---------------------------|
| Min. | - | Minute                    |
| HFCS | - | High fructose corn syrup  |
| HFC  | - | Hydrofluorocarbon         |
| HMF  | - | 5- Hydroxymethyl furfural |
| GMS  | - | Glucose syrups            |
| BFS  | - | Bee feeding syrups        |
| SS   | - | Sucrose syrups            |

**LIST OF FIGURES:**

| <b>FIG<br/>No.</b> | <b>TITLE</b>                    | <b>PAGE<br/>NO.</b> |
|--------------------|---------------------------------|---------------------|
| <b>8.1</b>         | <b><i>Adulterated honey</i></b> | <b>18</b>           |
| <b>8.2</b>         | <b><i>Pure honey</i></b>        | <b>18</b>           |
| <b>8.3</b>         | <b><i>Pure honey</i></b>        | <b>18</b>           |
| <b>8.4</b>         | <b><i>Adulterated honey</i></b> | <b>18</b>           |

## CHAPER: 1 INTRODUCTON

### 1.1 What is Adulteration?

**Adulteration** is a legal term meaning that a food product fails to meet the legal standards. One form of adulteration is an addition of another substance to a food item in order to increase the quantity of the food item in raw form or prepared form, which may result in the loss of actual quality of food item. These substances may be either available food items or non-food items. Among meat and meat products some of the items used to adulterate are water or ice, carcasses, or carcasses of animals other than the animal meant to be consumed.

### 1.2 Honey adulteration.

#### **Honey**

Honey is defined as the excretions of insects sucking on the living parts of plants. Honeybees are the most well-known plant-sucking insects and can collect and transform honey, and deposit, dehydrate, store and leave honey in the honeycomb to ripen and mature. Honeybees collect pollen and nectar from a variety of flowering plants and convert it into the wax and honey. Only worker honeybees forage for food, consuming as much nectar from each flower as they can. After foraging, worker honeybees return to the hive/comb and pass the collected nectar to the other worker honeybees. This worker holds the nectar on her tongue until the liquid evaporates, creating honey. The honey is then stored in a cell within the hive/comb

It was apparent that the most common **adulterants** in **honey** were sugars from high fructose corn syrup (HFCS), corn sugar syrup (COSS), inverted sugar syrup (ISS) and cane sugar syrup (CASS).

### 1.3 High-fructose corn syrup (HFCS)

HFCS also known as glucose-**fructose**, isoglucose and glucose-**fructose syrup**, is a sweetener made from **corn starch**. As in the production of conventional **corn syrup**, the **starch** is broken down into glucose by enzymes.

#### 1.4 Corn sugar syrup (COSS)

COSS is sold commercially as either light or dark **corn syrup**. Light **corn syrup** has been clarified and decolorized; it is **used** in baked goods, jams and jellies, and many other food products. Because it does not crystallize when heated, it is particularly valued as an ingredient in candies.

#### 1.5 Inverted sugar syrup (ISS)

**Inverted sugar syrup**, also called **invert syrup** and **invert sugar**, is an edible mixture of two simple sugars glucose and fructose that is made by heating sucrose (table sugar) with water. It is thought to be sweeter than table sugar, and foods that contain invert retain moisture better and crystallize less easily than do those that use table sugar instead. Bakers, who call it *invert syrup*, may use it more than other sweeteners.

#### 1.6 Cane sugar syrup (CASS)

**Cane syrup** is used in a lot of bars but often not in its most authentic form. Typically, bartenders will source organic **cane sugar** in its crystallized form, and the results taste similar to a **simple syrup**. But anyone who has made **cane syrup** from actual **cane** juice will tell you it will transform your cocktails

#### 1.7. ADULTRATION HONEY ANALYSIS

When exposed to any kind of heat flame should remain unburned. To perform this test, try dipping a matchstick/cotton bud in honey and then light it. If it burns, that means the quality of your honey is pure

##### 1.7.1 Perform the heat test

Pure honey, when exposed to any kind of heat or flame should remain unburned. To perform this test, try dipping a matchstick/cotton bud in honey and then light it. If it burns, that means the quality of your honey is pure. Adulterated honey might not show consistent results or burn properly, due to the fact that moisture or fermentation which might affect its quality and taste.

### **1.7.2 Water test**

Another really simple and effective way to test if the quality of your honey is pure or not is to do the water test. The properties of "pure honey" would ensure that it does not completely dissolve in water, once mixed and would instead, require some efforts to dissolve. If your honey is laced with sugar or glucose, it would leave a white trace at times.

### **1.7.3 Thumb test**

The Thumb Test Place a small amount of honey on your thumb and check if spills or spreads around like any other liquid. If it does, it may not be pure. Pure honey is thick while impure honey will be runny. Pure honey sticks to the surface it is applied to and doesn't drip away.

## **1.8. Type of Honey**

Blossom honey:

Honeydew honey

Monofloral honey

Multifloral honey (polyfloral):

## CHAPTER 2: REVIEW OF LITERATURE

**Blanka Zábrowská, Lenka Vorlová, (2014).** A large number of methods focus on the determination of a limited number of indicators, which is insufficient to detect the current methods of honey adulteration. It is no longer possible to focus on one indicator only, but a wide range of quality indicators need to be determined in order to detect honey adulteration, while monitoring a combination of such indicators and, in response to new data, revising existing legislation. It is also necessary to develop new methods and standards capable of detecting the presence and level of indirect adulteration of honey.

**Chris Luke, Stacy Atchison Barbara, Van Renterghem, (2009).** Different parameters, such as Tg and enthalpy changes, can be used to detect the effects of certain adulterants. DSC provides valuable information about the thermal behavior of honey and facilitates detection of adulteration in commercial honey, as well as honey's physicochemical and structural properties. DSC is an analytical technique that is capable of accurate and precise measurements and can be applied to routine analysis.

**Tatjana Pavlova, Ivan Dimov, Atjana Kalevska, Gjore Nakov (2019).** The physicochemical properties of honey are an important indicator of the quality and origin of honey. The physical properties and chemical composition of honey from different sources have been carried out by many researchers. This paper presents an overview of the quality characteristics that honey has on the basis of data from the available literature. In its chemical composition, honey is a mixture of carbohydrates (mainly glucose and fructose) and water, and a smaller content of organic acids, minerals, vitamins, enzymes, proteins, polyphenols and other substances. The chemical composition of honey effects of its physical

characteristics such as crystallization, viscosity, hygroscopicity, electrical conductivity, optical properties, surface tension, and color.

**Alemu Girma Tura, Dechasa Bersissa Seboka (2020).** Honey adulteration one of common food adulteration such as milk, coffee powder, butter and pepper powder in Ethiopia. There are many reasons why honey is adulterated more often. Some of the consumers are unaware of the problem, others have no access to methods of identification and the rest are due to carelessness. Adulteration in the honey items can cause unexpected effect on health without consumer knowledge. The consumer should avoid buying honey from unknown local traders. Both local and branded honey handling, stores, transportation should be inspected by government bodies. In Ethiopia sugar is the most adulterant in honey. Consumers or merchants traditionally identify this added sugar by taste and solidification whereas government checks through laboratories. There are also some people who identify adulterated their local honey by their experience. This traditional method has limitation because some honey is crystallized naturally. According to the obtained data from different literatures some of physicochemical characteristics of honey depend on floral source and difficult to detect in simple methods. The pollen in honey can be identified using a microscope, and gives a guide to the plants from which bees has been collecting. Experts are able to determine the geographical origin of honey by the pollen it contains before detection. Honey adulteration is the issue of all aspect of honey chains to maintain its quality and safety. That is government by develop and implement policy on honey adulterate; private food industries by monitoring and moral responsibility on produced honey; traders by selling or buying quality honey through good handling and consumers by understand adulteration issue.

**M.A. Rios-Corripio, M. Rojas-López & R. Delgado-Macuil (2012).** The adulteration of pure honey with several types of standard sugar solutions, glucose, fructose and sucrose, and also with cheap syrups, corn, inverted sugar and cane sugar, was determined by using ATR–FTIR spectroscopy and the multivariate methods PCA, PLS. The use of both two- and three-dimensional PCA score plots make possible the discrimination of the type of adulterant contained in honey in an

easy way. In addition to that, optimized SEC and SEP suggest a good predictive capacity of the model employed in this study, which were applied in standard sugars and in cheap syrups that are used as adulterants in honey.

**Anoushka Prajapati, Akanksha Agarwal, Manju, (2019).** Food is an essential part of human life. Anything intrigued with it enters the food chain. May it be beneficial or harmful? Adulterant are the negative entities associated with our daily intake of food. This problem is more serious in developed and under developing countries. It is the need of the hour that we start differentiating between what's healthy and what's harmful. Honey being the third most adulterated substance in the world and one of the most common utility of any household needs to be unadulterated. The presented paper suggests a way to get rid of the harmful effects of the adulterated honey. With future research and developments these results can be more accurate and it is even possible to check the amount of adulteration.

## **CHAPTER 3: AIM AND SCOPE**

### **3.1 AIM:**

To elucidate and evaluate the adulteration of honey from the food quality analysis.

### **3.2 OBJECTIVES:**

1. To evaluate the Adulteration of honey
2. To estimate the quality of honey
3. To estimate the honey Ppurity
4. To estimate the fiehe's test used it quality of honey
5. To estimate the honey (Sucrose, Glucose, maltose)
6. To evaluate the biochemical compounds by HMF
7. To estimate the Adulteration of honey methods

## **CHAPER 4: MATERIALS AND METHODS.**

### **4.1 Adulteration Method**

Commercial honey adulteration is typically classified as direct, indirect, and blending. As discussed earlier, the authors in one paper categorized the honey adulteration into two types; direct and indirect. The direct addition of sugar syrups is a post-production procedure of adulteration occurs by overfeeding the bees during the main nectar period with honey, chemicals, and industrial sugars to recover more honey from hives. Blending is another honey adulteration procedure, which can be explained as mixing pure and high-quality honey with cheap and low-quality honey. However, the syrup or sugar residues of some reported studies are identical to the natural residues in the honey. Therefore, the detection of these adulterants have proved difficult, and scientists have to discover new methods to distinguish the differences between pure and adulterated honey.

### **4.2 Direct Adulteration**

Direct adulteration of honey is commonly performed by the direct addition of a certain amount of sucrose syrup into the honey. The source of sucrose syrup could be sugar beet, HFCS, maltose syrup, or industrial sugar syrups (glucose and fructose) obtained from heat, enzyme, or acid treatment of starch. Direct adulteration causes harm to consumers and pure honey producers. According to a study done by, to make direct adulterated honey, pure honey was mixed with different concentrations (7%, 15%, and 30%) of date and inverted sugar syrups. The authenticity of the samples was estimated by applying a multivariate analysis (PCA and LDA) into the physicochemical and rheological analysis. Amiry et al, on the other hand, performed direct adulteration of honey with synthesized sugar. Adulteration was done by mixing glucose powder with distilled water and then adding it to the pure honey in laboratory design experiments. The honey adulteration was detected by an optical microfiber sensor in this research.

Moreover, Irawati et al. added the two rice syrups directly with a ratio of 1.2:1 to the pure honey to provide a mixture with a viscosity close to the honey. Furthermore, three-dimensional fluorescence spectra (3DFS) and multivariate calibrations were employed as a detection method for honey authenticity.

#### **4.3 Indirect Adulteration**

Indirect adulteration of honey is the incorporation of sugars into honey via bee-feeding. In this manner, low-quality honey, chemicals, and industrial sugars were incorporated into the honey during a natural process that happened in the bee's digestive system. During indirect honey adulteration, an extreme amount of sugar syrup was fed to the bee colonies in the main nectar flow period. One paper described the indirect adulteration as follows: the colonies were settled in empty beehives with bees, brood, and honey frames. Standard bee-feeding methods were applied in the early spring. The sucrose syrup as adulterants were presented to each colony to ensure the growth and strength of the forage worker bee population of colonies for the main nectar flow season. The honey and honeycomb frames of all colonies were taken at the end of the nectar flow season. After settling the bees in the hives, no more syrup was provided to the colonies.

11 of 2 Furthermore, other researchers developed a method analyzing the biochemical properties to distinguish indirect sucrose syrup (SS) adulterated honey from pure blossom honey. According to the result of this study, adulteration of honey by SS has no significant effect on the content of honey, since over 95% of the fed SS to the bees was converted to fructose and glucose. During the initial step of the bees' SS feeding, the sucrose content was hydrolyzed into its building blocks. However, after extending the intensive feeding duration, the bees start to convert and store the glucose into maltose and malt triose oligosaccharides. The amount of sucrose present in pure honey owes little to the activity of invertase enzymes present in pure honey. This is the enzyme in charge of breaking down the sucrose into its building blocks and reducing the sucrose content of pure honey. Hence, it can be concluded that high sucrose levels in a honey sample might be due to the adulteration of honey. According to the experiment done by on indirect adulteration of honey, prolonged SS feeding will have identical results as with artificial direct adulteration. Furthermore, the extended prolonged SS bee-feeding reduces the

nutritional properties and quality of the honey in the same manner of direct honey adulteration.

#### **4.4 Blending**

In this method, high quality (pure and rare) honey is mixed with cheaper honey that has lower quality and nutrition. Adulteration of pure honey with synthetic honey has become much more prevalent in recent years. In China and Venezuela, a well-known fraud in the honey industry consists of blending the costly acacia honey with rape honey (cheaper honey) to increase the marketing profit since the light amber color of rape honey is highly similar to that of the yellow-colored acacia honey. Acacia honey originates from *Robinia pseudacacia* blossoms and is commonly consumed nectar honey because of its mild aromatic and clear aspect and light yellowish color, and because it does not crystallize. In contrast, rape honey is sweeter, with a light amber color, and easily crystallizes. Hence, as the collection of *M. favosa* honey has steadily increased since 1985, it is the perfect target for blending with the cheaper honey of *A. mellifera*. In this study, acacia honey was blended with different concentrations (5–50%, w/w) of rape honey, and the simple method of liquid chromatography-electrochemical detection (LC-ECD) was used to detect the adulteration. Since the honey adulterants and adulteration methods have been discussed earlier, detection methods to investigate the authenticity of honey become the priority in the food safety of honey. These detection methods must be capable of distinguishing between pure and adulterated honey to assist the authorities in regulating the market and protect the consumer's right.

#### **4.5 Detection Method in Honey Adulteration**

The addition of sugar to honey is the common fraud in honey adulteration. Sugar addition always has been coupled with thermal treatment to produce a homogenous mixture to sell it as pure honey to the consumers. During applying heat to the honey, HMF can be produced as an intermediate compound via the Maillard reaction. So, the amount of HMF is an indicator of honey adulteration. There is a possibility of HMF formation at low temperature and can be elevated by the increase of applied temperature or storage. High-performance liquid chromatography (HPLC), gas

chromatography with mass spectrometry (GC-MS), micellar electro kinetic capillary chromatography (MEKC), and voltammetry are among the methods that applied for the determination of HMF in adulterated honey. A physiochemical analysis is another application to differentiate pure honey from adulterated honey. Honey adulterated with fructose and saccharide showed a lighter color (*L* values), while pure honey shows darker red (*a* values) and yellow (*b* values) color. Moreover, there is a decreasing trend in pH values and an increasing trend in water activity (*a<sub>w</sub>*) with the addition of fructose and saccharides to the pure honey .*Foods* **2020**, 9, 1538 12 of 21 In addition to HMF, diastase enzymatic activity can also be used for detecting sugar adulteration . Enzymatic activity (diastase and invertase) is one of the simplest and most applicable analytical methods, though it is not always conclusive and must be paired with other analytical methods to generate a reliable result. As a comparison between these two enzymatic activities, invertase is a better marker for the quality control of honey, since it degraded much faster during the heating up procedure of honey, compared to amylase . Moreover, Differential Scanning Calorimeter (DSC) can be used to determine the thermal properties of honey influenced by the direct addition of sugar syrup adulterants. Furthermore, the conventional polymerase chain reaction (PCR) can detect several genes of rice molasses, which has been used in the direct adulteration of honey. Furthermore, the standard curve attained from real-time PCR can quantify the rice molasses' DNA amount and calculate the exact level of adulterants. Several detection techniques have been developed in recent studies with the aim of detection of direct honey adulterated with sugar, such as high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) for the detection of CS and HFCS, HPLC, and stable carbon isotope ratio analysis (SCIRA) for the detection of HFS , GC-MS for the detection of HFCS , cavity ring-down spectroscopy (CRDS) and isotope ratio mass spectrometry (IRMS) for the detection of CS , three-dimensional fluorescence spectroscopy (3DFS) for the detection of RS , Raman spectroscopy for the detection of HFCS , matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) for the detection of IS and CS and headspace-gas chromatography coupled to ion mobility spectrometry (HS-GC-IMS) for the detection of CS . However, ultrahigh-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF-MS) was applied for indirect adulteration of honey, through bee feeding with multi-class sugar syrups,

such as HFCS, RS, and IS. Hence, the liquid chromatography-electrochemical detection (LC-ECD) and laser-induced breakdown spectroscopy (LIBS) methods were used for the detection of acacia honey blended with rape honey. The chemometric analysis is widely applied to the detection method as a helpful tool for the reduction of samples' complexity and classifies them into groups based on their similarities. The most commonly used chemometric techniques are the principal component analysis (PCA) , orthogonal projection to latent structure discriminant analysis (OPLS-DA) , partial least square-linear discrimination analysis (PLS-LDA) , and partial least squares regression (PLSR), represents the most recent detection methods for the identification of honey adulteration based on sugar adulterants.

## CHAPTER 5: RESULTS

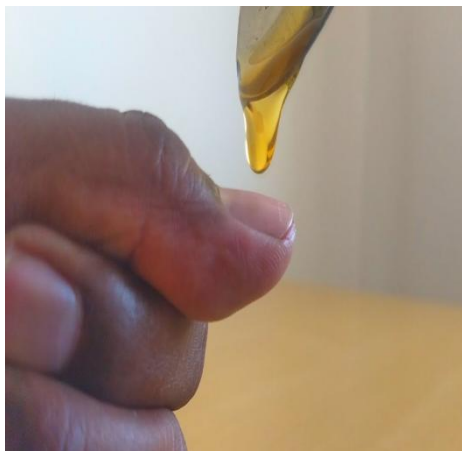
On this experiment we find out the adulterated and purified organic honey using four tests by the collected sample. First collected honey have tested on perform the heat test on this honey have taken by using cotton dipped on sample if have taken and burn by using match stick or Bunsen burner it shows the good unadulterated honey and adulterated honey. On second "water test", Here the collected sample honey have mixed with water when it mixed it was adulterated and no watery mixed it was unadulterated by this basis the test have been done and results have been showed be lower, we have find the both adulterated and unadulterated samples. At last, the final test was thumb test it has done by our thumb using collected samples. Place a drop of sample on thumb, if it get of loas it was adulterated flows honey is the unadulterated organic good honey. by this procedure we have find the results using collected sample. On the heat test, if burns due to the adulterated of jaggery on honey. on second, if mixed may be due to high watery content.



**Fg.8.1 Adulterated honey**



**Fg.8.2 Pure honey**



**Fg.8.3 Pure honey**



**Fg.8.4 Adulteration honey**

## CHAPTER 6: SUMMARY AND CONCLUSION

The significant impact of honey adulteration on market loss, reducing the quality of honey, shows the importance of studies to investigate different honey adulterants, adulteration methods, and detection methods. Moreover, this fraud has an adverse impact on the honey production industry and market by reducing the trust of consumers on this valuable product. According to this review, all six studied sugar adulterants (cane sugar, corn syrup, palm sugar, invert sugar, rice syrup, and inulin syrup) have health disadvantages toward human health based on their LD50 value and internal organ toxicology. The kidney and liver are the main organs that fail due to the consumption of sugar-adulterated honey. Diabetes, CKD, and AKI are the direct results of honey adulteration stated by in vivo histological examination. These diseases have a noticeable impact on human daily life and *Foods* 2020, 9, 1538 15 of 21 social health. Hence, the discussed detection methods can be used to identify honey adulteration via chemometric methods. While these techniques require specific laboratory knowledge and equipment, rapid and accurate detection methods, with ease of public accessibility to save customer's expenses and time, and guarantee their safety, are absent. As has been discussed in this review, more prompt actions must be taken by authorities to prevent the production, trading, and marketing of adulterated honey and discover the other harmful honey adulterants available on the market. The overall result demonstrates that honey adulteration is a threat to food safety, food security, and ecological sustainability of this important and valuable product.

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