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HMF as a Quality Indicator in *Garcinia indica* Fruit Juice Concentrate

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Abstract

Garcinia indica is commonly found fruit in India and known for its therapeutic applications. In Particular,its fruit juice concentrate is used as a refreshing drink and for certain therapeutic uses. However, this concentrate contains high amount of sugar and the way it is processed may result in undesirable products and quality deterioration. This study investigates the presence of 5-Hydroxymethylfurfural (5-HMF) in the fruit concentrate which is essentially formed from sugar dehydration, especially in the food which undergoes thermal treatment.The three types of samples used for the study are syrups of the fruit prepared domestically, available commercially and the fruit juice without sugar. Concentration of 5-HMF in the above mentioned three samples is 316.224 mg/kg 147.840 mg/kg and 19 mg/kg respectively. 5 -HMF in sugar-containing samples is above maximum tolerance level - 20 mg/kg (EU) and 25 mg/l (IFFJP). This is clearly due to high proportion of sugar in these concentrates and the processing method. These results raise questions upon its quality and warn about the potential risk of formation of 5-HMF.The study further investigates the effects of HMF on its therapeutic value.

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Keywords

Garcinia indica,
5-HMF,
Maillard reaction,
Quality.

Introduction

Nutritional factors are widely considered to be critical for human health. Diet rich in fruits and vegetables provides a broad range of nutrients and reduces the risk of various degenerative ailments. A plethora of study has been carried out on their nutritional and epidemiological importance. However, the impact of processing on the nutritional quality of fruits and vegetables is largely ignored in such studies[1]. Processing may impact content, activity, and bioavailability of bioactive compounds.

Food processing has been practiced since ancient times to preserve the freshness, taste, texture, appearance and nutritional value. With the advent of technologies, it has become one of the largest sector of foodindustry. According to FSSAI manual for food safety regulators, nearly every food preparation

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process reduces the amount of nutrients in food. In particular, processes that expose food to high levels of heat, light, and/or oxygen cause the greatest nutrient loss and result into many undesirable properties which deteriorate the quality as well as the biological value of food[2].

5-HydroxyMethyl-2-Furfural (HMF), the product of thermal treatment of foods that contain carbohydrates is a well-known indicator of quality deterioration[3-4]. This cyclic aldehyde is formed through the dehydration of hexoses and hexuloses in an acidic environment, or as a result of Maillard reactions and caramelisation. The presence of HMF in foods is due to high temperature during heat treatment and inappropriate and long-term storage. It reduces the nutritional value of food[4-8]. Maillard reactions represent an interesting research area for the implications in food technology, nutrition, and health.

HMF is considered to be an irritant to eyes, upper respiratory tract, skin and mucous membranes and damages striated muscles and viscera by combining with proteins, causing the accumulation of poisons in the body[9-10] which may lead to mutagenicity and carcinogenicity[11-12]. Although, its toxicological studies and experiments are limited to mice and rats and very little effortsare made to explore its potential risk to human health, the National Institute of Environmental Health Sciences nominated HMF for toxicity testing considering its widespread exposure through food, and evidence of carcinogenic potential of other members of this class[13].

In fact, regulations for HMF in foods legislation worldwide have been mainly limited to honey which is 80 mg/kg according to Codex Alimentarius (Alinorm 01/25, 2000) and 40 mg/kg according to European Union (EU directive 110/2001). The Food Safety and Standards Authority of India (FSSAI) have accepted the guidelines by Codex for honey but no specific standards are given for HMF in fruit juices. European Fruit Juice Association's (AIJN) code of practice (AIJN, 1996) has fixed maximum HMF level of 20 mg/kgin fruit juices. The International Federation of Fruit Juice Processors (IFFJP) has recommended maximum concentrations of 5-10 mg/l and 25 mg/kg in fruit juices and concentrates, respectively[16]; and the European Union has set a limit of 20 mg/kg 5-HMF for juices made for children[17].

Garcinia indica is a fruit-bearing plant commonly found in the western region of coastal southern India. Locally, it is called as Kokum and well-known for its rich medicinal and nutritional properties[18]. The studies have shown that it's fruits are a rich source of protein, tannin, pectin, sugars, fat, organic acids like (−)-hydroxycitric acid, hydroxycitric acid lactone and citric acid; the anthocyanins, cyanidin-3-glucoside and cyanidin-3-sambubioside; and the polyisoprenylatedphenolicsgarcinol and isogarcinol[19-21]. Preclinical studies have shown that kokum and / or some of its phytochemicals possess antibacterial, antifungal, anti-ulcerogenic, cardioprotective, anticancer, chemopreventive, free radical scavenging, antioxidant and anti-obesity effects[22-27].

Kokum squash or kokum concentrate is used in preparing a drink (sharbat) which is bright red in colour. It improves digestion and cools the body during summer. Traditionally, it is prepared by adding large amount of sugar in the fruit rinds and providing solarheat to convert itinto syrup. Sugar and heat treatment together improves shelf life and palatability. However, the severity of process and storage may lead tothe formation of a large amount of HMF which may lower its value with respect to safety concern. Many studies have reported the prevalence of HMF in fruit juices and concentrates due to processing and poor storage conditions[6, 28-33].

This studyaims to determine the HMF content in domestically prepared *Garcinia indica* syrup as well as syrup available in the Indian market. It is believed that the observations of this study may contribute to further research onthe effect of HMF on the medicinal values of this exotic plant as well asconsumer health and well-being.

Materials

The samples collected for this study include both domestically prepared syrup (traditional method), commercially available syrup (collected from pharmaceutical store of local market).

Sample Description

S1: Domestically Prepared Syrup of *Garcinia indica* Containing Sugar

It is prepared by mixing fresh *Garcinia indica* fruit rinds with sufficient amount of sugar in air tight glass jar. The glass jar is further placed in sunlight till all the sugar gets converted into concentrated syrup. Traditionally, it is prepared in the month of April-May and stored for long period.

S 2: Commercial Available Syrup of *Garcinia indica* Containing Sugar

It is collected from local market and used after 6 months of its manufacturing date.

S 3: Domestically Prepared Syrup of *Garcinia indica* Without Sugar

It is a salt preserved juice of fresh fruits and prepared by adding small amount of salt into fruit pulp.No sugar is added. It is also stored for long period and used as flavouring agent for many traditional food preparations.

All the samples were used after six months storage.

All the chemicals used for analysis are A.R. grade.

Methods

All the samples were analysed for the HMF content, sugar content, titratable acidity, pH, % moisture, total ash and sulphated ash. Two parallel analyses were carried out on each sample for each parameter.

HMF is determined spectrophotometrically by Winkler's method[34]. Two grams (±0.01g) of each syrup were dissolved in 10 ml water in a volumetric flask. Two Tubes containing 2 ml of the diluted syrup solutions and 5.0 ml of p-toluidine solution were prepared for each of the syrup. To one tube of syrup 1 ml of 0.5% barbituric acid solution was added (sample) and to another 1 mL water (Reference). The absorbance of all the solutions were measured at the wavelength of 550 nm by using spectrophotometer (SHIMADZU, UV-1800). The HMF value was then determined by using the proposed formula for the method.

Sugar content is determined spectrophotometrically by DNSA (dinitrosalicylic acid) method[35]. A series of standard solutions of sucrose (200 to 1000µg/mL) were prepared. Test solutions were prepared from sample by proper dilution. To each of the solution 0.5 ml of DNSA reagent is added and kept in a boiling water bath for 15 minutes followed by addition of 0.5 ml of 40% sodium potassium tartrate. Absorbance of each solution is measured at wavelength 540 nm against blank. Calibration curve method is used for quantitative estimation of sugar.

pH is measured by using pH meter (Equiptronics, EQ 621). Total acidity is determined by direct titration (BS 1741:1963) with 0.1M NaOH using phenolphthalein indicator. Percentage moisture is determined by heating 2 gm of syrup samples in silica crucible in oven at 105+2 ^{0}C till constant weight is obtained. Further it is ignited at 550 ^{0}C in muffle furnace to determine total ash. Sulphated ash is also determined for the analysis.

The effect of storage and temperature is also studied by determining HMF content at different temperatures and period.

Results

HMF Content and Parameters Responsible for HMF Formation

Formation of HMF is very common when carbohydrate-rich food undergoes thermal treatment[36]. The factors that influence the formation of HMF in food are (a) carbohydrate content, (b) physico-chemical properties (pH, total acidity), (c) thermal treatment, (d) water activity, and (e) long-term storage. In general, monosaccharides (i.e. fructose or glucose) are the basic substrates for HMF production. Thus, along with HMF, sugar content, total acidity, pH, moisture, and ash values of the *Garcinia indica* concentrate samples were also measured. The data obtained is shown in table 1.

Effect of Storage and Temperature

Due to heat treatment and increased dry matter, juice concentrates are more susceptible to non-enzymatic browning. Since the accumulation of HMF in the juice is described asan autocatalytic reaction its concentration at the beginning of a storage period may have an influence of its further increase[37,38]. Thus, a juice concentrate prepared by application of heat treatment probably has a higher HMF formation rate during storage as shown in table 2.

Table 1: Determination of concentration of 5 HMF, Sugar and pH of the concentrate sample

Sample	5-HMF (mg/kg)	Sugar (gm/kg)	pH	Total Acidity (meq/kg)	Moisture (%)	Total Ash (%)	Sulphated Ash (%)
S 1	316.224 + 0.041	1000	3.08	220	15.61	2.57	3.06
S 2	147.840 + 0.101	750	2.95	120	22.52	3.11	4.63
S 3	19.392 + 0.008	100	2.65	900	77.41	6.64	8.89

Table 2: Study of influence of storage and temperature on HMF formation in *Garcinia indica* fruit juice concentrate samples

Temperature No. of Weeks	Room temperature	37^0C	45^0C
Sample 1			
1	383.616 + 0.056	396.288+ 0.120	397.824+ 0.081
2	384.576 + 0.061	407.232+ 0.211	414.336+ 0.044
3	386.304+ 0.148	422.400+ 0.089	426.240+ 0.010
4	401.280+ 0.049	442.752+ 0.057	462.336+ 0.091
Sample 2			
1	211.162+ 0.018	371.328+ 0.067	377.088+ 0.035
2	220.032+ 0.099	386.112+ 0.043	389.568+ 0.061
3	249.792+ 0.021	411.264+ 0.100	420.480+ 0.027
4	293.760+ 0.050	415.872+ 0.072	425.856+ 0.080
Sample 3			
1	20.544+ 0.011	48.576+ 0.032	111.552+ 0.064
2	23.040+ 0.052	56.064+ 0.141	116.352+ 0.096
3	28.416+ 0.081	71.424+ 0.095	145.728+ 0.059
4	34.560+ 0.129	79.872+ 0.033	157.440+ 0.067

Discussion

The results in table 1 indicate that there is a significant effect of sugar concentration on the formation of HMF in the samples. The lowest HMF values for the S3 (19 mg/kg) is less than 20 mg/kg - maximum level established by AIJN for juices[15] decreased HMF level may be due to less sugar content.

The other two samples, S1 and S2 were found to have very high concentrations of HMF which are 316.284 mg/kg and 147.830 mg/kg respectively. The values clearly indicate that concentration of HMF in both the samples are too high and far beyond the maximumtolerance level[15]. Both the samples contain a very high proportion of sugar coupled with the heat treatment. In fact, sugar serves as preservatives for such concentrates; however, the results indicatethat it is also one of the obvious reasons for HMF formation[39]. The above results are obtained for the samples which are six months old; in fact, these concentrates are stored over the years and used. According to Kus *et al.,* formation of HMF is very common in concentrated fruit juice than in fresh juice, because the rate of the reaction depends on the amount of total soluble solids present in the juice or concentrate in addition to concentration process of fruit juices, dehydration of fruits, and storage at improper temperature[40,41]. Thus, this study is very important as regards to the quality standards of these preparations and their safety for therapeutic usage. However,the risk of browning reactions can be reduced by applying low temperature, vacuum, proper storage condition.There is a need for increased awareness about processing and storage hazards, especiallyfor locally prepared food.

During storage of the samples at different temperature, increase of HMF content was observed in all the samples (Table 2) including a sugarless sample (S3). Formation of HMF during storage in all samples and at all temperatures bears linear with time. Similar results were reported by Aslanova *et. al.,* for the study of different fruit jam samples[42]. The rate of the reaction was directly related to temperature. Thus, 5-HMF can be used as an indicator of heat stress and storage changes.

The juice concentrate has high demand among consumers. In this regard, such a high accumulation HMF cannot be ignored and further study is needed to find out its impact on the quality of this concentrate and thus public health. People should be made aware of physical and chemical changes that take place during processing of food and storage, specifically locally prepared food.

Conclusion

HMF formation in *Garcinia indica* fruit concentrate was investigated. *Garcinia indica* fruits are having a therapeutic and nutritional importance. In particular, its concentrate is used as a refreshing and digestive drink. This study brings into notice the high accumulation of HMF in this concentrate which is a result of decomposition of sugar due to thermal treatment in the acidic environment and long storage. In addition, this work demands further study on the effects of HMF accumulation on the therapeutic and nutritional value of these fruits and thus the quality of juice concentrate.

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Conflict of Interest

The author declares that there is no conflict of interest.

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