Effect of Fermentation Process on Ashgourd Juice

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Abstract: Ashgourd (*Benincasa hispida*) is valued for its nutritive and medicinal properties and further value addition is being attempted by fermentation process. In the present study, effect of fermentation was studied by using ashgourd as a substrate for nutrients and flavour components formation. Ashgourd fermented beverage was developed by using commercially available wet yeast and the changes in vitamins profile and flavouring compounds were evaluated after the fermentation process and it was compared with the raw ashgourd juice. Fermentation showed positive response indicating that, thiamine, riboflavin, niacin, pyridoxine and vitamin C were increased by 76µg, 7µg, 171µg, 459µg and 1.5mg respectively per 100 ml of the beverage in comparison to fresh juice. Fermentation showed many flavour components development in comparison to raw ashgourd juice and the fermentation process had a positive response on volatile component formation. Therefore fermentation improved the formation of nutritional and flavour components.

Keywords: Ashgourd, fermentation, vitamins, flavour compounds.

1. INTRODUCTION

Ashgourd (*Benincasa hispida*), a member of the family Cucurbitaceae is one of the familiar crops that are grown primarily for its use as a vegetable and usually recognized for its nutritional and medicinal properties especially in Asian countries. As a rich source of functionally important bioactives and therapeutics such as triterpenes, phenolics, sterols, glycosides and soluble dietary fiber, the vegetable has been widely used for therapeutic treatments [1-3]. However, it is necessary to exploit the vegetable into various value added products which will give more remunerable returns to the growers and benefits the consumers with respect to nutritional and therapeutic importance and made available commercially. Effective use of such strengthful vegetable can help in increased awareness of their value and promote efforts to conserve them. In India, ashgourd is used in curries or is coated with sugar or syrup and eaten as a sweet and recently Majumdar *et al.* [4-6] have reported on the stabilization of ashgourd juice and ashgourd blended juice and Devaki and Premavalli [7] optimised the fermentation process conditions for ashgourd juice with dry yeast by utilizing the statistical software RSM and reported that fermentation process improves the quality in terms of nutrients. Fermentation is the oldest form of preservation, essentially consisting of transformation of the simple raw materials into value added products by utilizing the phenomenon of the growth of microorganisms or their activities on various substrates. The microflora formed during fermentation aids in better digestion and thus improves the action in the digestive system. Fermented foods and beverages enhance the pleasure of eating and their nutritional roles include indirect contributions through subjective enhancement of appetite, aids in digestion, produce beneficial enzymes, offer better nutrition and help to absorb vitamins and minerals more effectively from foods. The scope of food fermentation ranges from producing alcoholic beverages, fermented meat, milk and vegetable products to genetic engineering producing super foods to carry out efficient fermentation to treatment and utilization of waste and overall producing nutritious and safe products with appealing qualities [8-10]. The area of fermentation is very well known and the process is not only preservation of the product but also its mode for nutrient formation but still the work on nutrients formation in vegetable juice has not been covered so far and thus, there is a need to develop vegetable based fermented juices. Though vegetables are often used for fermentation in other countries [11, 12] it is less commonly used in India. Vegetables specially green colour vegetables, carrots etc. being good source of vitamins and minerals, further availability can be improved by fermentation process [13, 14]. Considering the traditional use of ashgourd, subtle flavour and taste, yeast fermentation is preferred than lactic fermentation. Horiuchi *et al*. [15], Rakin *et al*. [16] and Jose-Maria *et al*. [17] have also reported use of yeast in respect of onion, beetroot and carrot fermentation. Cheigh *et al*. [18] have reported yeast and lactic acid mixed culture for kimchi fermentation. Rakin *et al*. [14] have proposed a combination of yeast and lactic culture for beetroot and carrot juice fermentation. Kuboi [19] has used yeast for the

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production of fermented ginger beverage. But the researchers have not focused on minor nutrients formation such as vitamins as well as flavour changes and only few reports are available on the effect of these components. Hence, in the present study, an attempt has been made to develop ashgourd fermented beverage by alcoholic fermentation using commercially available wet yeast. Wet yeast is used only in the preparation of bakery products and in the current study it was utilized in the preparation of the functional beverage by fermentation process. Therefore, the present investigation was carried in the direction of studying the effect of fermentation process using ashgourd as a substrate for nutrients and flavour components formation.

2. MATERIALS AND METHODS

2.1. Materials

Good quality raw materials i.e. ash gourd (*Benincasa hispida*), wet yeast and sugar were procured from local market. All the chemical and reagents used for the analysis were Analytical Reagent grade. For HPLC analyses HPLC grade solvents and standards were procured from M/s SK Chemicals, Burdick and Jackson, HPLC certified solvents and M/s Sigma Aldrich, St.Louis, USA.

2.2. Raw Material Processing

In order to observe the effect of fermentation and thermal treatment in ashgourd juice on nutritional and flavour components four groups of samples were prepared viz (a) ashgourd raw juice – without blanching; (b) ashgourd raw juice – with blanching; (c) ashgourd fermented beverage – without heating and (d) ashgourd fermented beverage – with heating.

Ashgourd was de-skinned, removed the rind portion, cut into pieces and extracted the juice using a juice extractor (RayLons Metal Works, Bombay, India) and this juice was used as first sample i.e., ashgourd raw juice – without blanching. For second sample i.e., ashgourd raw juice – with blanching, the cut pieces of ashgourd was blanched at 90°C for 3 mins and extracted the juice. In order to see the effect of fermentation on ashgourd juice, the blanched juice was processed further using sugar syrup in the ratio 1:1. The sugar syrup was prepared by mixing sugar and water in the ratio 1:1. Commercially available wet yeast was used as starter culture for fermentation and the colony count was $18X10^{10}$cfu/g. One percent wet yeast was added to the mixture of ashgourd juice and sugar syrup and was fermented at room temperature (18-33°C) for 3 days. After fermentation the fermented beverage was filtered in muslin cloth immediately and centrifuged to get a clear beverage. The obtained beverage was used as sample c i.e., ashgourd fermented beverage – without heating. To see the effect of thermal treatment on fermented beverage, the centrifuged beverage was poured in polypropylene bottles and the bottles were sealed and pasteurized for 20mins at 85ºC and cooled. All the four samples were analysed for nutritional and flavour components.

2.3. Analytical Evaluation

The raw and fermented samples were analysed for total phenols based on calorimetric oxidation/reduction reaction and antioxidants estimation was determined in terms of free radical scavenging capacity using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Sandra *et al*., [20]. HPLC analysis on reverse phase C_{18} analytical column was carried out to estimate water soluble vitamins i.e., thiamine, riboflavin, niacin, pyridoxine and vitamin C [21] and alcohol with isocratic elution of solvent orthophosphoric acid and water at 0.8ml/min, with UV detector (215nm) [22]. Flavour analysis was carried out by GC/MS analysis**.** Microbial analyses were carried out according to APHA [23]. The analysis for all the parameters was carried out in triplicate and the average value has been reported.

3. RESULTS AND DISCUSSIONS

Ashgourd is considered as one of the underutilized horticultural crops, as the crop is neither grown commercially on large scale nor traded widely. The importance of ashgourd as a vegetable crop has been long recognized due to its high nutritional value, medicinal properties and innumerable use but being used only for curries and poojas as sacred vegetable. Hence, in the present study ashgourd fermented beverage was developed by using commercially available wet yeast and the changes in functional parameters, vitamins profile and flavouring compounds were evaluated after the fermentation process and it was compared with the raw ashgourd juice. Total phenols and antioxidants were considered as functional parameters. B vitamins such as thiamine, riboflavin, niacin, pyridoxine and vitamin C were considered as nutritional parameters. The changes in flavour components were studied in raw and fermented beverage of ashgourd.

3.1. Effect of Fermentation on Functional and Nutritional Parameters of Ashgourd Juice

Total phenols was 7mg/100ml in raw juice, where as after fermentation process it increased to about 16mg/100ml and in antioxidant activity too there was marginal increase after fermentation process (Table **1**). Total phenols content increased two folds more than recorded for raw ashgourd juice. Abbas [24] reported that yeasts can synthesize a number of bioactive compounds which can serve as antioxidants. Several *Candida* and *Saccharomyces* species produce compounds such as caroteniods, citric acid, glutathione and tocopherols with interesting antioxidant properties while Cruz *et al*. [25] reported that the formation of these substances can be induced in yeasts grown under stressing conditions. Further total phenols as separated by LCMS analysis, epigallocatechin gallate, gallocatechin gallate, epicatechin and catechin hydrate were found in raw ashgourd juice, while epicatechin and catechin hydrate were present in ashgourd fermented beverage (Table **2**). Though two components were found in fermented beverage however, the concentration has been found to be more than double in fermented beverage. In order to understand the effect of fermentation on vitamin profile, the raw and blanched juice of ashgourd was studied. The fresh juice extracted from raw ashgourd (a) without blanching and (b) with blanching was injected to HPLC and the vitamin profile is presented in Table **3**. In few of the earlier reports, some of the authors have reported that ashgourd contains 27mg of vitamin C, 0.02mg of thiamine, 0.05mg of riboflavin and 0.4mg of niacin [26]; 1.35mg of vitamin C, 0.02mg of riboflavin and 0.46mg of niacin [27]; and 68mg of vitamin C, 0.02mg thiamine, 0.31mg riboflavin and 0.2mg niacin [28] per 100g edible portion of vegetable, where as in the present study, the fresh juice contained 52µg thiamine, 8 µg riboflavin, 260 µg niacin, 221 µg pyridoxine and 0.9mg vitamin C per 100 ml of juice in the first sample i.e. ashgourd raw juice – without blanching. The second sample i.e., blanched raw juice contained 44µg thiamine, 6 µg riboflavin, 221 µg niacin, 184 µg pyridoxine and 0.5 mg vitamin C per 100 ml of juice. It was observed that blanching the vegetable reduced the vitamins to about 8 µg, 2 µg, 39 µg, 37 µg and 0.4 mg in thiamine, riboflavin, niacin, pyridoxine and vitamin C respectively. From the above results there was

Table 1: Effect of Fermentation on Functional Parameters of Ashgourd Juice

Parameters	Raw juice	Fermented beverage
Total phenols* (mg/100ml)	7 ± 0.2	16 ± 0.06
Antioxidant ** (%)	4 ± 0.03	4.4± 0.12

*Total phenol expressed as mg/100ml gallic acid equivalents.
**DPPH free radical scavenging activity for 1ml sample extract.

Table 2: Effect of Fermentation on Total Catechins of Ashgourd Juice

Total catechins	Raw juice	Fermented beverage
Epigallocatechin gallate	√	-
Gallocatechin gallate	√	-
Epicatechin	√	√
Catechin hydrate	√	√

Table 3: Effect of Fermentation on Vitamin Profile of Ashgourd Juice

Vitamins (per 100ml)	Raw		Fermented beverage	
	Without blanching	With blanching	Without heating	With heating
Thiamine (B_1, µg)	52 ± 0.21	44 ± 2	128 ± 7.5	80 ± 0.8
Riboflavin (B_2, µg)	8 ± 0.15	6 ± 0.4	14.9 ± 1.1	5.2 ± 0.2
Niacin (B_3, µg)	260 ± 3.5	221 ± 4.16	431 ± 11.0	320 ± 0.6
Pyridoxine (B_6, µg)	221 ± 0.58	184 ± 1.0	680 ± 1.73	620 ± 0.6
Vitamin C (mg)	0.9 ± 0.01	0.5 ± 0.01	2.4 ± 0.18	1.4 ± 0.1

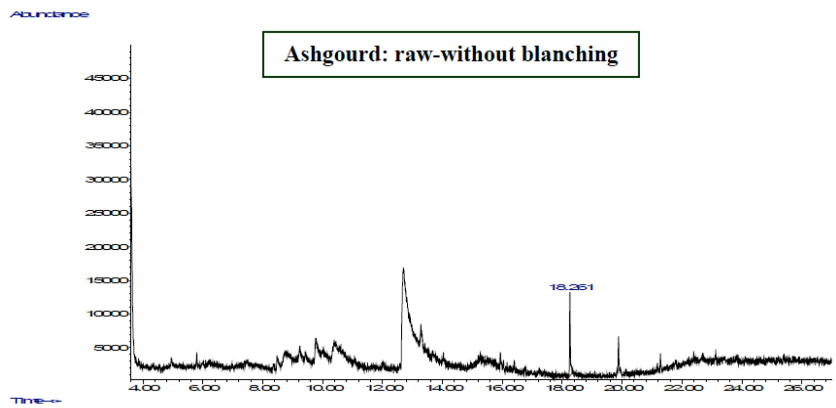
decrease in all the selected vitamins after blanching, but after fermentation, the vitamins concentration increased. After fermentation process the vitamins values were 128µg thiamine, 14.9µg riboflavin, 431µg niacin, 680µg pyridoxine and 2.4mg vitamin C per 100ml of the beverage. Therefore, it is noteworthy to mention that fermentation process helped in the formation of all the vitamins when compared to raw and blanched juice and thus had a positive influence on the vitamin formation in the ashgourd fermented beverage. Abbas [24] has reported that among the vitamins and other enzyme cofactors that are accumulated and/or synthesized by yeast are thiamine, nicotinic acid, pyridoxine and pantothenic acid. Afanaseva *et al*. [29] reported that sauerkraut juice had high amounts of vitamins such as C, β-carotene, thiamine, and riboflavin. Montano *et al*. [30] reported that on a dry basis, the fermented product was found to have a higher content of riboflavin, α-tocopherol and but a lower thiamine level than the unfermented product. Further, in order to stabilise the beverage with increased shelf life thermal process was followed and it was observed that pasteurization decreased 9 to 65% of vitamins and the values ranged 80 µg thiamine, 5.2 µg riboflavin, 320 µg niacin, 620 µg pyridoxine and 1.4 mg of vitamin C per 100ml of the beverage. However, when compared to the fresh juice, both fermented beverage as such and pasteurised had higher quantity of vitamins reflecting the advantage of fermentation process. Biosynthesis of B vitamins such as folacin, vitamin B_6, pantothenic acid was also studied by Hozova *et al.* [31] during fermentation (8 to 9 days at 20±2°C) and storage (4°C) of cut cabbage samples and the authors also found that after 40 hrs of fermentation, folacin content in samples increased from an initial level of 0.077mg/kg to 0.460mg/kg. Vitamin B_6 contents with initial value 14.6 mg/kg varied during fermentation for all samples. Rakin *et al*. [14] also reported that the fermentation of beetroot and carrot juices, with addition of brewer's yeast autolysate, increased the content of vitamins of the B group in fermented bioproducts which mainly originated from the brewer's yeast, as their presence in fresh beetroot and carrot is negligible. In the present study, as the starter culture used was yeast source alcohol formation was expected and in the present sample as estimated by HPLC the beverage had 1.7% of alcohol.

3.2. Effect of Fermentation on Flavour Components of Ashgourd Juice

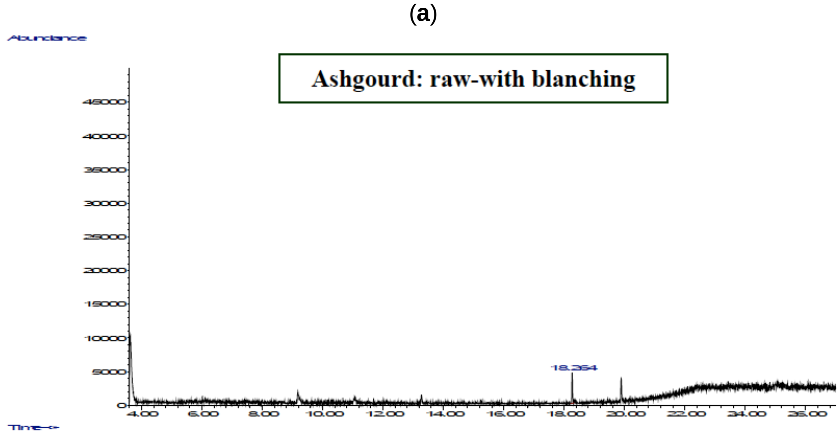
Fermented foods generally preserved have pleasant flavour with good keeping quality under ambient conditions. An important indicator of quality of fermented food is volatile substances created during fermentation [32-38]. In turn, determination of changes in the contents of flavour components such as aldehydes, organic acids and their esters, and alcohols in fermented beverages with fermentation performed in

Table 4: Effect of Fermentation on Flavour Components of Ashgourd Juice

Component	Raw		Fermented beverage	
	Without blanching	With blanching	Without heating	With heating
Hexadecanoic acid	√	√	√	√
1-tetra Decanol / Loxanol V	-	-	-	-
2,4-bis (1,1-dimethyl ethyl)Phenol	-	-	-	-
2-Ethyl Hexanol	-	-	-	-
2-Furan methanol	-	-	√	√
3,5,-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one	-	-	√	√
5 Methyl 2, Furancarboxaldehyde	-	-	√	√
5-hydroxy methyl 2 furancarboxaldehyde	-	-	-	√
9,12,15 – Octadecatrienoic acid	-	-	-	-
Chondrillsterol	-	-	-	√
Decanoic acid	-	-	√	√
Linoleic acid	-	-	√	√
Octadecanoic acid	-	-	√	√
Phenyl Ethyl Alcohol	-	-	√	√
Tetradecanal	-	-	-	-



(**a**)



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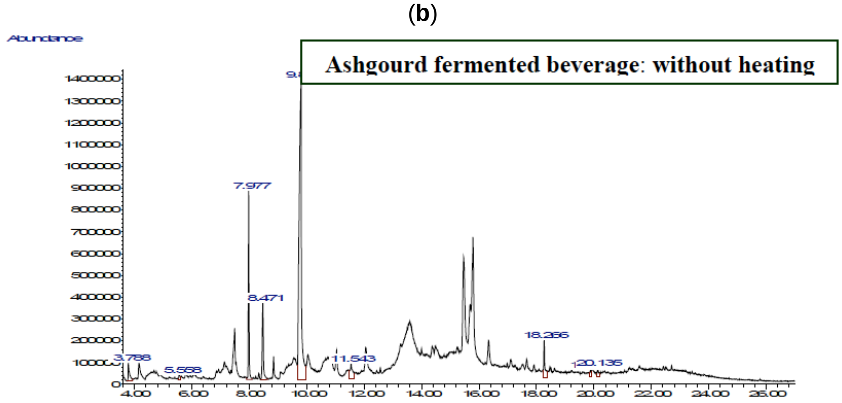
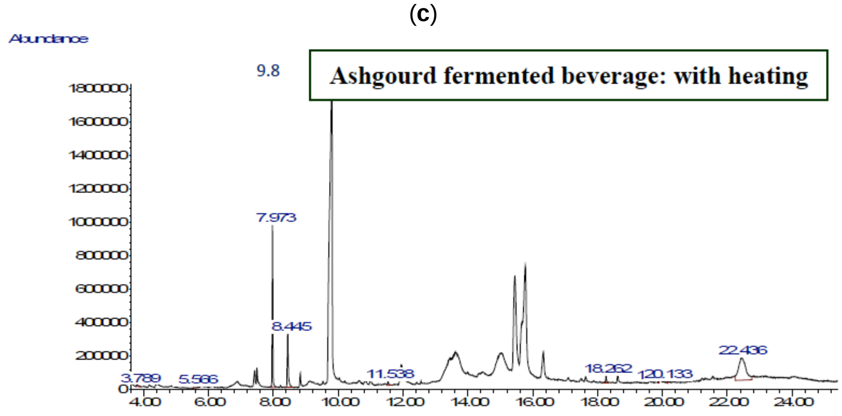


(**c**)



(**d**)

Figure 1: Flavour components of ashgourd juice and fermented beverage; **1a**: ashgourd- raw, without blanching; **1b**: ashgourd-raw, with blanching; **1c**: ashgourd fermented beverage – without heating; and **1d**: ashgourd fermented beverage with heating.

different ways and under various conditions is very important. GC/MS analysis was carried out to identify the flavour components in the fresh samples and fermented beverage after fermentation process. In order to identify the flavour components developed during fermentation process, the developed beverage was compared with the raw ashgourd juice. Some volatile compounds have been identified by the researchers in ashgourd fruit are aliphatic alcohols and carbonyl compounds being the major class [39]. The identified compounds are (E)-2-hexenal, n-hexanal, n-hexyl formate, (E, E)-2, 4-heptadienal and (E, E)-2, 4-nonadienal. Some other detected volatile components are (Z)-3-hexenal, (E)-2-heptenal, and 1-octen-3-ol [39, 40, 41]. The analysis of the volatile compounds of beverages from this fruit shows the presence of 2, 5-dimethylpyrazine, 2, 6- dimethylpyrazine, 2, 3, 5-trimethylpyrazine, 2-methyl pyrazine and 2-ethyl-5-methyl pyrazine [40, 41]. Maarse [39] claimed that the beverage prepared from this vegetable has different aroma, and the analysis of the volatile compounds indicated the presence of pyrazine, which is believed to be formed by Maillard reaction during the extraction of juices. Mingyu *et al*. [27] identified 18 volatile compounds using GC/MS with 2-aminohexanoic acid, 2-amino-3-cyano-propanoic acid, 2-aminobutanoic acid, 2-amino-4- hexenoic acid and 3-cyclohexenyl-1-glycine being considered as unusual substances. In the present study, GC/MS analysis was carried out in 4 samples (Table **4**) of ashgourd juice viz, raw juice without blanching (Figure **1a**); raw juice with blanching (Figure **1b**); fermented beverage as such (Figure **1c**) and fermented beverage with heating (Figure **1d**) and the peaks were identified from the NIST library. In the first sample i.e., raw ashgourd juice without blanching was injected and it was found that one compound was identified at the retention time of 18.26 mins and it was identified as hexadecanoic acid. In the blanched juice, it was found that hexadeconoic acid decreased and it may be due to partial volatility during heat treatment The quantum of volatile compounds formed during fermentation depends mainly on the fermented must composition, fermentation conditions and strain of yeast that was used [42]. The effect of fermentation on the flavour components has been evaluated and it was observed that 8 peaks were formed (Figure **1c**), and the identified compounds were 2-Furan methanol; 5 Methyl 2, Furancarboxaldehyde; Phenyl Ethyl Alcohol; 3,5,-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one; Decanoic acid; Hexadecanoic acid; Octadecanoic acid; Linoleic acid with the retention time of 3.79; 5.57; 7.98; 8.496; 11.5; 18.264; 20.129 and 19.88 mins respectively. For the development of ashgourd fermented beverage yeast was used as starter culture, therefore alcohol formation was observed in the fermented sample thereby, many alcoholic compounds could be identified. Ku *et al.* [43] carried out fermentation of ginger paste by *Lactobacillus plantarum* and *Lactobacillus brevis* and studied the sensory attributes, gingerol content and volatile components in both fresh ginger and fermented ginger. The authors reported that the fermented ginger had increased levels of alcohol components. Ashgourd fermented beverage was processed in sealed bottles. During processing, 10 peaks were found showing relatively higher formation of volatile compounds (Figure **1d**). The compounds identified were 2 - Furan methanol; 5 Methyl 2, Furancarboxaldehyde; Phenyl Ethyl Alcohol; 3, 5, - Dihydroxy - 6 – methyl - 2, 3 – dihydro - 4H – pyran – 4 - one; 5 - hydroxy methyl 2 furancarboxaldehyde; Decanoic acid; Hexadecanoic acid; Linoleic acid; Octadecanoic acid and Chondrillsterol and the retention time were 3.789; 5.556; 7.973; 8.445; 9.82; 11.538; 18.262; 19.884; 20.133 and 22.436 mins (Figure **1d**). In thermally processed fermented beverage two more volatile components such as 5 - hydroxy methyl 2 furancarboxaldehyde and Chondrillsterol were generated as compared to just fermented ashgourd juice. Therefore, fermentation showed many flavour components development in comparison to raw ashgourd juice and the fermentation process had a positive response on volatile component formation.

4. CONCLUSION

Effect of fermentation on ashgourd vegetable was studied for its nutritional and flavour components and the results revealed that fermentation showed positive response indicating that, thiamine, riboflavin, niacin, pyridoxine and vitamin C were increased in comparison to fresh juice. However giving thermal treatment decreased vitamins when compared to fermented beverage but, when compared to the fresh juice, both fermented beverage as such and pasteurised had higher quantity of vitamins reflecting the advantage of fermentation process. Fermentation formed 9 more flavour components in comparison with fresh juice. Therefore fermentation improved the formation of nutritional and flavour components.

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