

Losses in flavour of Indian potatoes: Influence of storage temperature

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ABSTRACT

Fresh potatoes are preferred for cooking over stored potatoes due to the superior taste of freshly harvested potatoes since with the increase in storage duration, quality as well as taste of potatoes are known to deteriorate. Therefore, the aim of the present study was to investigate the effect of storage (for 180 days at 4 °C and 12 °C) on concentration of flavouring compounds, particularly umami 5' nucleotides *viz.* adenosine monophosphate (AMP) and guanosine monophosphate (GMP) along with changes in carbohydrates. AMP+GMP content ranged from 2.19 to 4.69 µg/g FW (fresh weight) in freshly harvested raw and microwaved tubers of 11 potato cultivars. The content of umami 5' nucleotides increased to appreciable amounts during microwave cooking (3.52 to 8.92 µg/g FW). During storage at 4 and 12 °C, flavouring compounds ranged from 0.29 to 2.39 µg/g FW and 0.34 to 2.92 µg/g FW in raw tubers and from 0.91 to 5.64 µg/gFW and 2.00 to 4.28 µg/gFW in microwaved potatoes, respectively. On an average, there was 19% and 10% decrease in starch content whereas 112% and 69% increase in reducing sugars and 90% and 57% increase in sucrose content, at 4 °C and 12 °C storage, respectively. It is known that the quality and taste of potatoes deteriorates during storage due to starch hydrolysis leading to reducing sugar accumulation, but this study has revealed that it is not only the sugar accumulation but also the decrease in flavouring compounds *viz.* AMP+GMP which results in poor taste of stored potatoes. Hence, sugars accumulation and deterioration of flavouring compounds together leads to poor taste of stored potatoes.

Keywords: potato, flavouring compounds, adenosine monophosphate, guanosine monophosphate, storage, starch hydrolysis.

INTRODUCTION

There is a huge demand of fresh potatoes in the market due to the unique flavour of fresh potatoes. Potato tuber flavour is of great importance to consumers and it is now well known that cooking enhances the flavour of potatoes (Raigond *et al.*, 2014). Flavour consists of taste that is due to nonvolatile compounds e.g umami compounds, aroma which is due to volatile compounds and texture that is mainly the mouth feel. All these compounds interact during processing to give flavour to the final form of product. Food flavour is of great interest because consumers are depending on better-tasting food. Flavour of food depends on the nature of the food as well as flavouring compounds present in it. The concentration of flavouring compounds are influenced not only by plant genotype, but are also affected by storage and production environment (Jansky 2010). The main flavour precursors present in raw potato are amino acids, sugar, RNA and lipids. Flavour precursors react to produce the Maillard reaction compounds as well as sugars, lipid and RNA degradation products that contribute to specific flavour (Duckham *et al.*, 2001).

Various types of interactions and transformations take place during cooking/processing of potatoes that result in a pleasant and characteristic 'potato flavour'. Potato contains umami compounds that are known to enhance the flavour and mouth feel, giving the impression of creaminess and viscosity to savory dishes (Del Valle *et al.*, 2006). Umami is the fifth basic taste, is an inexplicable, delicious taste sensation that differs from sweet, sour, salty and bitter tastes by providing a meaty, savory sensation. Major umami compounds present in potato are glutamic acid, aspartic acid, guanosine 5'-monophosphate (GMP) and adenosine 5'-monophosphate (AMP). Glutamate is the most significant umami amino acid, whereas 5'-GMP is the most significant umami 5'-nucleotide (Yamaguchi *et al.*, 1971). Along with umami compounds, large number of volatiles produced during cooking also contributes to potato flavour, but there is no clear cut identification of those volatiles that are mainly responsible for cooked potato flavour (Morris *et al.*, 2007). Higher levels of 5'-ribonucleotides (AMP and GMP) are present in potatoes compared to other vegetables (Solms and Wyler 1979). These compounds are present in low

quantities in raw potatoes and increased to manifolds during cooking due to enzymatic hydrolysis of RNA.

To maintain round the year supply of potatoes to market and processing sector, potatoes are generally stored at low temperatures. Potatoes are either being stored at 2-4 °C to 10-12 °C for up to 6-7 months in cold stores as per their destined use. It is a well known fact that storage deteriorates the quality as well as taste of potatoes. During storage, temperature is the major determinant of tuber quality and taste. Potatoes are being stored at low temperatures for long periods which lead to 'senescent sweetening'. Whereas storage at low temperatures (<7 °C) leads to 'low temperature sweetening'. Both type of sweetening are resulting from the conversion of starch to sugars. Free radicals are generated during both type of stresses which leads to lipid peroxidation that result in loss of membrane integrity and as a result amyloplast membranes also become leaky (Kumar and Knowles, 1993; O'Donoghue *et al.*, 1995). Leaky amyloplast membranes are the major reason behind the starch hydrolysis during storage. The quality of potato tubers for fresh market and processing depends on the storage conditions and potato cultivar. High reducing sugars content in potato cultivars is an undesirable characteristic. Potato products such as chips and French fries prepared from such cultivars results in dark coloured products with bitter taste due to the 'Maillard reaction' that occurs due to reaction between reducing sugars and nitrogenous compounds at higher frying temperatures (Habib and Brown 1957).

Till date, most of the research has been focused on storage studies with respect to changes occurring in carbohydrates, antioxidants and shelf life of potatoes. Potato flavour was not given much priority because of difficulty in assessment and quantification of flavouring compounds. Hence, no information has been generated on the effect of storage on the level of umami compounds in Indian potato cultivars. Therefore, the present study was planned to quantify the effect of storage on the concentration of umami 5'-nucleotides in raw as well as microwave cooked potatoes and also observe changes in the carbohydrates during storage.

MATERIAL AND METHODS

Sample Preparation

The experiments were conducted at ICAR- Central Potato Research Institute, Shimla. Potato crop was raised at CPRIC, Modipuram, India (29° 4' N, 77° 46' E, 237 masl) during winters in 2013-14 using standard package of practices and were utilized for the present study. Freshly harvested tubers of 11 potato cultivars varying widely in

keeping and processing quality *viz.* Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3, Kufri Frysona, Kufri Himsona, Kufri Jyoti, Kufri Sherpa, Kufri Khasigaro, Kufri Bahar, Kufri Lalima and Kufri Lauvkar were used. Tubers were sampled at fresh harvest or after 180 days of storage (DOS) at 4 °C and 12 °C in walk-in-chambers. These storage temperatures are generally used by the commercial potato cold storage. Flavouring compounds (AMP+GMP) were estimated from raw and microwave cooked potatoes of these cultivars using High Performance Liquid Chromatography (HPLC). Microwaving of potatoes was done by using three medium sized tubers in a microwave oven for 10 min at 1,350 W. All the samples were analyzed in triplicates. Biochemical analysis *viz.* starch, reducing sugars and sucrose was done from freshly harvested (control) raw tubers and stored tuber (180DOS) following standard methods.

Biochemical Procedures

Flavouring compounds (AMP+GMP)

Reagents: Standards of high purity *viz.* AMP and GMP, perchloric acid, potassium dihydrogen phosphate, potassium hydrogen diphosphate were procured from Sigma Chemical Co (USA). All the reagents were dissolved in deionized water (MilliQ) and filtered through 0.45 µm filter.

Analysis of 5'-nucleotides (AMP + GMP)

5'-nucleotides were extracted with the method described by Raigond *et al.*, (2014). Tuber tissue (0.5 g) from raw as well as microwave cooked tubers were rapidly frozen in liquid Nitrogen and homogenized into powder. For extraction of 5'-nucleotides, chilled 5% perchloric acid (10 ml) was added to the powder and the sample was incubated in ice bath for 1 min. The extraction mixture was centrifuged at 4 °C for 10 min at 15000rpm. Supernatant (8 ml) was quickly neutralized to pH 6.5 to 6.8 with the addition of 5 mol/L potassium carbonate drops. Neutralized supernatant was collected after centrifugation at 15000rpm for 10 min. Supernatant was filtered through 0.45 µm filter and out of the filtrate, 6 ml supernatant was stored at -20 °C for HPLC analysis.

HPLC Analysis

Mobile phases: Mobile phase 'A' was prepared by dissolving 0.06 mol/L di-potassium hydrogen phosphate and 0.04 mol/L potassium dihydrogen phosphate in deionized water and pH adjusted to 7.0 with 0.1 mol/L potassium hydroxide. Mobile phase 'B' was 100% acetonitrile (HPLC grade, Merck, India).

HPLC Conditions: A reverse phase column (125 × 4 mm Purospher RP-18e, Merck, Germany) was used on the

LaChrom HPLC system (Merck-Hitachi Darmstadt, Germany). Isocratic system was standardized for estimation of AMP+GMP using Mobile phase 'A' and 'B' in the ratio of 80: 20. Flow rate of the mobile phase was 1.2 ml/ min and injection volume was 20 µl. The retention time was about 1 min for AMP+GMP. AMP + GMP concentration in the samples was identified and quantified using ESTD method and the standard curve was prepared using AMP: GMP mixture in 1:1 ratio (Fig. 1).

Total Starch Content

Starch content was determined according to the modified method of McCready *et al.*, (1958). The samples (100 mg) were suspended in 6.5 ml of 52% perchloric acid and 5 ml of distilled water. The samples were incubated for 24 h at room temperature (25 °C). After incubation, the samples were centrifuged and residue was extracted with 6.5 ml of 52% perchloric acid and centrifuged again. Both the supernatants were combined and final volume was raised to 50 ml with distilled water. For colour development, 50 µl of sample and 950 µl of distilled water was boiled for 8 min in presence of 2 ml of anthrone-sulphuric acid reagent (200 mg anthrone in 100 ml chilled conc. sulphuric acid). Samples were cooled to room temperature and absorbance was recorded at 620 nm. Total starch was calculated from the standard curve using the following equation:

$$\text{Concentration of starch in Sample} = \left(\frac{\text{Absorbance}_{\text{sample}} - 0.026}{12.23} \right) \times 0.9$$

Reducing Sugars Content

Sugar content was determined by the method developed by Somogyi (1952). Fresh tissue samples (10 g) were made protein free by using lead acetate and potassium oxalate. The sample was filtered and volume was raised to 20 ml with distilled water, and 100 µl of extract was mixed with 900 µl of distilled water. After adding 1ml Nelson alkaline reagent, the samples were boiled for 20 min and cooled in chilled water to stop the reaction. Nelson's Arsenomolybdate reagent (1 ml) was added and vortex mixed. To these samples 7 ml of distilled water was added and optical density was measured at 620 nm. Reducing sugars were calculated from the standard curve using the equation:

$$\text{Concentration of sugars in sample} = \left(\frac{\text{Absorbance}_{\text{sample}} - 0.006}{5.72} \right)$$

Sucrose Content

Sucrose content was estimated with the method described by van Handel (1968). The content was measured by the addition of 100 µl of 30% potassium hydroxide to the samples (100 µl extract + 900 µl distilled water). The samples were boiled for 10 min and cooled in chilled water. After bringing the samples to room temperature, 3 ml of 0.15% anthrone solution prepared in 76% sulphuric acid was added. The samples were incubated at 40 °C for 15 min and optical density was measured at 620 nm. Sucrose was calculated from the standard curve using the equation:

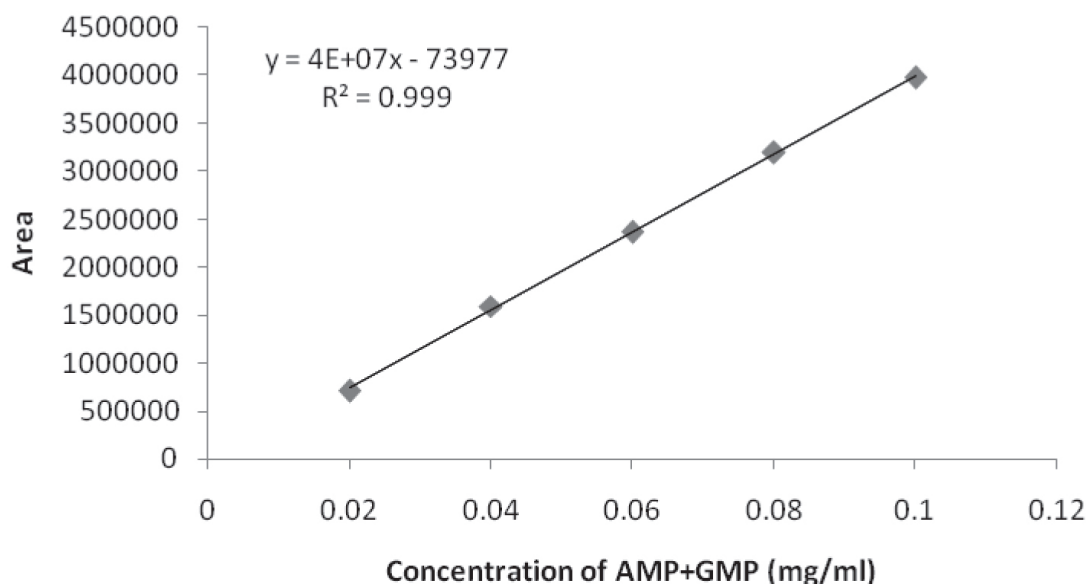


Fig. 1: Standard curve of AMP+GMP standard mixture.

$$\text{Concentration of sucrose in sample} = \left(\frac{\text{Absorbance}_{\text{sample}} + 0.109}{10.08} \right)$$

Statistical Analysis

All the data (three replications of each treatment) was subjected to statistical analysis using MSTAT 4.0C software and ANOVA was calculated at 5% level of significance.

RESULTS AND DISCUSSION

Effect of Cooking on Flavouring Compounds

The food flavour is gaining importance since consumers have preferences for better-tasting food. AMP+GMP content ranged from 2.19 to 4.69 µg/g FW and from 3.52 to 8.92 µg/g FW in freshly harvested raw and microwaved tubers of eleven potato cultivars, respectively, with mean value of 3.26 and 5.48 µg/g FW (Fig. 2). The content of

umami 5' nucleotides increased to appreciable amount during cooking. Before cooking these compounds were higher in Kufri Khasigaro (4.69 µg/g FW), whereas after microwave cooking the formation of these compounds was the maximum in Kufri Lalima (8.92 µg/g FW) followed by Kufri Sherpa (7.05 µg/g FW) and Kufri Khasigaro (6.15 µg/g FW). After cooking, cultivar Kufri Lauvkar (3.15 µg/g FW) contained the lowest concentration of these compounds followed by Kufri Jyoti (4.14 µg/g FW) and Kufri Chipsona 1 (4.20 µg/g FW). On an average, AMP+GMP content was non-significantly higher in table cultivars compared to processing cultivars. The variations in flavour of potatoes has been attributed to the factors like plant genotype, production environment, storage environment and the enzymes that react with them to produce flavour compounds (Jansky 2010). The variations observed in 5'-nucleotides in raw as well as microwaved

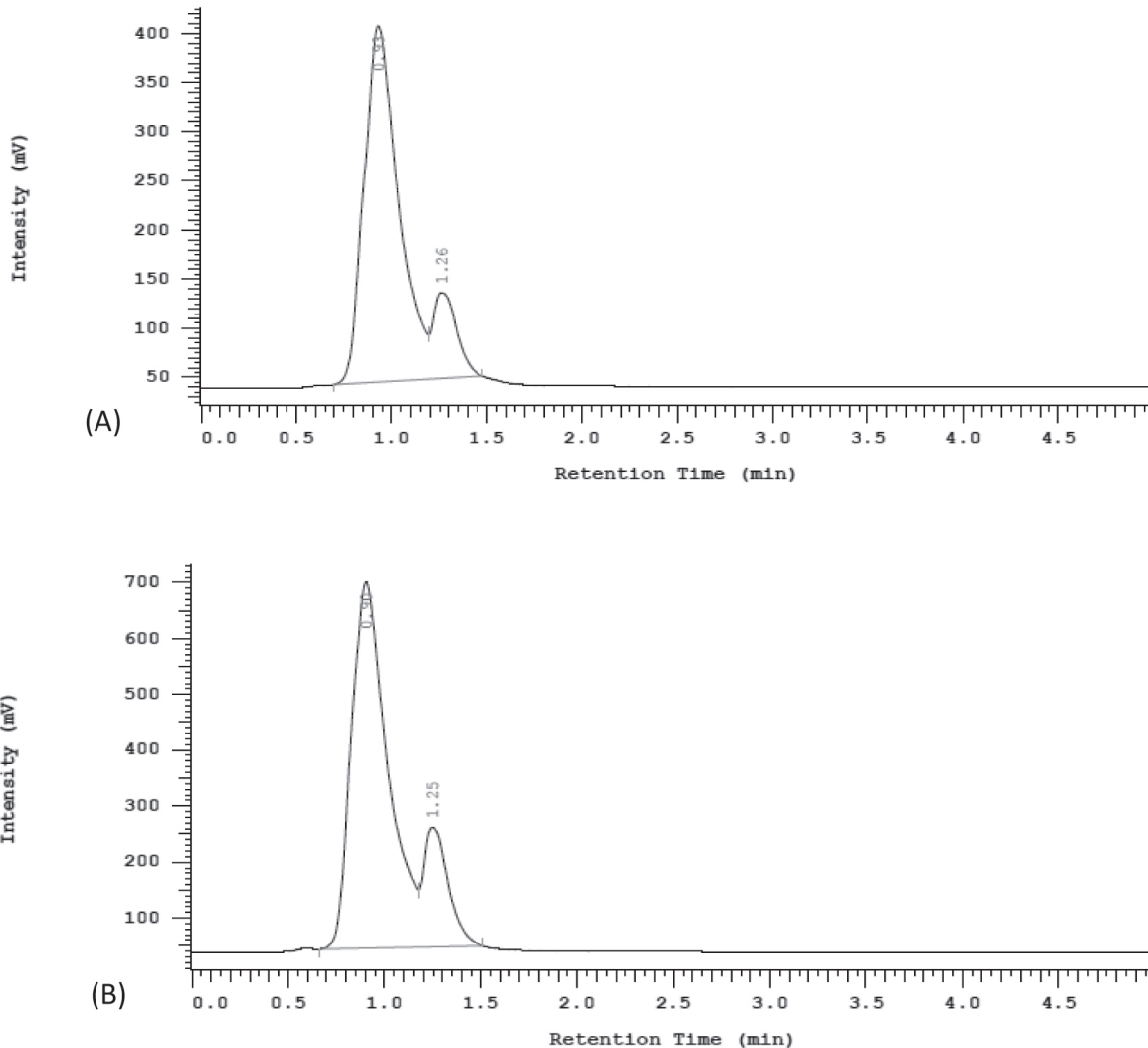


Fig. 2: HPLC chromatogram of AMP+GMP in (A) raw and (B) microwave cooked potato tubers.

tubers of eleven Indian potato cultivars was probably due to differences in the activities and types of enzymes that break down RNA. In accordance with the published literature (Morris *et al.*, 2007), concentration of 5'-nucleotides was low in raw tubers of all the cultivars. GMP and Glutamate interact synergistically so small increase in GMP may lead to enhanced flavour. The exact mechanism behind the rise in 5'-nucleotides after cooking is not clear. Previously, it was assumed that the accumulation of 5'-nucleotides in cooked tubers is due to the action of nucleases that breakdown RNA. But Morris *et al.* (2007) could not find any difference in the level of the enzyme activity in different cultivars showing different 5'-nucleotides concentrations. During microwave cooking, the tuber temperature increases relatively uniformly, with all parts reaching 100 °C within a few minutes of each other (Oruna-Concha *et al.*, 2002). Microwave-baked potatoes have lower levels of volatiles than oven-baked or boiled potatoes, probably due to evaporative cooling at the tuber surface and the loss of volatile compounds through co-distillation as water evaporates. GMP is the most significant nucleotide among the 5'-nucleotides (Yamaguchi *et al.*, 1971). Microwave cooking increased the level of 5'-nucleotides in all the cultivars.

Effect of Storage on Flavouring Compounds

Quality as well as taste of potatoes generally degrades with the prolonged storage. In raw tubers stored at 4 °C, the flavouring compounds AMP+GMP ranged from 0.29 to 2.39 µg/g FW and at 12 °C from 0.34 to 2.92 µg/g FW after six months of storage (Fig. 3). AMP+GMP content was the maximum in raw tubers of Kufri Himsona (2.39 µg/g FW) and Kufri Bahar (2.92 µg/g FW) stored at 4 and 12 °C, respectively. At 4 and 12 °C storage, Kufri Lauvkar (0.29 µg/g FW) and Kufri Frysona (0.34 µg/g FW) contained least content of these compounds, respectively.

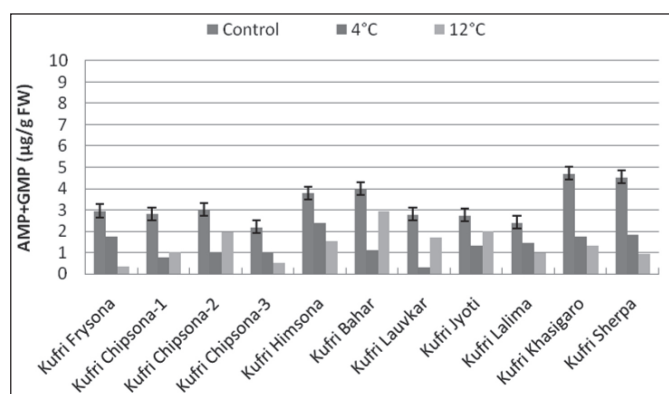


Fig. 3: Effect of storage (180 DOS) on flavouring compounds of raw potatoes.

In all the tested cultivars, the content was significantly higher in freshly harvested tubers and decreased with storage period. Content was almost three times higher in freshly harvested raw tubers compared to stored tubers. On an average, tubers stored at 12 °C contained non-significantly higher flavouring compounds compared to 4 °C storage. Average decrease in content of flavouring compounds of raw tubers was 59.20% and 57.36% at 4 and 12 °C storage, respectively.

Freshly harvested microwaved tubers contained flavouring compounds ranging from 3.52 µg/g FW to 8.92 µg/g FW and average content was 5.48 µg/g FW (Fig. 4). After almost six months of storage, the content of flavouring compounds decreased and content ranged from 0.91 to 5.64 µg/g FW and 2.00 to 4.28 µg/gFW at 4 and 12 °C, respectively, in microwave cooked tubers. At 4 and 12 °C storage, the average content was 2.43 and 3.05 µg/g FW, respectively. Average decrease in flavouring compounds during storage was 55.66% and 44.34% at 4 and 12 °C, respectively. More decrease was reported at 4 °C compared to 12 °C storage. HPLC chromatograms of freshly harvested and stored tubers of Kufri Lalima have been shown in Fig. 5. In both raw as well as cooked tubers the content of flavouring compounds were significantly higher in freshly harvested tubers compared to stored tubers. Morris *et al.*, (2010) reported negligible difference in umami 5' nucleotides during storage of steamed tubers up to 3 months, but observed decrease in EUC (equivalent umami concentration) due to decline in glutamate and aspartate. In accordance with our results they could also get genotypic differences in these compounds. Genotype is not the only factor that affects flavour, but factors such as fertilization, stage of maturity, storage conditions and method of processing are also known to influence the flavouring compounds and sensory quality to great extent (Dresow and Bohm 2009).

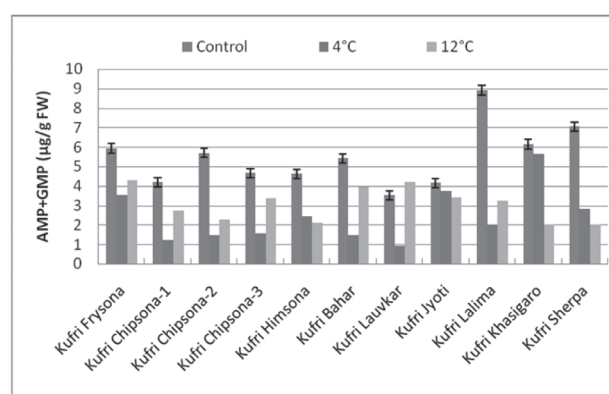


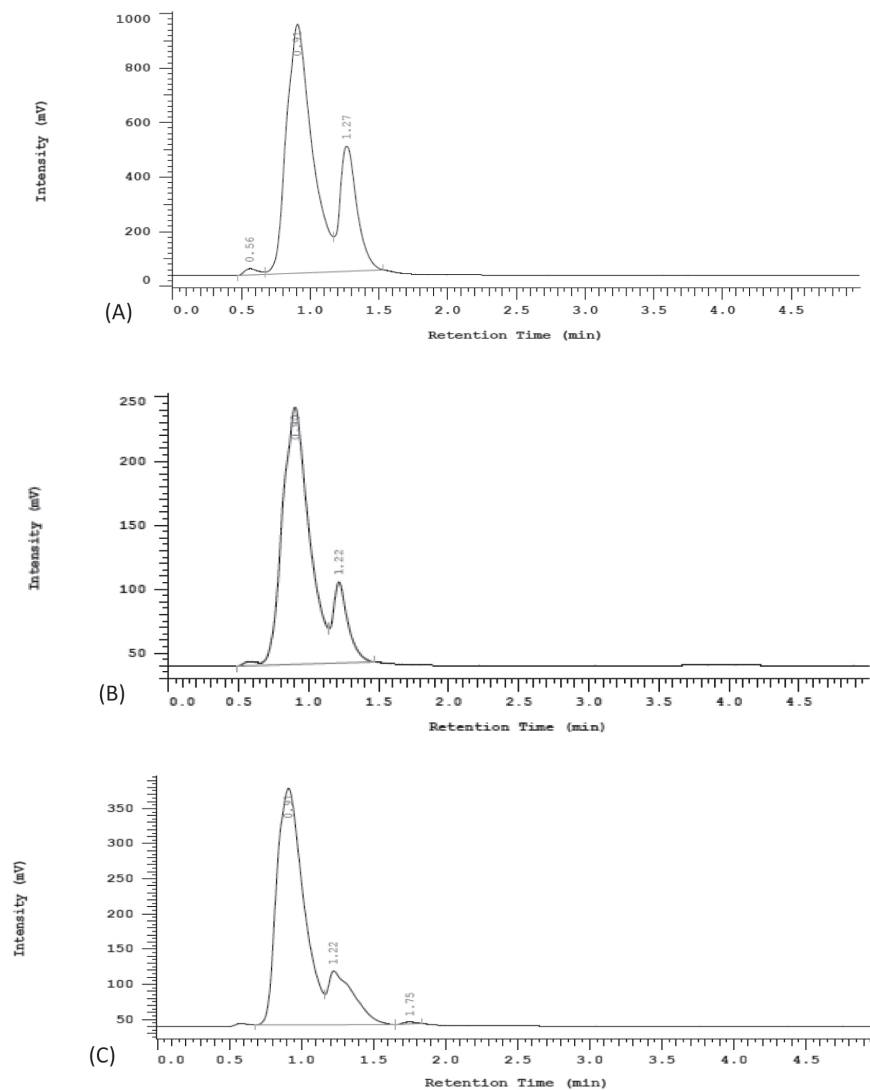
Fig. 4: Effect of storage (180 DOS) on flavouring compounds of microwaved potatoes

Table 1: Effect of storage (180 DOS) on carbohydrate content

Cultivars	Starch Content			Reducing sugars			Sucrose content		
	C	4 °C	12 °C	C	4 °C	12 °C	C	4 °C	12 °C
K. Frysona	66 ^{a-e}	54 ^{d-g}	58 ^{c-g}	372 ^{g-k}	887 ^{ab}	743 ^{a-f}	141 ^{kl}	567 ^a	331 ^{bcd}
K. Chipsona 1	61 ^{a-g}	52 ^{efg}	60 ^{c-g}	170 ^k	901 ^{ab}	798 ^{a-d}	121 ^l	247 ^{e-i}	169 ^{jl}
K. Chipsona 2	69 ^{a-e}	53 ^{efg}	63 ^{a-g}	155 ^k	814 ^{a-d}	607 ^{a-h}	117 ^l	208 ^{g-k}	205 ^{g-k}
K. Chipsona 3	77 ^{ab}	60 ^{b-g}	61 ^{a-g}	216 ^{jk}	785 ^{a-e}	647 ^{a-h}	176 ^{ij}	368 ^b	299 ^{b-f}
K. Himsona	58 ^{c-g}	47 ^g	54 ^{d-g}	374 ^{g-k}	639 ^{a-h}	531 ^{c-i}	206 ^{g-k}	152 ^{kl}	158 ^{kl}
K. Bahar	67 ^{a-e}	58 ^{c-g}	63 ^{a-g}	338 ^{h-k}	877 ^{abc}	437 ^{c-k}	154 ^{kl}	188 ^{h-i}	155 ^{kl}
K. Lauvkar	73 ^{abc}	53 ^{efg}	58 ^{c-g}	639 ^{a-h}	939 ^a	807 ^{a-d}	139 ^{kl}	342 ^{bcd}	312 ^{b-e}
K. Jyoti	67 ^{a-e}	48 ^g	60 ^{c-g}	351 ^{g-k}	933 ^a	867 ^{a-d}	162 ^{kl}	209 ^{g-k}	188 ^{h-i}
K. Lalima	65 ^{a-f}	55 ^{d-g}	57 ^{c-g}	697 ^{a-g}	800 ^{a-d}	652 ^{a-h}	203 ^{h-k}	319 ^{b-e}	285 ^{c-g}
K. Khasigaro	78 ^a	65 ^{a-f}	67 ^{a-e}	522 ^{d-j}	660 ^{a-h}	434 ^{e-k}	121 ^l	358 ^{bc}	220 ^{fj}
K. Sherpa	71 ^{a-d}	55 ^{d-g}	66 ^{a-e}	426 ^{f-k}	774 ^{a-f}	577 ^{b-h}	153 ^{kl}	264 ^{d-h}	339 ^{bcd}
Mean	68	55	61	387	819	652	154	293	242

Different letters indicate significant differences between cultivars ($P < 0.05$)

C: Control (without storage)

**Fig. 5:** HPLC chromatogram of AMP+GMP of (A) freshly harvested, and stored and microwaved tubers (B: 4°C, C: 12°C) of Kufri Lalima

Starch Hydrolysis During Storage

Starch content ranged from 58 g/100 g DW (Kufri Himsona) to 78 g/100g DW (Kufri Khasigaro) in freshly harvested tubers with an average of 68 g/100 g DW. Starch content was comparatively higher in cultivars *viz.* Kufri Chipsona-3 (77 g/100g DW), Kufri Lauvkar (73 g/100g DW), and Kufri Sherpa (71 g/100g DW). Starch content decreased during storage in all the tested cultivars and decrease was more at 4 °C compared to 12 °C. During ageing and low temperature storage, membrane damage facilitates the enzyme access to starch granules, speeding up the starch breakdown and resulting in sugars accumulation. Reducing sugars content ranged from 155 mg/100gFW to 697 mg/100gFW in freshly harvested tubers with average content of 387 mg/100gFW. Processing cultivars with low reducing sugars are desirable to avoid product darkening caused by 'Maillard reaction'. The processing cultivars contained lower reducing sugar content compared to table cultivars and the mean content was 257 mg/100 g FW and 496 mg/100g FW in processing and table cultivars, respectively. After storage, the sugar accumulation was observed in all the cultivars and sugar differences between cultivars were almost non-significant. Cultivars Kufri Lauvkar and Kufri Jyoti contained higher sugars content after storage at both the temperatures, whereas, sugars accumulation was least in Kufri Khasigaro. Sucrose content ranged from 117 mg/100g FW (Kufri Chipsona-2) to 206 mg/100g FW (Kufri Himsona) in freshly harvested tubers with an average content of 154 mg/100g FW. On an average, there were 19 and 10% decrease in starch content, whereas, it resulted in 112 and 69% increase in reducing sugars and 90 and 57% increase in sucrose content at 4 °C and 12 °C, respectively. Low temperature storage leads to starch hydrolysis. One major reason behind the poor quality of stored tubers is membrane leakage due to lipid peroxidation. 'Senescent sweetening' term is used for accumulation of reducing sugars during prolonged/low temperature storage, is likely due to the progressive degeneration of amyloplast membranes, which facilitates the enzymic hydrolysis of starch (Sowokinos *et al.*, 1987). This phenomena occurs due to the progressive loss of amyloplast membranabne integrity.

CONCLUSION

The present investigation revealed that microwave cooking enhances the formation of flavouring compounds and these compounds increased to manifolds after microwave cooking. It is generally considered that the taste of potatoes deteriorates due to the starch hydrolysis and reducing sugar accumulation during low temperature as well as long term

storage. However, this study has clearly revealed that it is not only the reducing sugars accumulation which results in the poor taste of stored potatoes, but flavouring compounds also deteriorate during storage, almost up to 50% compared to fresh potatoes and hence contribute to reduction in the taste of potatoes.

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