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Guava spread (*Psidium guajava*) and Matcha (*Camellia sinensis*) spread: A potential anti-inflammatory product

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Abstract
Background: Excessive amount of prolonged high-intensity exercise is characterised by an increase in pro-inflammatory markers resulting in decreased sports performance and recovery period. Dietary intervention like inclusion of antioxidant/anti-inflammatory foods is used in combating inflammation. Guava fruit and matcha tea are rich in in vitamin C, polyphenols and catechins which are potential candidates that can be explored for their anti-inflammatory properties.
Objective: The objective was to develop an organoleptically acceptable bread spread using *Psidium guajava* (Guava) and *Camellia sinensis* (matcha). Sensory and proximate analysis of the most acceptable variant was evaluated. Further quantification of the anti-inflammatory components i.e., ascorbic acid, polyphenols, catechin and potassium levels of the most acceptable variant was carried out using standard protocols.
Materials & Methods: 3 variants of bread spread were developed using guava as a base with varying concentrations of matcha. A descriptive sensory score card was used to conduct sensory evaluation of the spread. AOAC methods were followed for proximate and potassium estimation. Folin-Ciocalteau (F-C) method was used for antioxidant estimation and HPLC protocols were followed for the estimation of vitamin C and catechin.
Result: Three spreads were developed, GMS, GMS1 and GMS2, with GMS serving as control with only Guava and GMS1 and GMS2 with matcha tea powder in varying amounts. 30 semi-trained sensory panel members conducted sensory analysis of the spreads. The Guava spread variant 2 i.e., (GMS1) with 10g of guava and 0.3g of matcha was the most acceptable with an overall acceptability score of 8.2±0.72. The energy, moisture, carbohydrates, protein, fat and dietary fibre content of GMS1 /100g was 260±10kcal, 56.8±0.1g, 21.19±1.0g, 3.75±0.1g, 16±0.1g and 15±1.0g respectively. Potassium content of GMS1 was found to be 331.34±0.1mg/100g, ascorbic acid and catechin content was 30.33 ± 0.2 mg /100gand 110 ± 0.1 mg/100g respectively with a total polyphenol content of 7.66 ± 0.3 mg/100g.
Conclusion: The developed Guava spread GMS1 variant was organoleptically acceptable with appreciable concentrations of polyphenols, catechin, vitamin C and potassium therefore can be used to deal with excessive training induced inflammation.

Keywords: Exercise, inflammation, inflammatory markers, anti-inflammatory, antioxidants, Catechin, polyphenol, potassium, spread, guava, matcha

Introduction
Physiological stress due to intense exercise and training regime, mode of exercise, duration and lack of proper rest and recovery can induce muscle tenderness, soreness, swelling, damage, reduced strength, range of motion and inflammation [1]. The inflammation so induced coupled with oxidative stress ensuing increase in ROS and lipid peroxidation and efflux of myocellular proteins can negatively affect sports performance [1, 2].
Excessive amounts of prolonged high-intensity exercise are characterised by an increase in pro-inflammatory cytokines markers like IL-6, IL-1β, and TNF-α resulting in delayed recovery ultimately affecting performance [3]. Ample recovery time, proper sleep, rest, nutritious diet, and inclusion of antioxidant rich foods can be a key strategy in combating exercise induced inflammation.
Food is a source of micronutrients i.e., vitamins & minerals as well as non-nutritive bioactive constituents called phytochemicals that help modulate immunomodulatory and inflammatory processes in the human system. Fruits and vegetables are abundant source of these vitamins, minerals and bioactives, therefore are a mandatory component of a balanced diet

consequently recommended by all international nutrition bodies to consume fresh fruits and vegetables to maintain overall health [4].

Vit B6, folic acid, Vit B12, Vit C, Vit D, Vit E, Zinc, selenium and carotenoids omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), Quercetin, Hesperetin, Catechin, Genistein, Curcumin, Resveratrol, Carotene, Lutein are some phytochemicals that have anti-inflammatory properties and widely studied in context to inflammation associated with obesity, diabetes, arthritis and cancer [5, 6, 7, 8].

Against this background the aim of this research work was envisioned and an organoleptically acceptable product namely bread spread possessing anti-inflammatory properties was developed using *Psidium guajava* (Guava) and *Camellia sinensis* (matcha) with the intention of alleviating exercise induced inflammation.

Material and Methods

Materials

All the raw materials needed i.e., Guavas, Matcha powder, almonds, hazelnuts & walnuts, sugar, butter were bought from a local market in Mysore.

Guava matcha spread preparation

Guava matcha Spread (GMS) was prepared by extracting pectin rich pulp by boiling guavas in water and removing the seeds by straining through a muslin cloth. To the pulp containing pectin (100g), matcha powder (with 2 variations GMS1 & GMS2 containing 0.3g & 0.5g respectively), almonds (5g), hazelnuts (5g), walnuts (3g), butter (10g), icing sugar (6.6g) and lemon juice (5ml) were added, cooked till desired consistency, removed from fire and allowed to cool at room temperature before storing them in air tight glass bottles till further analysis. GMS without matcha powder served as control/standard product against which the variations were sensorially scored.

Sensory Analysis

Guava matcha Spread was sensorially analyzed for descriptors namely appearance, consistency, color, aroma, taste and overall acceptability by a semi trained panel (n=30) with the help of an individual score card.

Proximate analysis

The most acceptable variant of the product was subjected to further analysis *viz.*, moisture, ash, carbohydrate, protein, fat, dietary fiber, ascorbic acid, total polyphenols, catechin, and potassium.

Moisture

Moisture was estimated as the percent difference between fresh sample to dried sample. Ash was estimated from moisture free sample by ashing it in a muffle furnace at 550◦C for 6 hours (AOAC 2016), and expressed in percentage [9].

Protein

Kjeldahl method was used to estimate protein as the nitrogen content of the sample based on quantifying the conversion of nitrogen to ammonia using titrimetric method (AOAC 2016) [10]. Fat analysis was done using SOCS PLUS method based on solvent extraction principle [9].

Carbohydrate

Total carbohydrate content was determined using Anthrone method which involves hydrolyzing carbohydrates into simple sugars using dilute hydrochloric acid followed by dehydration of glucose to hydroxymethyl furfural in hot acidic medium. The compound formed gives blue-green color complex in presence of Anthrone reagent which is measured spectrophotometrically at 630nm [11].

Fiber

Defatted dried sample was used to estimate dietary fiber. The sample is treated with glucosidases and proteases to hydrolyze starch and proteins followed by solvent treatment to precipitate fiber and to remove hydrolyzed/depolymerized protein and glucose (from starch). The residue is filtered; washed with solvents dried and weighed. One duplicate is analyzed for protein and the other used for ash determination. TDF is calculated as the weight of the filtered and dried residue less the weight of the protein and ash [9].

Anti-inflammatory components

Total polyphenols: Polyphenols are the most abundant and widely distributed group of bioactive molecules present in plants with wide range of health benefits. It was estimated using Folin-Ciocalteau (F-C) method. The F-C reaction is an antioxidant assay based on electron transfer, which measures the reductive capacity of an antioxidant [12].

Ascorbic acid: Vit C is a white crystal, water soluble, vitamin with antioxidant potential, and was estimated using reversed-phase high-performance liquid chromatographic method which involved using tetrabutylammonium hydroxide as an aqueous phase and the mobile phase consisted of a mixture of the aqueous phase–methanol (80:20, v/v, pH 6.0 adjusted by phosphoric acid) [13]

Potassium: Potassium was estimated using AAS method potassium in solution is atomized to flame and the flame excites atom of potassium causing them to emit radiation at specific wavelength. The amount of radiation emitted is directly proportional to concentration and it is measured in a flame photometer with suitable filter, which transmits only potassium wavelength (768 nm red filter) [14].

Catechins: They are phenolic compounds ascribed with antioxidant activity, and RP HPLC method with a phenyl-based stationary phase and gradient elution was used to determine its content in bread spread [15].

Statistical analysis

Data of organoleptic characteristics, nutrient analysis and anti-inflammatory components, of the formulated products and were subjected to statistical analysis namely mean, standard deviation and one-way ANOVA.

Results and Discussion

Spread is an edible paste that is usually consumed by spreading it onto bread or cracker with a help of knife - like butter, so as to increase the palatability and flavor profile of the otherwise bland tasting bread, toast, cracker or such other foods. Margarine, nut and seed spreads like peanut butter, yeast extracts, spreadable processed cheese are some

examples of commonly consumed spreads. Novel trends in the field of product development has led to exploring unconventional sources for making conventional food products. Fruits are a store house of anti-inflammatory compounds and elements like antioxidants, flavonoids, flavones, anthocyanins, vitamins and minerals hence potential candidates for making spreads.

Table 1 shows the comparative sensory score of the three guava matcha spread i.e., GMS, GMS1 and GMS2. The products were assessed for sensory attributes *viz.*, appearance, consistency, color, aroma, taste and overall acceptability. GMS1 fared better on all the parameters hence used for further analysis. GSM1 and GSM2 scored almost similar on appearance 7.8±0.76 and 7.5±0.68 which could be attributed to the addition of matcha powder. Sensory analysis is a crucial step in product development as it determines the success or failure of a product in the market and also provides scope to improve upon sensory experience of the customers.

Table 1: Comparative sensory analysis score of the Guava matcha spread.

Product	Appearance	Consistency	Color	Aroma	Taste	Overall acceptability
GMS	7.3±0.9	6.7±1.11	6.6±1.29	5.7±1.46	6.7±1.35	6.7±0.81
GMS1	7.8±0.76	7.9±0.66	7.9±1.02	7.5±0.9	8.3±0.87	8.2±0.72
GMS2	7.5±0.68	7.3±0.74	7.1±1.04	5.8±1.55	6.9±0.92	6.8±0.84

Proximate Analysis

Fruit spreads are made by blending sugar with whole fruit, concentrated fruit juice, or processed fruit juice. Some breakfast spreads don't contain a lot of polyphenols and vitamins to alternative workplace merchandise.

The term "proximate analysis" refers to a technique for estimating the amounts of macronutrients in food samples. These figures are generally reported as nutritional information and are typically displayed on the labels of finished food products, although they are also decided upon throughout the product development and production process. Table 2 shows the proximate content of the most accepted spread variant i.e., GMS1 with 0.3g matcha.

Table 2 & Fig 1: The proximate and energy content of GMS1

Sample	Moisture (g)	Carbohydrate (g)	Protein (g)	Fat (g)	Dietary fibre (g)	Energy (kcal)
GMS1 (100g)	56.8 ± 0.1	21.19 ± 1.0	3.75 ± 0.1	16.1 ± 0.1	15 ± 1.0	260 ± 10

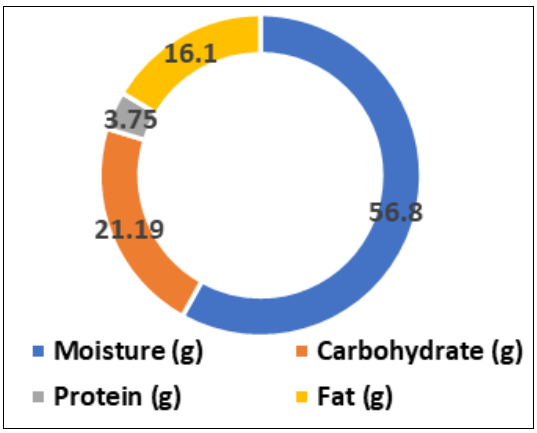

Fig 1: The proximate composition of GMS1

Guava is a treasure house of micronutrients and phytochemicals hence included as superfruits but underutilized commercially except for juices and jellies. Fruits are high in moisture content and this being one of the reasons for their perishable nature. Similarly, matcha powder, almonds, hazelnuts, walnuts, butter, icing sugar and lemon juice all contribute to the proximate value of the product. The moisture content of 100g guava was about 2.8 – 5.5g [25] and our product had 56.8 ± 0.1g per 100g sample. This is can be attributed to the addition of water to cook the guava.

The carbohydrate content was estimated to be 9.1 – 17 mg and fat 0.43 – 0.7g per 100g of the fruit [25]. The total energy content of the product was 260 kcal per 100 gm. Matcha is a type of powdered green tea and does not contribute much to total calorie whereas the nuts used namely almond, walnuts, Carbohydrates and fats provide energy and vital for work capacity and sports performance. Compared to carbohydrates fats are concentrated sources of energy. The carbohydrate content of the GMS1 was 21.19 ± 1.0g and fat was 16.1±0.1g per 100g. Commercial bread spreads are currently available with fat contents ranging from 80% to 90%. Spreads resemble margarine but have a lower fat content thereby making them healthier alternative. The nutritional and practical needs of the consumer must be satisfied by table spreads. A spread developed with broken riceberry rice with quinoa was found to have moisture, protein, fat, coarse fiber, ash and carbohydrates at 49.64, 3.83, 4.13, 1.81, 1.56 and 39.03% respectively [17].

The protein of content of 100g fruit was reported to be 0.1 – 0.5g [25]. The estimated protein content was 3.75 ± 0.1g/100g GMS1. The higher protein content of the developed product could be attributed to the cumulative protein value of the nuts used. Proteins especially have an important role in sports nutrition due to their muscle building property and anti-inflammatory properties. Leucine, a BCAA, reduces central fatigue and decrease muscle soreness [18, 19] thus helping in recovery.

Table 2: Anti – inflammatory components of the product

Sample	Potassium (mg)	Vitamin C (mg)	Total polyphenol content (mg)	Catechin (mg)
GMS1 (100g)	331.34±0.1	30.33 ± 0.2	7.66 ± 0.3	110 ± 0.1

Table 2 depicts the anti – inflammatory components of the developed product namely potassium, Vit C, total polyphenol and catechin. Dietary fibres, vitamins A, C, and folic acid, as well as several dietary minerals including potassium, copper, and manganese, are all abundant in guava [20]. *Camelia sinensis* or Matcha is storehouse of phytochemicals and contains four main catechins, i.e., (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) [21] that have antioxidant, anticancer, anti-inflammatory, and anti-hypercholesterolemia properties.

The analysed potassium content of the GSM1 was 331.34 mg/100g. Potassium, sodium, and calcium play integral roles in the electrophysiology of pain. Potassium ion channels are widely distributed in various body cells and regulate the cellular movement of potassium. They are abounding in neurons and muscles, especially in nociceptors (pain perception sensors) and potassium deficiency (cellular) is considered to cause inhibition (closure) of potassium ion channels which leads to increased nerve stimulation and irritability [22]. A spread development study carried out with jackfruit seed showed that one serving (21 g) provided 82 kcal and was evaluated only for minerals and vitamins *viz*., potassium (59 mg), phosphorus (8 mg), calcium (26 mg), magnesium (4 mg), thiamin (0.02 mg), riboflavin (0.02mg), niacin (0.2 mg), and vitamin C (2 mg) [23].

The Vit C, total polyphenol and catechin content of the product was 30.33 ± 0.2 mg, 7.66±0.3 mg, 110±0.1 mg per 100g sample. The capacity to give electrons makes vitamin C an important antioxidant micronutrient that also aids in immune protection. Polyphenols show a beneficial role in the prevention and the progression of diabetes, obesity, cardiovascular diseases and cancers, in which inflammation is observed [24]. Catechin through its activity to alter the pathway by NF-KB, Nrf-2, TLR4/NF-KB, COMT, and MAPKs shown anti-inflammatory, anti-diabetic, anti-cancer, activity [25].

Conclusion

The presence of antioxidants in the developed spread, makes it a suitable prework or post work out product for athletes as they can easily incorporate it into their diets to reduce inflammation and improve performance. The general public also can include it regularly in their diets. Guava fruit is readily available in Indian markets, and preparing a spread from it only requires a few basic cooking techniques. The cost to prepare 100g of the product (GSV2) is Rs. 41.09. Therefore, Fruits and edible leaves are an abundant source of bioactives therefore provide a great scope to develop antioxidant and anti-inflammatory ready to eat and use products.

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