



Enrichment of Bottle Guard-Muskmelon Smoothie with Chia Seed as a Source of Omega-3 Fatty Acids

Rajeev Kumar¹, Aanchal Srivastava²

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ABSTRACT

Background: This study evaluated the impact of the level of supplementation of chia seed on the nutritional and antioxidant capacities of smoothies made from a blend of bottle gourd-muskmelon.

Methods: Uniformly ripe muskmelon and bottle gourd were selected for extraction of pulp for preparing smoothie. Chia seeds were soaked in honey water for overnight to get uniformly swelled up and to absorb a slight sweet taste of honey. The blending ratio was used to combine the bottle gourd, muskmelon and chia seed.

Result: The results obtained in the bottle gourd-muskmelon smoothie had moisture content (92.9 ± 0.02 – $93.98 \pm 0.08\%$), crude protein (1.11 ± 0.02 – $1.55 \pm 0.01\%$) and reducing sugar (2.98 ± 0.01 – $6.02 \pm 0.05\%$). The DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) free radical scavenging activity of the samples were found (21.26 ± 1.14 – $70.23 \pm 2.34\%$). The total soluble solids TSS ($^{\circ}\text{brix}$) values showed high sugar content 13.95 ± 0.06 for bottle gourd-muskmelon (30:70) while the pH results showed a range between 5.14 ± 0.05 – 5.43 ± 0.04 for different combination of bottle gourd-muskmelon smoothie. The supplementation of chia seed had a considerable impact on the sensory properties as well as it would enrich the smoothie with omega-3 fatty acids to impart various health benefits.

Key words: Antioxidant, Chia seed, Fruit, Sensory, Smoothie.

INTRODUCTION

Fruits include a range of nutrients and a smoothie is a really useful and efficient way to benefit from the different kinds of nutrients that fruits offer. Smoothie is defined as beverages made from a blend of various fruits that are semi-liquid. It can be sub-divided into three main categories viz. fruit only, fruit with dairy product and functional smoothie (Srivastava *et al.*, 2019). Functional smoothies are considered as the recently available product in the market, usually containing probiotics amongst itself. There is already established market for smoothies in several developed countries; the United States and the United Kingdom generate about 2 billion USD and 141 million pounds (Aderinola, 2018).

Muskmelon (*Cucumis melo*) is an excellent, succulent, delectable and flavorful organic product well known for its nutritive and therapeutic properties. The nutrient content of muskmelon is low in calories, fat and sodium but good in potassium and vitamin C. It is also a great source of beta carotene, vitamin C but low in folic acid, iron and calcium. Muskmelon is a beautiful, juicy, tasty and delicious fruit that is well-known for its nutritional and medicinal qualities such as for the treatment of cardiovascular disorders, diuretic, stomachic and antitussive (Parle and Singh, 2011; Bhalekar *et al.*, 2022).

Bottle gourd contains every basic constituent that is required for wellbeing and quality human life. All of the minerals and essential amino acids needed for normal human health are found in bottle gourd (Rahman *et al.*, 2003). This fruit has historically been utilized as a general and cardiac toner. There has been reports that bottle gourds possesses diuretic, anti-inflammatory, anticancer and

¹Department of Dairy Technology, The Charutar Vidya Mandal (CVM) University, Anand-388 120, Gujarat, India.

²Department of Food Technology, Uttaranchal University, Dehradun-248 007, Uttarakhand, India.

Corresponding Author: Rajeev Kumar, Department of Dairy Technology, The Charutar Vidya Mandal (CVM) University, Anand-388 120, Gujarat, India. Email: rajeev.kumar267@gmail.com

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antioxidant qualities (Palamthodi and Lele, 2014). The bottle gourd juice helps in preventing untimely turning gray of hair, sleeping and urinary tract related disorders. It has high amount of choline among all vegetables which helps in improving memory (Deshpande *et al.*, 2008).

Chia seed has natural source of ALA (α -linolenic acid) which is found in the seeds of the chia plant (*Salvia hispanica* L.), a herbaceous plant in the Lamiaceae family whose seeds can be crushed to release their oil. The 40% of the weight of chia seed is made up of oil which has an ALA content of roughly 60% (Ixtaina *et al.*, 2011). In addition, it has a substantial amount of proteins, natural antioxidants, dietary fiber ($>30\%$), vitamins (including vitamin B) and minerals more than that of present in milk (Munoz *et al.*, 2012). The American Heart Association advises consumers to take omega-3 fatty acids by raising its consumption through food source (AHANC, 2000). Omega-3 fatty acids have been scientifically found to contain antiallergic, hepatic,

hypotensive, cardiovascular and antidiabetic effects (Ayerza and Coates, 2011).

Smoothie being a blend of a variety of fruits provides a good medium for obtaining the different micronutrients therein. Fruits are generally low in protein content; the resulting beverages (smoothies) would also be deficient in this vital nutrient. Blending method is generally used as an alternate to reduce under-utilization of fruits adding with other nutritional components. It may be attributed to alter dietary intake, taste preference and way of life of consumers. The demand of blended smoothies has been increasing with each passing year due to their health benefits and amazing taste. Therefore, to enhance taste, flavor, palatability, aroma and nutritive properties of bottle gourd, the present investigation is aimed to be convenient to blend it with highly nutritive fruit muskmelon as well as providing enormous health benefits of chia seeds.

MATERIALS AND METHODS

All raw materials for the preparation of smoothie were procured from the local market of Dehradun, Uttarakhand. DPPH was purchased from Himedia, Mumbai, India. All other chemicals used in the study were of analytical grade. The study was conducted at Department of Food Technology, Uttaranchal University, Dehradun.

Extraction of pulp

Uniformly ripe bottle gourd and muskmelon were selected for extraction of pulp for making smoothie. Fresh, tender and mature bottle gourd of uniform light green colour was subjected through various preliminary treatments such as cleaning, washing and trimming. After sorting of bottle gourds on the basis of uniform inner white colour, these were sliced into small pieces. Thereafter, pieces were taken for pulp formation in clean mixer grinder to obtain a homogenous blend. In case of muskmelon ripe and fully matured fruits were washed and extraneous parts were removed. Pulp was extracted mechanically through mixer grinder.

Soaking of chia seeds

Chia seeds were soaked in honey water for overnight to get uniformly swelled up and absorb a slight sweet taste of honey.

Preparation of smoothie

Smoothie was prepared from the bottle gourd-muskmelon pulp as per standard procedure adopted by Balaswamy *et al.* (2013). The amount of chia seeds and honey were kept constant 10 and 5 % in the smoothie. The remaining part of the smoothie was made up of bottle gourd and muskmelon pulp blend in different ratios (w/v) 30:70, 50:50 and 70:30. All smoothies were prepared without addition of water, sugar and citric acid. The smoothies were subjected to pasteurization temperature of 90°C for 5 min followed by storing at refrigeration temperature (4±1°C).

Total soluble solids (°Brix)

Level of complete dissolvable solids was dictated by utilizing a portable refractometer (Erma, EHB-32ATC) by putting a drop of test squeeze on the crystal of the refractometer and watching the occurrence of shadow of the example, the perusing on the scale communicated as °Brix.

pH

Concentration of H⁺ ions was determined by using a digital pH meter. The cleaned and dry electrode was earlier calibrated using buffer solutions of pH 4, 7 and 9. The electrode was rinsed with distilled water and then placed into the smoothie samples and readings were recorded.

Moisture, protein and reducing sugar

The proximate composition moisture content and protein of the smoothie were carried out using AOAC, (1995). Reducing sugars were evaluated by the strategy for Miller, (1959).

Estimation of Omega-3 fatty acid

1.5 g of smoothie sample was firstly extracted with 12 ml of chloroform: methanol (2:1, v:v). The samples were centrifuged at 9000 rpm for 10 min. The bottom chloroform layer was collected and filtered into the vials. To determine fatty acids profile, methyl esters were prepared by the method O' Fallon *et al.* (2007). Omega-3 fatty acids content in fat sample were analyzed in packed glass column using Gas Liquid Chromatography (GLC-2010 plus Shimadzu, Japan) equipped with flame ionization detector and temperature control modules.

Antioxidant activity

Antioxidant activity of guava samples was analyzed by DPPH radical scavenging method (Wani *et al.*, 2018). Guava fruit extract (0.1 mL) was added with 3.9 mL DPPH. The reaction mixture containing sample with DPPH was kept for about 30 min. in dark and absorbance was taken at 517 nm using UV Spectrophotometer (Systronic-AU2701). The results were expressed as (% inhibition) by the equation given below:

% DPPH inhibition activity=

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Microbiological analysis

Total plate bacterial counts, mold and yeast count were determined according to the procedures described in ISO 4833-1 and ISO 6611 respectively. The pour plate technique was used for total bacterial count using agar medium and incubating the plate at 30°C for three days. Mold and yeast count was determined using by yeast extract glucose chloramphenicol agar medium. The plates were incubated at 25°C for 5 days.

Sensory evaluation

The sensory evaluation includes ten panelists who have prior experience in evaluating fruits, vegetables and their

products through sensory analysis. The 9-point hedonic scale was used to analyze the samples where 9 being exceedingly like and 1 being extremely disliked. The following qualities were evaluated: overall acceptability, color, consistency, taste and smell. Every sensory test was carried out in the sensory analysis room, which had separate booths with standard illumination. The testing area's circumstances were all managed to minimize distractions and guarantee panelists' comfort in making decisions (Ranganna, 2003).

RESULTS AND DISCUSSION

Total soluble solids (TSS)

The TSS of bottle guard and muskmelon were found to be 5.12 ± 0.043 and 15 ± 0.045 . The TSS of different blends varied from 11.13 ± 0.08 – 13.95 ± 0.06 °brix (Table 1). The bottle gourd-muskmelon smoothie (30:70) contains highest amount of soluble solids as compared to other blends. The data in Table 1 shows difference in total soluble solids of different bottle gourd-muskmelon smoothie. The variation which is being observed may be due to variation of TSS in raw material. As compared to bottle gourd, muskmelon has high total soluble solids. There was little variation in the product's soluble solids content when it was kept at 4°C. Ribeiro *et al.*, (2020) reported the soluble solids content in at 25°C after 15 days showed a little decline then after remains steady for 90 days. Palamthodi *et al.*, (2019) reported TSS (9.9 ± 0.13) in bottle gourd-jamun (30:20). The gradual increase in the TSS content during the storage period might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides (Kadam and Lele, 2017).

pH

The pH of bottle guard and muskmelon were found to be 6.9 ± 0.015 and 6.48 ± 0.03 . The pH of various blend ratios was found to be significantly same. The pH of smoothies turns to be slightly towards acidic side as compared to fresh pulp of bottle gourd and muskmelon due to the presence of chia seeds as well as honey. The pH of chia seeds is usually 3.5 while that of honey in which these chia seeds were soaked is generally 3.9. Due to the very little amount of chia seeds being incorporated pH doesn't alter as much. Acidity is an important quality factor related

to the flavour of product. High acidity in fruit beverages prevents the growth of microbes and helps to maintain the color and flavour. These pH values were however less acidic when compared with 4.36–4.41 reported by Babajide *et al.* (2013) for spiced cucumber and pineapple drink.

Reducing sugars

The information in Table 1 uncovers the noteworthy contrast in reducing sugar content. The reducing sugar of bottle guard and muskmelon were 7.92 ± 0.045 and 5.01 ± 0.056 . There was significant change seen in reducing sugars. Reducing sugar content of blends increased gradually with increase in ratio of bottle gourd. Reducing sugars were recorded greatest in 70:30 mixed smoothies while least reducing sugars were recorded in 30:70 mix. The increase in reducing sugars may be because of conversion of non-reducing into reducing sugars and hydrolysis of polysaccharides. Byanna *et al.* (2013) reported the increase in reducing sugar in sweet orange and pomegranate blended ready to serve (RTS) beverage.

Moisture content

The Moisture content (%) of bottle guard and muskmelon were 96.02 ± 0.04 and 90 ± 0.02 and the moisture content in smoothie combinations (30:70, 50:50 and 70:30) had found to be 92.9 ± 0.02 – 93.98 ± 0.08 (Table 1). Slight variation in moisture content of smoothies might be due to the influence of the drying process, temperature and humidity. The natural acidity and sugar content of fruit also influences the moisture content of smoothies. Moisture content in the smoothies define growth of undesirable microorganisms, food hazards, critical control points, standards of various preserved foods (Fontana, 2000). Smoothies made from the blends of pineapple, banana and apple with supplementation of *Moringa oleifera* leaf had moisture content in the range of (49–79%) (Aderinola, 2018).

Protein content

The protein content in bittergourd, muskmelon and chiseeds were found as 1.4 ± 0.06 , 0.40 ± 0.04 , $16 \pm 0.23\%$. The bottle gourd: muskmelon (30:70, 50:50 and 70:30) combinations had 1.11 ± 0.02 , 1.3 ± 0.04 and $1.55 \pm 0.01\%$ protein content respectively. Chiseed has been found to have much protein content that enhanced the nutritional makeup of smoothies. There was an increase in protein with the addition of whey in muskmelon juice (40:60)

Table 1: Physico-chemical characteristics of bottle gourd-muskmelon smoothie.

Parameters	(Bottle gourd: Muskmelon 30:70)	(Bottle gourd: Muskmelon 50:50)	(Bottle gourd: Muskmelon 70:30)
TSS (°brix)	13.95 ± 0.06	$12.50^b \pm 0.09$	$11.13^a \pm 0.08$
pH	$5.43^a \pm 0.04$	$5.32^a \pm 0.05$	$5.14^a \pm 0.05$
Reducing Sugars (%)	$2.98^a \pm 0.01$	$4.24^b \pm 0.06$	$6.02^c \pm 0.05$
Moisture (%)	$92.9^a \pm 0.02$	$93.45^b \pm 0.04$	$93.98^b \pm 0.08$
Protein (%)	$1.11^a \pm 0.02$	$1.3^a \pm 0.04$	$1.55^a \pm 0.01$
Antioxidant activity (% DPPH inhibition)	$70.23^c \pm 2.34$	$42.15^b \pm 1.67$	$21.26^a \pm 1.14$

Values are mean $\pm$ standard error (n=3); values in the same row with different letters are different at $p < 0.05$.

recorded high protein value (2.00 ± 0.32 g) than control sample (0.56 ± 0.15) (Krishnaprabha *et al.*, 2024). Braide, (2012) reported total crude protein content was noticeably higher supplementation of *Moringa oleifera* leaf in smoothie.

Omega-3 fatty acid content

The results for the chia seed in this study (Table 2) are in agreement with values reported by Marineli *et al.* (2015) who observed for palmitic acid, stearic acid, oleic acid, linoleic acid and α -linoleic acid of 7.64, 2.76, 6.39, 19.54, 63.41 % respectively. The fatty acid composition palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid in smoothie were found to be as 0.04 ± 0.01 , 0.12 ± 0.01 , 0.37 ± 0.02 , 0.81 ± 0.03 and $3.52 \pm 0.11\%$ respectively. Eicosanoids formed from omega-6 polyunsaturated fatty acid are thought to be pro-inflammatory, while those derived from omega-6 polyunsaturated fatty acid are thought to be anti-inflammatory, according to Patterson *et al.* (2012). Derewiaka *et al.* (2019) investigated similar observations in yogurt supplemented with 2% chia seed.

DPPH antioxidant activity

Antioxidants have been shown in studies to help prevent or lessen the effects of free radicals, which have been linked to a number of illnesses, including as diabetes, high blood pressure and cancer (Aderinola, 2018). Foods containing bioactive phenols and flavonoid chemicals have anti-

inflammatory and anti-oxidant qualities, among other health-promoting qualities (Sun-Waterhouse *et al.*, 2010). In contrast to medications, which are frequently costly and may have adverse effects, diets (foods, including beverages) that are essential to human survival offer a viable and sustainable alternative method of preventing or reducing some of the problems caused by free radicals. The free radical scavenging property (DPPH) of the smoothie's bottle gourd: muskmelon 30:70 $70.23 \pm 2.34\%$ indicating their antioxidant ability (Table 1). The antioxidant activity of the sweet and spicy bottle gourd-mint-lime (80:15:5) RTS drink with 2% chia seeds had the antioxidant activity of 17.88%. The 50:50 blend of pineapple and carrot juice was reported with the antioxidant activity 54.16% (Owolade *et al.*, 2017).

Microbiological analysis

The total bacterial count, yeast and mold count of all smoothie samples were found to be less than 10 cfu/g at storage condition of 4°C. The microbial increase was reported only from the 8th week onwards. Gerardo *et al.*, (2017) found similar observations purple seedless grapes (45%), cucumber (35%), beet root (12%) and broccoli (8%) containing 2.32 and 2.72 log cfu g⁻¹ at 4°C and 30°C on the 75th day. According to Codex Standard (CX STAN, 2001) the acceptable levels for total bacterial count, yeast and mold count are 100 cfu/g and 50 cfu/g, respectively. Smoothie underwent heat treatment as well as it contains honey, which has antimicrobial activities, may also be the likely cause of the longer shelf life and slower proliferation of micro-organisms (Rani *et al.*, 2024).

Sensory evaluation of smoothie

The quality is the ultimate benchmark of the desirability of food product. Among various elements which influence the quality of products, sensory characteristics may be considered as a major factor and these are accountable to change during storage. The flavour data is graphically presented in Fig 1 and significantly varies between 6.8-7.4. It is clear from the data that bottle gourd-muskmelon (30:70) blend was liked moderately followed by 70:30 and

Table 2: (%) Omega-3 fatty acid in chia seed and smoothie.

Fatty acids	Chia seed	(Bottle gourd: Muskmelon 30:70)
		Smoothie
Palmitic acid (C16:0)	7.64 ± 0.02	0.04 ± 0.01
Stearic acid (C18:0)	2.76 ± 0.03	0.12 ± 0.01
Oleic acid (C18:1)	6.39 ± 0.10	0.37 ± 0.02
Linoleic acid (C18:2)	19.54 ± 0.40	0.81 ± 0.03
Linolenic acid (C18:3)	63.41 ± 0.31	3.52 ± 0.11

The values are mean $\pm$ standard error (n=3).

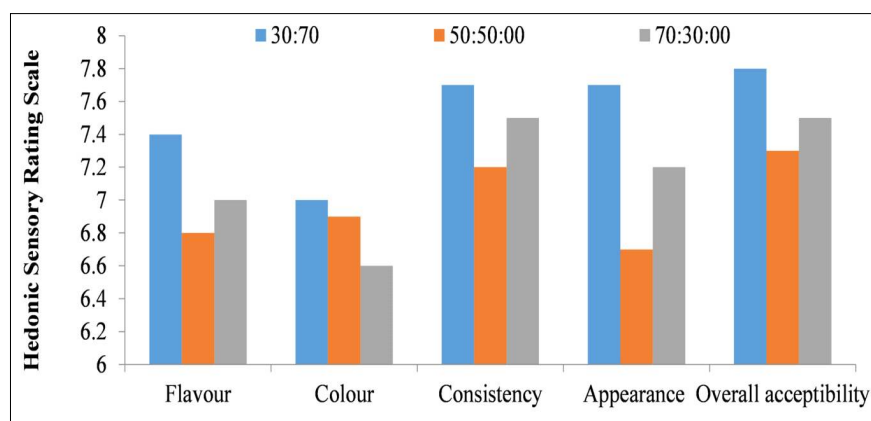


Fig 1: Effect of different blend ratio on sensory evaluation of smoothie.



Fig 2: Bottle gourd: Muskmelon smoothie (30:70, 50:50 and 70:30).

50:50. The colour data revealed that 30:70 bottle gourd-muskmelon blend was more liked with respect to 70:30 blends with minimum score of 6.6 (Fig 2). The perusal of consistency data (Fig 1) portrayed that 30:70 ratio of bottle gourd and muskmelon was liked moderately by the panel of nine judges. However, 50:50 blends failed to express its consistency as compared to other blends, which was slightly liked by the panel of judges. It is clear from the appearance data that 30:70 blends emerged to be liked moderately by the judges as compared to other blends. While uncertainly other blends failed to appear more attractive. The overall acceptability data revealed that bottle gourd and muskmelon (30:70) blend was overall acceptable in the attributes of sensory. The blend was given 7.8 points in hedonic scale which was found to be liked moderately by the panel of judges. 50:50 and 70:30 blends were also found acceptable by the panel of judges with sensory scores 7.3 and 7.5 respectively.

CONCLUSION

The TSS decreased significantly in bottle gourd-muskmelon smoothie as the ratio of muskmelon decreased. The percentage of reducing sugars increased significantly with increase of bottle gourd pulp ratio. The pH, moisture and protein content of the various blend ratios were found to be significantly same in bottle gourd-muskmelon smoothie. DPPH antioxidant activity significantly increased as the muskmelon ratio increase in the smoothie. Based on the sensory evaluation data smoothie made from bottle gourd and muskmelon (30:70) blend was found to be more acceptable in colour, flavour, consistency, appearance and overall acceptability followed by 70:30 and 50:50. The study revealed that consumption of formulated smoothie had tremendous antioxidant activity. Furthermore, omega-3 enrichment of smoothie adds functional value in the smoothie that may impart various health benefits while consumption.

Conflict of interest

Authors declare no conflict of interest.

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