APPLIED BIO-SYSTEMS TECHNOLOGY

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Effect of Particle Size of Oat (*Avena sativa*) Flakes on Physicochemical and Sensory Properties of Oat Incorporated Drinking Yoghurt

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Abstract

Background: Consumption of oats (*Avena sativa*) has increased steeply over the last few years due to the multiple health benefits shown by its constituents, including dietary fibre. Accordingly, numerous functional foods have been formulated incorporating oats. The aim of this study was to determine the optimum particle size of oat flakes for the development of oat incorporated drinking yoghurt.

Methods: Drinking yoghurt was formulated incorporating oat flakes of particle sizes 850-425 µm, 425-180 µm and <180 µm. Physicochemical parameters of the formulated drinking yoghurts, including pH, Titratable Acidity (TA), Total Soluble Solids (TSS), degree of syneresis and firmness were determined for 21 days. The sample that showed the best sensory attributes and physicochemical properties was analysed for proximate composition and microbial safety.

Results: The sensory attributes of the drinking yoghurts with oat flakes of three different particle sizes were not significantly different ($P>0.05$). The particle size of oat flakes affected the physicochemical properties of drinking yoghurts. In fact, the yoghurt with oat flakes of the smallest size showed the highest titratable acidity, TSS and firmness in the drinking yoghurt ($P\leq0.05$). The variation of the physicochemical properties of the yoghurts with time followed a similar pattern. In fact, the pH decreased, TA increased, while TSS decreased with time ($P\leq0.05$). The selected drinking yoghurt, which was prepared incorporating oat flakes of size range 850-425 µm and 300 ppm of potassium sorbate, showed a shelf life of 14 days at 4 °C. It exhibited similar sensory attributes other than taste to a popular drinking yoghurt from the market.

Conclusions: Drinking yoghurt incorporated with oat flakes of 850-425 µm size range showed a better taste and nutritional profile, than regular yoghurt.

Keywords: Drinking Yoghurt, Oat Flakes, Particle Size, Physicochemical Properties, Sensory Properties

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INTRODUCTION

The formulation of novel foods and beverages with increased nutrient content has shown an upward trend worldwide allowing the consumers to have easy access to a balanced diet [1]. This approach is much favourable since unbalanced diets could lead to numerous non-communicable diseases such as diabetes, hypercholesterolemia and cancer. Unfortunately, the incidence of non-communicable diseases has shown a steep increase in numerous countries in the last two decades, highlighting the importance of formulating healthy foods and beverages [2].

The popularity of fermented dairy products is increasing among people around the globe continuously mostly as a result of extensive product diversification. Yoghurt drink, which is a ready-to-drink beverage produced from yoghurt of low viscosity, is an exceedingly successful fermented beverage developed quite recently. Regular consumption of yoghurt could lead to numerous health benefits including enhanced lactose tolerance, hypocholesterolemic effects, and anticancer properties. Further, it is also capable of stimulating the immune system and controlling gastrointestinal infections [3]. Drinking yoghurt, which shows numerous health benefits and of high popularity, is indeed an excellent beverage for fortification with nutrients. Like many non-fermented beverages fortified with nutrients [4-5], drinking yoghurt also could be fortified with numerous nutrients, including vitamins [6], minerals [7-8], and insoluble dietary fibre derived from different cereals such as oats [9] to provide further health benefits.

Oat (*Avena sativa*) is a cereal, rich in nutrients. Dietary fibre including β-glucan, high amounts of tocopherols and polyunsaturated fatty acids are the main nutrients responsible for the functional attributes of oats [10]. Health benefits of oats are many. Regular consumption of oats can reduce the blood low-density lipoprotein (LDL) cholesterol level, decrease the risk of cardio vascular diseases, and reduce the blood glucose level thereby declining the risk of type-2 diabetes mellitus. Further, it can lessen the risk of gastrointestinal disorders and cancer. Dietary fiber is responsible for most of the health benefits of oats [11]. Total dietary fibre intake by an adult should be approximately 28-36 g per day and it is highly essential for maintaining the health of the digestive system [2].

However, the daily dietary fibre intake by people of numerous countries is lower than the recommended level, highlighting the need for fibre fortified food products [2, 12]. Catering to the need of fiber fortification, Malki *et al.* [13] has formulated a set yoghurt incorporating oat flakes. Accordingly, incorporation of oats or its fractions into drinking yoghurt will be an appealing way of developing a functional food, which supplies the daily dietary fibre need.

In addition to functioning as an excellent source of dietary fibre, oat starch has been reported to function as a thickening or gelling agent in food formulations [14]. The characteristic large setback viscosity of oat starch explains the formation of thick gels by this thickener [15]. Thus, oat flakes, which are moderately processed oats that show easy gelatinization, may function as thickening agents for yoghurt products. However, sedimentation of the large particles may be problematic. Hence, powdered oat flakes may be a promising thickening agent for drinking yoghurt.

The particle size of oat flakes used in drinking yoghurt preparation may directly cause changes in sensory attributes like mouthfeel, sweetness and texture. This can also influence certain physicochemical properties like pH, titratable acidity, total soluble solids, syneresis and firmness of the final product. With this background, the present study was conducted to investigate the effect of the incorporation of three different particle sizes of oat flakes (850-425 µm, 425-180 µm, <180 µm) on the physicochemical and sensory attributes of drinking yoghurt.

METHODOLOGY

Materials

Oat flakes were purchased from Stassen Exports Pvt. Ltd., Colombo, Sri Lanka. Fresh cow milk of acceptable organoleptic and microbial quality was obtained from a reputed local supplier (Kothmale Holdings PLC, Sri Lanka). Sucrose, milk solids (Nestle Lanka PLC, Sri Lanka), potassium sorbate (INS No. 202) and drinking yoghurt were purchased from a local retail market. The starter culture with *Lactobacillus bulgaricus* and *Streptococcus thermophiles* microorganisms was procured from the Veterinary Research Institute, Gannoruwa, Sri Lanka.

Preparation of Oat Incorporated Drinking Yoghurt

Fresh milk (1000 mL) was pasteurized at 80 °C, while stirring continuously. Sucrose (90 g), milk solids (10 g) and oat flakes of reduced particle size obtained through milling and sieving (5 g) were mixed into the heated milk, which was then homogenized at 90 °C for 15 min. After cooling the sample down to 50 °C, potassium sorbate, which is a permitted preservative according to the Sri Lanka standard SLS 824:1989, was added such that the final concentration was 300 ppm (300 mg/kg) [16].

At 42 °C, the yoghurt starter culture was added (according to the recommendation of the Veterinary Research Institute, Sri Lanka) and the sample was stirred for the complete dissolution of the starter culture. Then, the sample was incubated at 42 °C for 4-5 h until a soft curd was formed and the pH of the sample reach to pH 4.6. The incubated sample was refrigerated overnight at 4 °C, and the curd was broken by swirling 40 times with a handheld stirrer to form a homogeneous product. The sample was refrigerated at 4 °C, until further analysis.

Three different particle sizes of oat flakes were used separately as thickening agents in the preparation of three different types of drinking yoghurts (Table 1).

Table 1: Three Different Sizes of Oat Flakes used in Drinking Yoghurt

Treatment	Size of Oat Flakes (µm)
T1	850-425
T2	425-180
T3	<180

Physicochemical Properties

Physicochemical variations of the three different types of oat incorporated drinking yoghurts (treatments) stored at refrigerated conditions (4 °C) were observed for 21 days. The pH was determined using a digital pH meter (EZODO, Taiwan). Titratable Acidity (TA) was determined by titrating aliquots of yoghurt samples with 0.1 N NaOH using phenolphthalein as the indicator as recommended by Association of Official Analytical Chemists (AOAC) [17]. Total Soluble Solids (TSS) content was obtained by a handheld refractometer (ATAGO N-46, Japan) and expressed as Brix%. The parameters stated above were monitored at 3-day intervals. Syneresis was measured for a period of 21 days at 7-day intervals, along with the Texture (firmness), which was measured using a texture analyser. All the analyses were performed in triplicate [18].

Sensory Evaluation

The sensory evaluation was carried out using thirty (30) untrained panellists. The panellists were asked to access nine parameters: colour, mouthfeel, odour, sweetness, sourness, taste, texture and overall quality, and purchasing intension of the drinking yoghurts, using a 5-point hedonic scale.

A second sensory evaluation was carried out comparing the best treatment from the study with a popular drinking yoghurt from the market (M). Similar to the first sensory analysis, the panellists were asked to access the aforementioned sensory parameters [19].

Proximate Analysis

Proximate analysis was carried out for the best sample selected based on the physicochemical and sensory analyses. It was compared with the drinking yoghurt from the market (M). The moisture content, ash content, total solid and solids-non-fat content were analysed according to the standard methods recommended by AOAC [17]. The crude fibre content was determined according to Weende method [20]. Total fat content was determined by the Soxhlet extraction method, while the crude protein content was determined by the Kjeldhal method [17]. The total carbohydrate content was determined according to a standard formula [21]. Analyses were performed in triplicate.

Microbiological Analysis

Microbiological analysis was carried out for the best drinking yoghurt (treatment) selected according to the sensory and physicochemical properties. Plate count agar was used for the determination of total viable counts. Analysis was carried out according to SLS 824 [16] for 21 days at 7-day intervals using yoghurt samples refrigerated at 4 °C. Microbial counts were obtained in 24 h.

Statistical Analysis

Data of sensory evaluation were analysed by using the Friedman test, while parametric data were analysed using one-way Analysis of Variance (ANOVA) and two-way ANOVA in OriginPro (version 9) software. Mean comparison was carried out using Fisher LSD method at $p \leq 0.05$ significance level. Data was expressed as Mean ± SD (SD: Standard Deviation).

RESULTS AND DISCUSSION

Physicochemical Analysis

The variation of physicochemical parameters of the three different drinking yoghurts (treatments) with storage time is shown in Figures 1 – 5.

pH

As shown in Figure 1, the pH of all treatments decreased significantly (*P≤0.05*) with time until 21 days. This decrease may be due to the excessive sugar fermentation by the lactic acid producing microorganisms [22]. Overall, the pH of treatments T1, T2 and T3 were not significantly different. However, significant differences (*P≤0.05*) among the treatments were observed at each time interval. According to Sri Lanka Standard SLS 824: 1989 [16], the pH of drinking yoghurt should be pH 4.5 and the pH of the treatments showed approximately similar values. Maintaining this optimum pH is important, since pH higher than 4.5 may facilitate the growth of pathogenic organisms, while pH lower than 4.5 may increase alcoholic aroma and acidic taste in yoghurt. The decrease of pH and increase of TA have been observed in many other yoghurt preparations [23].

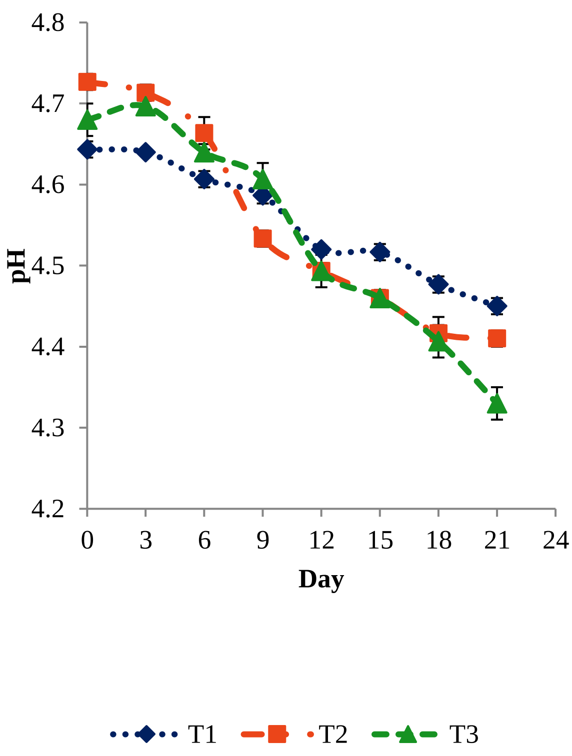

Figure 1: Variation of pH of Oat Incorporated Drinking Yoghurts with Time (Mean ± SD)

Note: T1: Oat flakes of size 850-425 µm incorporated yoghurt, T2: Oat flakes of size 425-180 µm incorporated yoghurt, T3: Oat flaks of size <180 µm incorporated yoghurt

Titratable Acidity (TA)

As shown in Figure 2, TA of all treatments increased significantly (*P≤0.05*) with time. The increase in TA with time is due to the fermentation of lactose, producing lactic acid [22]. The TA of T3 was significantly higher than that of T1 and T2, which were not significantly different based on the post-hoc analysis of means (Table 3). The water holding capacity of oat flakes may increase with reducing particle size due to higher release of fibre and starch that bind water. It may lead to lower water activity of T3, than T1 and T2. The higher acidity of T3 may have resulted, mainly, due to its higher water holding capacity than T1 and T2. The lower amount of free water molecules of T3 to dilute the lactic acid formed is reflected by its higher TA, compared to T1 and T2.

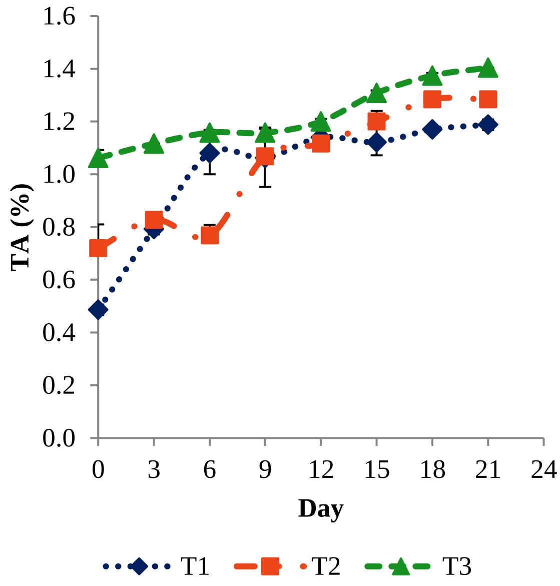

Figure 2: Variation of TA (%) of Oat Incorporated Drinking Yoghurts with Time (Mean ± SD)

Note: TA: Titratable Acidity, T1: Oat flakes of size 850-425 µm incorporated yoghurt, T2: Oat flakes of size 425-180 µm incorporated yoghurt, T3: Oat flaks of size <180 µm incorporated yoghurt

Total Soluble Solids (TSS)

As expected, TSS of all three treatments decreased with time (*P≤0.05*) as shown in Figure 3. The main reasons for this decrease are the consumption of sucrose by the microbes as an energy source and conversion of sugars into lactic acid through fermentation [24]. The TSS of the yoghurts increased with decreasing particle size of oat flakes. In fact, TSS of three treatments in the increasing order was: T1<T2<T3, while the mean values were 21.2%, 22.9% and 23.6%, respectively. This variation may be due to the higher degrees of leaching out of soluble material from oat flakes of lower particle size, due to their high surface area [25]. Further, the high degree of intact complexation of amylose to lipids in large oat flakes may have retarded leaching of soluble material, in addition to amylose, to the medium thereby showing lower TSS values [26].

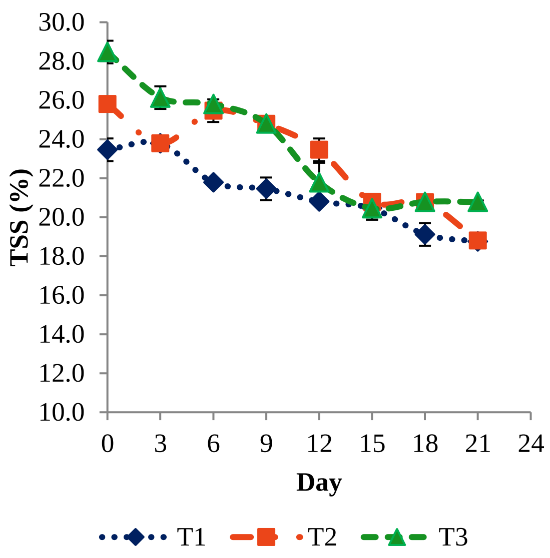

Figure 3: Variation of TSS of Oat Incorporated Drinking Yoghurts with Time (Mean ± SD)

Note: TSS: Total Soluble Solids, T1: Oat flakes of size 850-425 µm incorporated yoghurt, T2: Oat flakes of size 425-180 µm incorporated yoghurt, T3: Oat flaks of size <180 µm incorporated yoghurt

Syneresis and Firmness

Syneresis indicates the amount of whey separation. Overall, the syneresis of the three treatments was not significantly different at *P≤0.05* level (Figure 4). However, the syneresis in the decreasing order was: T1≥T2≥T3, indicating that the syneresis of T1 was greater than that of T3. However, the mean difference between T1 and T3 was 0.41% only. As it was mentioned previously, oats show pronounced swelling and leaching of soluble glucan during gelatilinization [27]. The T3 treatment, which consisted of the smallest size of oat flakes may have leached out more soluble glucan, amylose and amylopectin than T1, affecting the yoghurt microstructure leading into a higher water holding capacity and lower degree of syneresis [28].

Syneresis increased in all treatments with storage time and this may be due to loosing of casein network in the yoghurt gel [29]. Although many reasons such as those discussed above may explain the low degree of syneresis of T3, the overall results show that syneresis was not significantly different among the three treatments, as mentioned previously. However, the texture (firmness) of the three treatments was significantly different (*P≤0.05*). T3 showed a higher firmness than T1 and T2, which showed similar firmness levels (Figure 5). It may be due to the distinct effects of oat flakes of different particle sizes on the microstructure of yoghurt and varied water holding capacities of the three treatments. Favourably, the firmness of all treatments showed no significant variations with storage time (*P>0.05*).

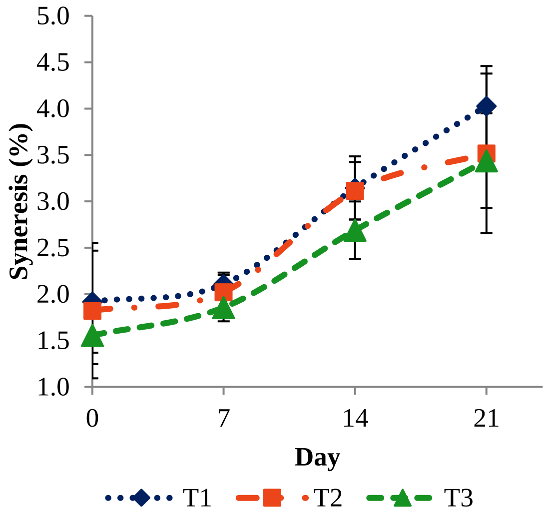

Figure 4: Variation of Syneresis of Oat Incorporated Drinking Yoghurts with Time (Mean ± SD)
Note: T1: Oat flakes of size 850-425 µm incorporated yoghurt, T2: Oat flakes of size 425-180 µm incorporated yoghurt, T3: Oat flaks of size <180 µm incorporated yoghurt

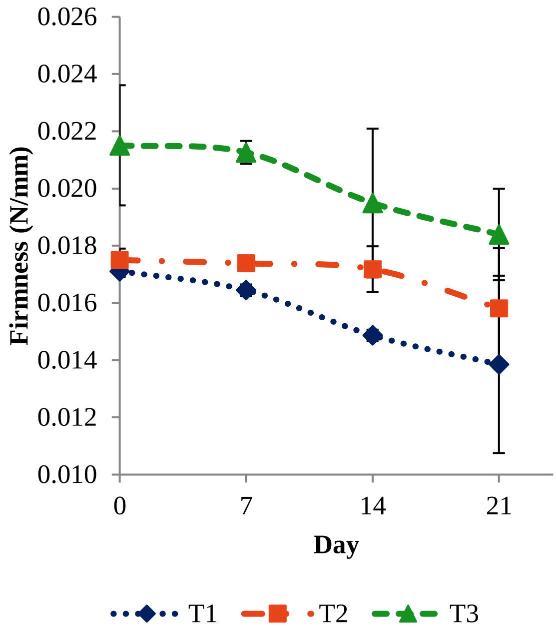

Figure 5: Variation of Firmness of Oat Incorporated Drinking Yoghurts with Time (Mean ± SD)
Note: T1: Oat flakes of size 850-425 µm incorporated yoghurt, T2: Oat flakes of size 425-180 µm incorporated yoghurt, T3: Oat flaks of size <180 µm incorporated yoghurt

Sensory Evaluation of Oat Incorporated Yoghurts

Interestingly, the four different types of yoghurt samples used in this study (C-control with no oat flakes, T1, T2 and T3) showed similar sensory attributes. Favourably, the medians of the four samples were 4 on a scale of 1 – 5 for all attributes, except sourness. The medians for sourness of T1 and T2 were 4, while those of T3 and C were 3 and 3.5, respectively. Accordingly, the purchasing intension was similar for all samples (*P=0.27*) as shown in Figure 6.

The presence of milk components retards the absorption of water and leaching out of amylose from starch granules, leading to reduced gelatinisation of oat starch [30]. However, the incorporation of instant oat flakes, which can be gelatinized easily, in yoghurt must have contributed to the favourable sensory attributes shown by the yoghurts. Unlike thermodynamically stable emulsions, kinetically stable macroemulsions like yoghurt benefit from biopolymers in the aqueous phase for increasing the stability of the food [31-32]. Hence, the incorporation of gelatinized oat flakes most probably has led to enhancing the stability and sensory attributes of, especially, mouthfeel, texture, and overall quality of the different types of yoghurts investigated in this study.

The particle size of oat flakes exhibited minimal effects on the sensory properties of yoghurts. Also, the oat flake incorporated yoghurts showed similar properties to regular yoghurt (control) of which the thickener was gelatin. Interestingly, the intension to purchase of both oat flakes incorporated drinking yoghurts and regular drinking yoghurt was similar, indicating the potential of the functional yoghurt to enter the yoghurt market in Sri Lanka.

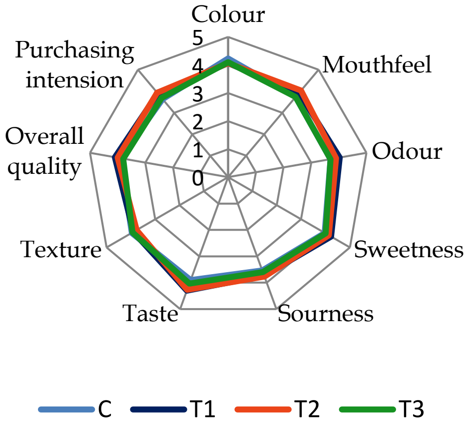

Figure 6: Mean Scores Obtained for Sensory Attributes and Purchasing Intention of Drinking Yoghurts

Note: C: Control, T1: Oat flakes of size 850-425 µm incorporated yoghurt, T2: Oat flakes of size 425-180 µm incorporated yoghurt, T3: Oat flaks of size <180 µm incorporated yoghurt

Selection of Yoghurt for Further Analysis

The type of drinking yoghurt with the largest particle size of oat flakes (T1) was chosen for further analysis, including microbiological analysis. This type of drinking yoghurt showed sensory properties similar to regular yoghurt and other types of oat incorporated yoghurts analysed in this study. The yoghurt with the smallest particle size of oat flakes (T3) was different to other types with respect to TA, TSS and firmness. However, as stated previously, the sensory attributes of T1, T2 and T3 were similar. T1 with the largest particle size range of oat flakes was chosen over T2 and T3 with smaller particle sizes of oat flakes, since the reduction of particle size requires energy [33].

Sensory Evaluation of Developed Oat Incorporated Drinking Yoghurt and Market Drinking Yoghurt

Interestingly, the sensory attributes of the selected treatment (T1) and market drinking yoghurt (M) were not significantly different except taste (Figure 7). In fact, the taste of T1 was better than that of M (*P=0.01*), with T1 and M having mean ranks of 1.73 and 1.27, respectively. The unique taste of gelatinized oat flakes appeared to have contributed positively to the taste of the oat incorporated drinking yoghurt.

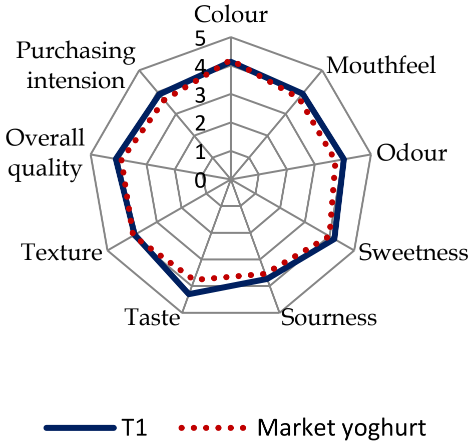

Figure 7: Mean Scores Obtained for Sensory Attributes of Yoghurts T1 and M

Note: T1: Oat flakes of size 850-42 5µm incorporated drinking yoghurt, M: Drinking yoghurt from the market

This result indicates that the oat flake incorporated drinking yoghurt may have similar or higher consumer acceptance compared to regular drinking yoghurt available in the market. However, the purchasing intension towards T1 and M were not significantly different (*P=0.14*).

Proximate Analysis

The proximate compositions of the selected oat flake incorporated drinking yoghurt (T1) and drinking yoghurt from market (M) are shown in Table 2. The proximate compositions of the selected oat drinking yoghurt (T1) and yoghurt from the market (M) showed significant differences with respect to the moisture, protein, fat, crude fibre, total solids, carbohydrate content, ash and solid non-fat contents.

T1 was significantly lower in the moisture content than M (*P=0.000*) because of added oat flakes as a solid bulk [13]. The protein content of T1 was significantly higher than that of M (*P=0.001*) because oat, containing globulin, is a rich protein source [34]. Also, the lipid content of T1 was significantly higher than that of M (*P=0.001*) because of the high lipid content of oats. In fact, oats contain much higher levels of lipids than other cereals which are excellent sources of energy and unsaturated fatty acids [35].

The fibre content of T1 was higher than of M (*P=0.004*). Regular drinking yoghurt does not contain any trace of dietary fibre. In contrast, T1 shows the presence of fibre because polysaccharides such as cereal β-glucan, arabinoxylans and cellulose are present in oats [35]. The carbohydrate content of T1 was significantly higher than that of M (*P=0.016*) mainly due to the presence of starch in oat flakes [35]. These results indicate the higher nutritional value of oat incorporated drinking yoghurt than regular drinking yoghurt available in the market.

The ash content of oat drinking yoghurt was higher than that of M (*P=0.024*) due to the presence of bran layers and alurone layers in oat particles [36]. According to the SLS standard, SLS 824 [16], yoghurt should contain a minimum 8.0% SNF. Accordingly, both T1 and M showed SNF values above 8%. However, the SNF value of T1 was higher than that of M (*P=0.040*). These results indicate the suitability of T1 as a drinking yoghurt product.

Microbiological Analysis

Microbial analysis was carried out for drinking yoghurt incorporated with oat flakes of particle size 850 – 425 µm (T1) stored under refrigerated conditions (4-8 °C). Total plate count increased up to the 14th day after which it started to decrease (Table 3). The increment of the level of acidity, which was reflected in the reduction of pH of the medium with time, may have partly caused the reduction of bacterial growth. Also, exhaustion of nutrients in the medium may have caused the reduction of bacterial count after day 14 [37]. Thus, oat drinking yoghurt (T1), which was prepared using 300 ppm of potassium sorbate and stored at 4 ºC, is suited for consumption within 14 days from the production, according to microbiological analysis.

Table 2: Proximate Composition of Oat Flake Incorporated Drinking Yoghurt and Market Drinking Yoghurt

Parameters (%)	**Oat Incorporated Drinking Yoghurt**	**Market Drinking yoghurt**
MC	73.27 ± 0.89^{b}	80.22±0.34^{a}
Protein	4.92±0.22^{a}	2.80±0.40^{b}
Fat	6.07±0.07^{a}	3.79±0.39^{b}
Crude Fiber	0.01±0.00^{a}	0.00±0.00^{b}
Ash	0.33± 0.03^{a}	0.27± 0.02^{a}
TS	26.75± 1.76^{a}	21.22±0.31^{b}
SNF	20.67±1.78^{a}	17.43±0.61^{a}
Carbohydrate	15.40± 0.98^{a}	12.93±0.41^{b}

Note: Means with different superscripts within each row are significantly different at 0.05 level.
MC: Moisture content, TS: Total solids, SNF: Solid Non-Fat

Table 3: Bacterial Count of the Developed Drinking Yoghurt with Storage Time

Day	Total Plate Count (CFU/g)
0	9.6×10^4
7	12.0×10^4
14	9.2×10^5
21	4.8×10^5

The level of potassium sorbate can be increased up to 1000 ppm according to SLS 824 [16] to enhance the shelf-life of the drinking yogurt developed in this study.

CONCLUSIONS

The particle size of oat flakes exhibited an impact on TA, TSS and firmness of drinking yoghurt. These changes may be attributed to changes in water holding capacity, surface area and the free water activity. Nevertheless, the sensory attributes of the three oat flake incorporated drinking yoghurts were similar. The drinking yoghurt with oat flakes of the largest size range (850 – 425 µm) was chosen for comparison with the market yoghurt considering that the other yoghurts require a higher energy input for size reduction. The taste of the selected drinking yoghurt was better than that of the market yoghurt. Further, it has a higher nutritional profile than market yoghurt and can be stored microbiologically safely for 14 days at 4 ^{0}C. These findings can be used for further development of oat incorporated drinking yoghurts.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

HD: Carried out the investigation and data curation, and wrote the manuscript. GP: Conceptualized, designed the research, supervised the study, performed statistical analysis and interpretation of data, and wrote the manuscript. AW: Designed the research and supervised the study. All authors read and approved the manuscript.

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