Effect of inulin on physico-chemical, sensory, fatty acid profile and microstructure of processed cheese spread

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Abstract To develop a functional processed cheese spread (PCS) different levels of inulin (0, 4, 6 and 8%) addition into PCS was studied with its physico-chemical, sensory and fatty acid profile and micro-structural quality. As the level of inulin addition increased moisture, a_w and titratable acidity, decreased. At the highest level of inulin addition (8%) sensory panelists reported a significant decrease in total sensory score. PCS with 6% insulin was found to have optimum from quantity. The addition of inulin in cheese spread decreased both total saturated fatty acid and unsaturated fatty acid and in unsaturated fatty acid, mono unsaturated fatty acid decreased; however, polyunsaturated fatty acids increased as compared to the control. Scanning Electron Micrograph of PCS containing insulin showed uniform distribution of insulin with diameter ranged 4–10 μm in the protein matrix.

Keywords Inulin · Processed cheese spread · Sensory · Fatty acid profile · Microstructure

Introduction

Countries worldwide are going through a nutritional transition and are now affected by double burden of nutritional problems. Under-nutrition and specific nutrient deficiencies continue to present a problem, and simultaneously imbalanced diets and chronic diseases are becoming alarmingly common. Non-communicable conditions such as cardiovascular diseases, diabetes, obesity, cancer, and respiratory diseases account for 5% of the 56.5 million deaths annually and 45.9% of the global burden of disease (WHO 2011). According to WHO (2011), it is estimated that up to 80% of coronary heart disease, 90% of type-2 diabetes, and 1/3 of cancers can be avoided by changing lifestyle, including diet. But, dietary habits are notoriously difficult to change and even more difficult to sustain, even if the positive health effects are known. So, the efforts can be made to fortify or enrich the conventional foods with the active ingredients which can therefore provide the related health beneficiary effects (Giri et al. 2013b, 2014b, c). Hence, the foods consisting of these active ingredients can offer a means of providing some health or well-being benefits in a way that requires negligible or smaller behavioural changes (diet pattern, eating habits etc.) by the individual consumer. Improved products may also provide dietary components that would otherwise be difficult to obtain in sufficient amounts from normal diets.

Inulin is a soluble dietary fibre forming a subset of nutraceutical ingredients that is increasingly used in food products. Basically inulin is a carbohydrate of fructan family with β (2 $\rightarrow$ 1) linked fructosyl residues mostly ending with a glucose residue. Asparagus, garlic, leak, onion, Jerusalem artichoke, chicory, etc. are often eaten as vegetables which are rich source of inulin. The average daily consumption of inulin and oligofructose has been

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estimated to be 1–4 g in the United States and 1–3 g in Europe (Van Loo et al. 1995). It has GRAS status in the US and in most of the European countries it is recognized as ‘natural food ingredients’. The recommended range of dietary fiber intake is 25–38 g/day (Slavin et al. 2001) however; there is no official Recommended Daily Allowance (RDA) for Inulin intake. Inulin supports a healthy circulatory (blood) system in that it lowers triglycerides, total cholesterol and LDL cholesterol, while increases HDL cholesterol, not only that it acts as prebiotic, increases mineral absorption in the colon, eliminates constipation and decreases cancers of the colon.

Butter, the traditional spread for bread is now avoided due to poor spreadability, high saturated fat and cholesterol content. Margarine produced on the hydrogenated oils basis have high levels of trans fatty acids which increase concentrations of LDL cholesterol and reduce HDL cholesterol (Karabulut and Turan 2006). The annual growth rate of cheese production in India is 10–15% and ~90% cheese is consumed as processed cheese and processed cheese spread (PCS). The PCS contains not only protein and fat in pre-digested form, but also calcium, phosphorus, riboflavin and other vitamins in a concentrated form. It also has health beneficial bioactive peptides, conjugated linoleic acid (CLA) and contains a lower amount of fat and higher amount of protein compared to any low fat table spread (Giri and Kanawjia 2014). Hence, cheese spread can provide nutritionally superior spread for bread and it can be incorporated with inulin to enhance its functionality.

In another study we found that there was significant beneficial effect of inulin incorporated processed cheese spread on lipid profile of blood serum and liver in rats (Giri et al. 2014a). The textural and melting properties of PCS containing different levels of inulin has also been evaluated (Giri et al. 2013a). Therefore, the present study was undertaken to assess the effect of inulin addition on physico-chemical, sensory and fatty acid profile of PCS.

Materials and methods

Raw materials and ingredients

Cheddar cheese

For manufacture of control processed cheese spread (CPCS) as well as inulin incorporated processed cheese spread (IPCS), both young (~1½ month ripened) and old varieties (~6 months ripened) of Cheddar cheese were used and of both the ages were obtained from the Experimental Dairy of National Dairy Research Institute, Karnal, Haryana, India. The chemical analysis of both

the varieties of cheeses was carried out which is presented in Table 1. The ripening index of cheese blend was found to be 29.8% which was suitable to manufacture PCS with excellent physico-chemical and sensory properties.

Inulin

Inulin (extracted from chicory root; Frutafit®HD, manufactured by Sensus, Netherlands) was procured from Premier Speciality Ingredients, Chennai, Tamil Nadu, India. The specification of inulin was dry matter—96.6%; composition on dry matter: inulin (DP2-DP60)—90.0%, fructose—3.2%, glucose—0.5%, sucrose—6.0%, average chain length (monomers)—9, ash—<0.2%.

Other ingredients

Iodized common salt (TATA Salt, Tata Chemicals Ltd., Mumbai) was procured from the local market for preparation of processed cheese spread. Tri-sodium citrate (Posy Pharmachem Pvt. Ltd., Ahmedabad) as emulsifying salt was used for PCS manufacturing.

Manufacture of processed cheese spread

A blend of Cheddar cheese (25% old Cheddar cheese and 75% young Cheddar cheese) was taken for the preparation of processed cheese spread. The amount of different ingredients such as water, salt and tri-sodium citrate as emulsifier to be added were calculated on the basis of final product (processed cheese spread). In this investigation each batch of 1 kg PCS was prepared. The young and old Cheddar cheeses were cleaned, quartered and grated. Cheese processing vessel (Stephy®, Stephy Industries, New Delhi) was used for manufacture of processed cheese spread. At first a portion of the grated cheese was taken into cheese processing cooker and to this calculated amount of salt and emulsifier was added after mixing into calculated amount of water. Then the remaining ground cheese was added and the contents in the vessel were heated with continuous stirring and scraping of the surface with a wooden ladle. When the mass become semi solid and homogeneous, inulin was added (0, 4, 6 & 8% inulin addition to PCS) and mixed thoroughly. Before adding to cheese spread, inulin was separately mixed with warm water (~50 °C) through running blender (Mincer Blade, Type-H-100, RPM-12000, Lee Handy™, Lumix Appliances, Ambala city, India) for 3 min. After straining it was mixed with the cheese spread. The content was heated to 85 °C for 3–5 min with continuous agitation. Thereafter, the heating was stopped and the hot product was filled

Table 1 Quality of Cheddar cheese used for preparation of processed cheese spread (Giri et al. 2014b, c)

Parameters	Young Cheddar cheese (~ 1½ month)	Old Cheddar cheese (~ 6 months)
Moisture (%)	39.5	37.0
Total fat (%)	29.0	30.0
Fat on dry matter (%)	47.9	47.6
FFA (µg/g)	17.8	20.9
Total protein (%)	24.8	25.9
Soluble protein (%)	6.3	11.1
Ripening index	25.4	42.9
Salt (%)	3.0	3.1
Ash (%)	3.5	3.7
Acidity (% lactic acid)	1.026	0.816
pH	5.3	5.8

Cheese blend (Young cheese:Old cheese::3:1) Ripening index = 29.8

$$\text{Ripening index} = \frac{\% \text{ soluble protein}}{\% \text{ total protein}} \times 100$$

immediately into sterile sample container. The PCS was allowed to cool to the room temperature and thereafter stored under refrigeration (4 ± 1 °C) (Giri and Kanawjia 2013a, b).

Analytical methods

Physico-chemical attributes

The moisture content of Cheddar cheese as well as PCS was determined by standard gravimetric method as described in IS: SP: 18 (Part-XI), 1981. The fat content in Cheddar cheese and PCS was determined by the Gerber method described in AOAC (1990). The total protein content of Cheddar cheese as well as PCS was estimated by Micro-Kjeldhal method (AOAC 1992). The salt and total ash content in Cheddar cheese and PCS samples was estimated as per the process delineated in IS: SP: 18 (Part XI), 1981. Titratable acidity of the PCS was determined according to AOAC (1995).

pH

Ten grams of PCS was mixed with 10 ml of distilled water and a slurry was prepared thereof. The pH of slurry was determined using microprocessor controlled pH Analyzer (Verson I, Labindia, New Delhi) with combined glass electrode. The pH meter was first calibrated at pH 9.2 and 4.0 and then standardized at pH 7.0 using buffer solution.

Water activity

Water activity was measured using water activity meter (Aqua lab, Model series 3TE, Decagon Devices, WA, USA). Prior to the measurement the samples were tempered at 25 °C.

Total dietary fiber content

The total dietary fiber content of the defatted, powdered, oven dried (105 ± 2 °C/overnight) functional PCS was estimated by the AOAC (2000) method, employing TDF-100A kit (Sigma, Missouri, United States).

Determination of fatty acid profile

Fatty acid profile of the product as well as the mixture of standard fatty acids was determined using Gas–Liquid Chromatography (GC Model 5765, NUCON, New Delhi) fitted with flame ionization detector, computer assisted programmable data processing, storing unit and a printer, using the method AOAC (2011) after converting them into their respective methyl esters.

Microstructure

Scanning electron microscopy (SEM) was used to determine microstructure of cheese spread samples. Samples were prepared according to method Karami et al. (2009). For this cheese spreads were spreaded on a flat and smooth surface and using a saving blade, spreaded cheeses were cut into approximately 5–6 mm³ blocks, immersed in fixative (2.5% glutaraldehyde and 2% paraformaldehyde made in 0.1 M sodium phosphate buffer; pH 7.2) for 3 h and freeze dried. To prevent any damages to fat globules, cheese spread samples were not washed using ethanol/ water combination and acetone. The samples were cut into approximately 1 mm thickness and mounted on the aluminum stubs by using double sided sticky carbon based tape and coated with gold for 200 s in sputter coater (Polaron SC7640, East Sussex, UK). The prepared samples were observed under SEM (Carl Zeiss EVO 40, Oberkochen, Germany) at 20 kV.

Sensory analysis

Sensory evaluation of processed cheese spreads was carried out using descriptive sensory score and it was performed by 10 trained panellists of the Institution, constituted based on their interest, performance, motivation, compliance and availability. Attributes evaluated were flavour, body and texture, colour and appearance as well as spreadability (Maximum Score: Flavour—45; Body and Texture—30;

Colour & Appearance—10; Spreadability—15; Total Sensory Score—100). Before profiling, two sessions were used to train the assessors in the definition of attributes of a standard identity processed cheese spread (Makhal et al. 2012, 2013c). The panellists were allowed to use water and bland crackers for palate cleansing between the samples. All the samples (~50 g), randomly coded using three digit numbers, were evaluated in each session and each panellist was given the samples (20 °C) in white 50 ml HDPE sample container always 2 h before or after the meals.

Statistical analysis

The effects of levels of inulin on the properties of PCS were analyzed by one way analysis of variance using IBM SPSS Statistics 20 software package. The data are presented as mean $\pm$ standard error (SE). When significant (5% levels) differences were observed, individual means were compared using Tukey Post Hock multiple comparison test (Makhal et al. 2011, 2013a, b; 2014).

Results and discussion

Physico-chemical characteristics

The physico-chemical characteristics viz. moisture, water activity (a_w), titratable acidity and pH of the products are presented in Fig. 1. It was observed that as the levels of inulin addition was increased from 0 to 8% in PCS, the moisture percentage decreased significantly ($p < 0.05$) from 58.2 to 55.6%. The decrease of moisture percentage was due to proportionate increase of total solids content in the cheese spread.

Water activity (a_w) indicated the activity of water to function as a reactant and solvent in the product. As inulin addition level increased from 0 to 8% in PCS, a_w decreased from 0.983 to 0.980. However, up to 6% inulin addition, a_w did not change significantly ($p > 0.05$) as compared to the control, however at 8% level a significant ($p < 0.05$) reduction in a_w was observed as compared to the CPCS. Inulin is very hygroscopic in nature. So, on addition of inulin at higher level, free water present in PCS was absorbed that resulted in decrease in a_w .

Titratable acidity of PCS declined from 0.984 to 0.591% as the levels of inulin was increased from 0 to 8%. At 4% addition of inulin, a sharp and significant ($p < 0.05$) decrease in the titratable acidity of PCS as compared to CPCS was observed; however, beyond that level there was no significant ($p > 0.05$) change. This reduction of titratable acidity was not well understood.

The pH of the CPCS and experimental PCS with different levels of inulin was 5.9. The addition of inulin did

not significantly change the pH of the PCS, though acidity decreased. This phenomenon might be due to high buffering capacity of the cheese due to different salts and acidic as well as basic amino acids present in the cheese spread (Fox and McSweeney 1998).

Sensory characteristics

The effect of inulin addition at different levels on sensory characteristics of the PCS with respect to flavour, body and texture, colour and appearance, spreadability and total sensory score is presented in Fig. 2.

Flavour

Flavour is the most important among all the sensory attributes, which generally decides the acceptability of any food product. Though, as the levels of inulin addition was increased from 0 to 8% in PCS, flavour score decreased from 41.5 to 40.7; however, this decrease was not significant ($p > 0.05$). Higher levels of inulin addition proportionately decreased the quantity of natural cheese, resulting a decrease in typical cheese flavour. However, it is fact that inulin has about 10% of sweetness of sucrose (Franck 2002). So, sensory panellists might like this functional cheese spread with sweet palate.

Body and texture

Body and texture is the indicative of consistency of the product. The body and texture score ranged from 28.5 to 25.0 among the control and all inulin incorporated experimental cheese spreads. Due to hygroscopic nature, inulin absorbed moisture from the cheese spread. The softness of the product decreased due to inulin addition. However, the body and texture score did not significantly ($p < 0.05$) reduced up to 6% inulin addition. Similarity between fat and gel behavior was suggested by Bot et al. (2004). According to Walstra and Jenness (1984) in fat like butter, structure is stabilized by van der Waal's attraction and According to André et al. (1996) inulin molecules are bound by intermolecular hydrogen bonds. However, at 8% inulin addition, panelist noticed a sharp reduction in softness, as a result significant ($p < 0.05$) decrease in body and texture score was observed. This could be substantiated with the scores of spreadability which was adversely affected at maximum level of addition of inulin. In addition to, at higher level of inulin addition in cheese spread, significant ($p < 0.05$) change of textural attributes viz., increase of firmness and work of shear and decrease of stickiness as compared to the control, might be the reason of decreased body and texture score. Not only that, lower body and texture may be attributed to interaction of fiber

Fig. 1 Effect of different levels of inulin on different physico-chemical characteristics of processed cheese spread; $n = 3$; different small alphabets indicate significantly different ($p < 0.05$); vertical bars indicate errors of means

with milk solids that led to weak gel formation which is also reported by Raju and Pal (2012) during addition of inulin in *misti dahi*.

Colour and appearance

The colour and appearance score of the control as well as inulin incorporated cheese spread samples ranged from 9.1 to 8.3. The colour and appearance score of all inulin incorporated cheese spreads was significantly ($p < 0.05$) lower; however, among all the inulin incorporated PCS there was no significant ($p > 0.05$) difference in colour and

appearance scores. The control processed cheese was shiny yellowish in colour because Cheddar cheese used in this study was made from cow milk, but due to addition of inulin, yellowness decreased significantly ($p < 0.05$) that resulted in dull colour and appearance.

Spreadability

Spreadability is a very important functional characteristic of any spread. In the current investigation, though up to 6% inulin addition, spreadability score was similar as compared to the control, but at 8% inulin addition, it decreased

Fig. 2 Effect of different levels of inulin on different sensory characteristics of processed cheese spread; $n = 3$; different small alphabets indicate significantly different ($p < 0.05$); vertical bars indicate errors of means

significantly ($p < 0.05$). Panelists recorded difficulty in spreading of PCS containing 8% inulin. The water binding property of inulin, at higher level (8%) may have reduced the softness of spread that caused poor spreadability.

Total sensory score

Total sensory score revealed that as the level of inulin addition increased up to 6%, total sensory scores did not significantly ($p > 0.05$) decreased; however, at 8% inulin addition a significantly ($p < 0.05$) decrease was observed due to increase in hardness and coarseness as well as poor

spreadability. Panellists noticed that 6% inulin added PCS (IPCS) was comparable with the control. So, cheese spread, with 6% inulin level was observed to be optimum.

It has been reported that at lower level of inulin addition in food, the rheological properties and sensory quality of the product did not affect strongly due to the natural or slightly sweet taste and the limited effect on viscosity (Meyer et al. 2011). To claim product with inulin, product should be added with inulin in the range of 3–6 g/100 g of product (European Commission 2007). The optimized product in the current investigation was under this regulatory range.

Table 2 Proximate composition of inulin incorporated (IPCS) along with control (CPCS) processed cheese spread (Giri et al. 2013a)

Constituents (%)	CPCS	IPCS
Moisture	58.2	56.1
Fat	19.0	18.0
Protein	17.4	15.4
Salt	2.0	1.9
Ash	3.4	3.2
Total dietary fiber	–	4.9

Table 3 Fatty acid composition of inulin incorporated (IPCS) along with control (CPCS) processed cheese spread

Fatty acids	% Fatty acid present in product	
	CPCS	IPCS
Butyric (C _{4:0})	ND	0.02
Caproic (C _{6:0})	0.07	0.06
Caprylic (C _{8:0})	0.13	0.12
Capric (C _{10:0})	0.50	0.49
Lauric (C _{12:0})	0.58	0.59
Myristic (C _{14:0})	2.09	2.03
Palmitic (C _{16:0})	6.31	5.93
Stearic (C _{18:0})	1.98	2.21
Oleic (C _{18:1})	7.04	6.09
Linoleic (C _{18:2})	0.17	0.19

ND not detected

Analysis of optimized product

Proximate composition

The composition of IPCS is depicted in Table 2 (Giri et al. 2013a). It was observed that cheese spread containing inulin caused reduction in fat and protein content from 19 to 18 and 17.4 to 15.4% respectively; however, the developed product contained high amount of dietary fiber (4.9%).

Fatty acid profile analysis

The addition of inulin on the fatty acid composition of the cheese spread is presented in Table 3. The study revealed that the CPCS contained a significant amount of linoleic acid (8.95 mg/g fat or 0.17% of product). The addition of inulin in the product slightly increased the linoleic fatty acid. It has been reported that increase in concentration of PUFA particularly CLA during processed cheese making. The proposed mechanism was that under anaerobic condition isomer of linoleic acid, CLA could be produced by the auto-oxidation of linoleic acid during heating (Ha et al. 1989). Like linoleic acid—butyric, lauric and stearic acid percentage increased in IPCS as compared to the CPCS, whereas all other fatty acid viz., caproic, caprylic, capric, myristic, palmitic and oleic acid percentage decreased due to inulin addition. This change in fatty acids components might be due to the interaction of inulin with specific fatty acids. It is fact that, the addition of inulin in cheese spread decreased total saturated fatty acid (from 11.66 to 11.45%) and total unsaturated fatty acid (from 7.21 to 6.28%) and in unsaturated fatty acid, MUFA decreased from 7.04 to 6.09%; however, PUFA increased from 0.17 to 0.19% as compared to the control.

Microstructure

A comparison was also made in this study between the microstructure of CPCS and IPCS using Scanning Electron Microscopy (Fig. 3). The fat droplets were uniformly distributed in CPCS and were 18–60 µm in diameter with irregular shape. However, it has been reported that microstructure of processed cheese product typically consisted of concentrated emulsion of discrete, rounded fat droplets of varying size (typically ~1–35 µm) in a hydrated protein matrix (Hennelly et al. 2006). The smaller fat globules most likely indicated more effective fat emulsion. In the current study, processed cheese spreads due to insufficient shearing (due to manual stirring) tended

Fig. 3 Scanning Electron Micrograph of control (a) and inulin incorporated (b) processed cheese spread; P protein matrix; F fat globule; I inulin

to show some irregular shaped and larger fat droplets. Similar observation has been made by Trivedi et al. 2008. Scanning Electron Micrograph of IPCS showed that fat globule size was 20–60 µm in diameter, whereas Inulin was uniformly distributed with diameter range 4–10 µm in the protein matrix.

Conclusion

In this study a functional PCS was developed with the incorporation of inulin which is a potent functional ingredient. As the levels of inulin addition was increased from 0 to 8% in PCS, physico-chemical attributes such as moisture, a_w and titratable acidity of the product decreased, whereas, sensory attributes, up to 6% inulin addition, were not significantly affected. The level of inulin addition was optimized at 6% level in PCS. Due to inulin addition, fat and protein content reduced in cheese spread (IPCS) as compared to the control sample; however, the developed product contained high amount of dietary fiber. The addition of inulin in cheese spread decreased both total saturated fatty acid and unsaturated fatty acid; however, PUFA increased as compared to the control sample. From Scanning Electron Micrograph of IPCS it was observed that Inulin was uniformly distributed in the protein matrix.

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