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UTILISATION OF POPPY SEEDS (*PAPAVER SOMNIFERUM*) BY REDUCING MORPHINE FOR NEW FOOD PRODUCT DEVELOPMENT

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ABSTRACT

Poppy seed (*Papaver somniferum*) has been used as an Ayurvedic herb for thousands of years. It consists of many painkiller alkaloids (such as morphine, codeine etc.) and rich in protein and linoleic acid which helps in lowering blood cholesterol. It has analgesic, calmative, bactericidal, sedation and antidiarrheal properties. Morphine content in poppy seeds recently has major concern due to intoxication in maximum limits. With the aim purpose to use poppy seeds at consumer level and bakeries, a significant reduction was observed by using an optimal treatment consist of washing, drying and grinding which will simultaneously improve the organoleptical quality of the product. A systematic method (washing, drying, grinding and baking) was used for the degradation of alkaloids. HPLC method was used to analyse alkaloids. Processed seed Processed seeds recipes (cake, biscuits, ladoo and tea) with 2.5%, 5%, 7.5% and 10% level and 100% value added recipes made from poppy seed oil were evaluated by 9-point hedonic scale by 15 semi trained panel members. Results have shown significant reduction of alkaloids i.e., morphine codeine, thebaine and narcotine were 0.178%, 0.038%, 0.041%, 0.002% respectively which were significantly ($p<0.5$) reduced to 0.046%, 0.009%, 0.002% and 0.001% respectively. Acceptability evaluation showed that incorporated recipes were more acceptable as compared to standard. The level of incorporation acceptable was 2.5% and 5% respectively while poppy seed oil recipes were equally accepted to standard. Bakery products showed significant reduction ($p<0.5$) in alkaloids due to grinding and heat treatment done at 200ºC. The conclusion drawn from the study explained the fact that bakery products prepared by incorporation of poppy seeds demands grinding to improve the aroma of the products as well as they are baked at high temperature decreases alkaloid level at 90%. Hopefully the traditional variety and craftsmanship of the high quality poppy seed bakery products and poppy seed oil based supplements will not suffer from risk assessments and exaggerated fear of the public.

Keywords: Alkaloids, Morphine, Thebaine, Narcotine, Codiene, *Papaver somniferum* and HPLC.

INTRODUCTION

Papaver somniferum (Opium Poppy) is cultivated as an erect annual herb (Bernath J, Nemeth E, 2009) in countries such as India, China, Czechoslovakia or Turkey. It is mainly grown for its opium and oilseed (Ozcan MM, Atalay C 2006). The latex (milky sap) of the opium poppy contains alkaloids, including the narcotic agent's morphine and codeine that have been used by man for the treatment of severe pain for generations. The latex permeates all parts of the plant, except the seeds and is to be found in the particular in the pericarp of the capsule (Efsa (European Food Safety Authority) panel on contaminants in the food chain (CONTAM), 2011).

Poppy seed is an important herb in ayurveda and has medicinal properties to treat various health condition (Aruna K, Sivaramakrishnan VM, 1992) .It has high nutritional value (protein upto 24%) and high amount of linoleic acid (Singh SP , et al, 1990, Singh SP , et al, 1995a, Singh SP , et al, 1999) which helps in lowering blood cholesterol in human system. Besides this it contains some properties such as analgesic, calmative, bactericidal, sedation and versatile member in pharmacological application (Qidwai W, et al, 2003). Its sedation effect may be a reason for improved sleep. However, it is worthwhile to mention that, a cumulative effect of protein from the seeds and sugar added to the drink may have stimulated the secretion of serotonin causing improved sleep during intervention period (Cohen HM, et al 2002, Nergis C, Otles S, 1999). Further, poppy seeds drink brought about a marked increase in serum B12 level, although it is not a good source for this nutrient (Zadeh SS, Begum K, 2011).

Poppy seeds are used as food in bakery products, on top of dishes, in fillings of cakes and in desserts and to produce edible oil (Sproll C, et al, 2006). Whilst the seeds of the poppy plant do not contain the latex, they can become contaminated with alkaloids as results of insect damage to the capsule, or through poor harvesting

practices. The federal institute for risk assessment (BFR) derived a provisional guidance value of 4 mg morphine/kg poppy seed based on a maximum daily intake of 0.38 mg morphine/person. The maximum value of 4 mg morphine/kg(Nencini P, 1997) poppy seed might have led to a nearly complete market shake out because a great deal of all products had concentration above the limit. To recalibrate this, alkaloid content of poppy seed samples and poppy seed containing foods can be reduced by several methods of pre- treatment and processing. Food processing may decrease the alkaloids content by up to about 90%. The most effective methods include washing, soaking and heat treatments, as well as grinding and combinations of these treatments. If the combinations are reduced by processing, the exposure would be up to 90% lower (Sproll C, Lachrnmeier DW, 2006).

POPPY SEED OIL

Poppy seed oil appears to be of good quality for human consumption since it is generally rich in polyunsaturated fatty acids (Ozcan MM, Atalay C ,2006 , Baydar H, Turgut l, 1999, Bozan B, Temelli F, 2003, Kryzmanski J, Jonsson R,1999, Luthra R, Singh N,1989). Poppy seed oil contains 50% of edible oil with a pleasant aroma and taste like almond oil. In the Papaver Somniferum the solvent extraction yields 36.8% and contains highest amount of linoleic acid (69.2%) than any other species(Azcan N, et al, 2004), which makes it good oil for nutrition, as a high % of linoleic acid is desirable for lowering the cholesterol content in the human system and thus prevents coronary heart trouble. Poppy seed oil is used widely for culinary purposes and seeds are from capsule which not has been sacrificed, they are free from narcotic compounds(Yadav HK, et al, 2008) and used as a cooking medium or as salad oil. It is mixed with olive oil and used as a salad oil. It has a high digestibility coefficient of about 96% at a daily intake of 50g. On hydrogenation, it yields a production similar to groundnut oil, which may also be useful for industrial purposes. Being it is high in essential fatty acids it is considered as good for human consumption(Erinc H, et al, 2009) as well as the oil cake is good fodder for cattle (Duke JA, 1989).

The study undertaken is an attempt to develop some products was incorporated by poppy seeds which have reduced amount of alkaloids after processing as well as its oil. The main purpose of this study was to utilise all the nutritional aspect present in the seed and its oil.

MATERIALS AND METHODS

A randomly selected poppy seeds sample from Kota, Rajasthan by local authorities was analyzed for proximal analysis, morphine, codeine, thebaine and narcotine.

CHEMICAL COMPOSITION

PROXIMATE ANALYSIS

The moisture was determined by hot air oven method (Sharma S, 2007). The protein level was determined by the Khjeldal method, according to official analytical chemists (AOAC, 1995). The ash was determined using NIN, 2003 manual (Raghuramulu N, et al 2003). Crude fibre were estimated by acid alkali method (Raghuramulu N, et al 2003), lipid were extracted in soxhlet apparatus with petroleum ether at 40-60 according to the method 3 75 Ai AOAC (1993) and carbohydrate by difference. Estimation of iron content was done by Wong's method (Raghuramulu N, et al 2003). Estimation of calcium was carried out by titrametric method (Sharma S, 2007).

PRE-TREATMENT OF POPPY SEEDS TO REDUCE THE MORPHINE CONTENT

The most feasible conditions to reduce the morphine content were found to be, the washing of poppy seed using hot water from the centralized hot water installation (around 60ºC) for some minutes. Our experiments allow deriving the guidelines for consumers and bakeries given in table 2, about the correct treatment of poppy seed. The poppy seed should be washed with water as described in table 2; afterwards the seed must be dried to prevent microbiological contamination, germination and rancidity. By the usage of the proposed treatment besides morphine content reduction the organoleptical quality of poppy seed was notably enhanced.

EXTRACTION SOLVENT

A powdered capsule (ca:150 mg) was weighed and placed in a 2ml eppendorf tube and extracted with a1.5ml solvent (5%AcOH + EtOH(1:1)) under sonication for 30 min. Then the mixture was stirred with vortex mixture for 1 min and centrifuged (18000xg, 10 min). The supernatant (20µl) was injected into the HPLC column.

ESTIMATION OF ALKALOIDS

The opium extract sample were analysed by paired ion reverse phase Chromatography following Lurie waters (Milford, U.S.A). High pressure liquid chromatography (model ALC/GPC-204) consisting of M 6000 A solvent delivery system, U6 K injector, µ Bondapack C18 column (4mnid x 30 cm), M 440 UV detector at 254 nm and 10 MV recorder (Houston Instruments, Houston, Texas) was used for analysis.

The opium samples for chromatography were prepared by titrating 75 mg of dry opium in 10ml of dimethyl sulphoxide (DMSO) for about 10 minutes. This was followed by ordinary filtration and then running the samples through waters samples classification kit, the injection volume was 5µl.

The mobile phase was constituted by methanol, glacial acid and triple distilled water (40:1:59) to 1 litre of which 1-heptanesulphonic acid (PIC reagent B7 manufacture by waters associates, U.S.A) was added to get 0.005 morality of 1-heptanesulphonic acid and 3.5 pH of the solution. The flow rate was 2ml/min, chart speed 1cm/min and attenuation 0.1 AUFS. The ambient temperature was 26±1ºC. Furthermore the method is qualified for the detection of Thebaine and Narcotine.

PRODUCT DEVELOPMENT

In the present study eight products were developed in two ways i.e. by incorporation of poppy seeds in various ratios (i.e. 2.5%, 5%, 7.5%, 10%) while another value added products were 100% product made

from poppy seed oil. Sensory evaluation was carried out by using 9 point hedonic scale with respect to various attributes namely appearance, colour, and flavour, texture, consistency, after taste and overall acceptability.

RESULTS AND DISCUSSION

The significant reduction (i.e. 90%) of alkaloids was seen after processing. The most effective methods include washing and soaking, heat treatments using temperatures at least above 135ºC, but preferably above 200ºC, lower temperatures (e.g. 100ºC) in combination with moisture or washing as well grinding and combination of the multiple treatments. (Sproll C, et al, 2006, Sproll C, Lachrnmeier DW, 2006, Bjerver K, et al, 1989, General J, et al, 2007, Lachenmeier DW, et al, 2010, Sproll C, et al, 2007)

Poppy seed containing foods go through several processes before being served. In case of bread, often whole untreated poppy seeds are commonly ground before adding on top of a dish or before using in bakery products (Efsa (European Food Safety Authority) panel on contaminants in the food chain (CONTAM), 2011). Poppy seeds are also used as poppy seed filling, which is a combination of ground poppy seeds, sugar, liquid (water or milk) and possible additional ingredients and spices. The poppy seed filling is usually heat treated before use in the food preparation (General J, et al, 2007). Thus poppy seeds in foods often go through a combination of different processing steps including grinding, mixing with liquid, and heat treatment and sometimes even with several heat treatment steps. Although a single processing step may not have a major reducing effect on the poppy seed alkaloid content, e.g. 10%-50% reduction due to baking of whole poppy seeds on bread(General J, et al, 2007) or 25-34% due to grinding of poppy seeds(Sproll C, et al, 2006), a combination of pre- treatment (e.g. processing of poppy seed filling) followed by heat treatment (e.g. baking) may reduce the poppy seed alkaloid content down to non-detectable quantities. By the combination of washing and drying on technical scale(Nencini P, 1997), achieved reductions of morphine concentrations also in highly contaminated batches of raw poppy seeds (original concentration varying from 50 up to 220 mg morphine/kg) down to concentrations below 4 mg morphine/kg without loss of quality and organoleptic properties.

The study undertaken is an attempt to develop some products, incorporated by poppy seeds which have reduced amount of alkaloids after processing. The main purpose was to utilise all the nutritional aspect present in the seed. In this way it could be proved to be the beneficial to heal various types of diseases such as cold, cough, depression, pain in the body, cancer, tuberculosis, diarrhoea, dysentery etc.

To assess the overall acceptability of the four developed products by incorporation of poppy seeds in various ratios (i.e., 2.5%, 5%, 7%, and 10%) panellist was asked to evaluate the products on the basis of 9 point hedonic scale where following attributes were estimated: appearance, flavour, colour, texture, consistency, after taste, overall acceptability.

POPPY SEED INCORPORATION

POPPY SEED TEA

The significant difference ($p \leq 0.05$) was found in all samples at 2.5, 5, 7.5, and 10% level. The mean scores for overall acceptability attributed ranged from 7.2 ± 0.86 to 8.5 ± 0.51. The highest value was reported for overall acceptance in PT3 i.e. 7.6± 0.72 as compared to the standard.

POPPY SEED BISCUITS

There was found to be no significant difference ($p \geq 0.05$) in all samples. The highest value for overall acceptability was reported in PB2 i.e. 8.3 ± 0.81 as compared to the standard.

POPPY SEED CAKE

For overall acceptability there was found to be no significant difference ($p \geq 0.05$) in the sample except PC4 which was significant different at ($p \leq 0.05$). The highest value for overall acceptability was reported in PC1 i.e. 8.3 ± 0.48 as compared to the standard.

POPPY SEED LADDO

There was found to be no significant difference between ($p \geq 0.05$) except PL4 at 10%. The most overall acceptable sample was found to be PL1 i.e. 8.2 ± 0.59 as compared to the standard.

All the incorporated recipes were equally accepted but poppy seed biscuits and cakes were most acceptable at 5% and 2.5% level respectively. Bakery products showed significant reduction ($p<0.5$) in alkaloids due to grinding and heat treatment done at 200°C.

Table 1: - proximate composition of poppy seeds

Nutrients (g/100g)	Standard	Test
Ash	5.2	5.8±0.15
Moisture	4.3	4.5±0.15
Crude fibre	6.4	7.3±0.15
Protein	18.0	16.5±0.15
Fat	42.0	49.0±1.00
Carbohydrate	37.0	33.7±0.20
Iron (mg)	10.4	10.2±0.20
Calcium (mg)	1438.0	1145±30.61*

*denotes mean difference is significant at 95% confidence interval ($p \leq 0.05$)

Table:-2 reduction method of morphine and codeine

Technological step	Description	Effect
Washing	Rinsing with water above 60ºc at least 2 min	Morphine reduction
Drying	Removal of water residues (e.g. 40ºc for 2 hour)	Prevention of microbiological contamination, germination, and rancidity
Grinding	The washed seeds must be ground using poppy mills	Further morphine reduction, improvement of the aroma
Baking	Heating at high temperatures around 200ºc.	Further thermal morphine degradation

Table 3:-the mean scores ± sd for different attributes of poppy seed tea

Attributes	Samples				
	Standard	Pt1	Pt2	Pt3	Pt4
Appearance	8.2 ± 0.48	7.5 ± 0.65*	7.3 ± 0.87*	7.5 ± 0.65	7.8 ± 0.68*
Flavour	8.1 ± 0.35	7.1 ± 0.63	7.3 ± 0.86*	7.3 ± 0.72*	7.2 ± 0.72*
Texture	8.1 ± 0.35	7.3 ± 0.97*	7.5 ± 0.91*	7.6 ± 0.81*	7.7 ± 0.96*
After taste	8.3 ± 0.56	7.6 ± 0.63*	7.0 ± 0.59*	7.5 ± 0.51	7.4 ± 0.82*
Overall acceptability	8.5 ± 0.51	7.2 ± 0.86*	7.1 ± 0.83*	7.6 ± 0.72*	7.4 ± 0.83*

Table 4:-the mean scores ± sd for different attributes of poppy seed biscuits

Attributes	Samples				
	Standard	Pc1	Pc2	Pc3	Pc4
Appearance	8.2 ± 0.59	8.0 ± 0.70	8.2 ± 0.70	8.2 ± 0.70	8.0 ± 0.79
Flavour	8.2 ± 0.67	8.1 ± 0.74	8.2 ± 0.67	7.9 ± 0.96	7.5 ± 0.99
Texture	7.4 ± 0.83	7.7 ± 0.79	8.1 ± 0.63*	8.0 ± 0.70*	7.8 ± 0.67*
Crumbness	7.7 ± 0.79	7.5± 0.83	7.2 ± 1.03	7.4 ± 0.91	7.3 ± 1.23
After taste	7.6 ± 0.72	8.2 ± 0.59	8.2 ± 0.67	8.2 ± 0.67	7.4 ± 0.91
Overall acceptability	7.6 ± 0.72	8.2 ± 0.59*	8.3 ± 0.81	8.2 ± 0.79	7.8 ± 1.01

Table 5:-the mean scores ± sd for different attributes of poppy seed cake

Attributes	Samples				
	Standard	Pc1	Pc2	Pc3	Pc4
Appearance	8.3 ± 0.72	8.2 ± 0.67	8.2 ± 0.45	8.0 ± 0.79	7.6 ± 0.81*
Flavour	8.4 ± 0.51	8.3 ± 0.89	8.1 ± 0.83	8.1 ± 0.83*	7.6 ± 0.81*
Texture	7.8 ± 0.83	8.2 ± 0.70*	8.0 ± 0.79*	8.2 ± 1.08*	7.7 ± 1.03
Crumbness	7.9 ± 0.70	8.0 ± 0.65*	7.8 ± 0.74	8.2 ± 0.96	7.8 ± 0.83
After taste	8.1 ± 0.74	8.0 ± 0.59	7.9 ± 0.79*	8.1 ± 1.06	7.5 ± 1.18*
Overall acceptability	8.2 ± 0.56	8.3 ± 0.48	8.2 ± 0.77	8.2 ± 0.94	7.8 ± 0.83*

Pc= poppy seed cake, *denotes mean difference is significant at 95% confidence level ($p \leq 0.05$)

Table 6:-the mean scores ± sd for different attributes of poppy seed laddo

Attributes	Samples				
	Standard	Pl1	Pl2	Pl3	Pl4
Appearance	8.4 ± 0.63	8.4 ± 0.50	8.0 ± 0.92	8.4 ± 0.73	8.1 ± 0.63
Flavour	8.4 ± 0.73	8.0 ± 1.06	7.2 ± 1.06*	8.2 ± 0.94	7.7 ± 0.79*
Texture	8.2 ± 0.77	8.0 ± 1.06	7.1 ± 1.06*	8.3 ± 0.89	7.8 ± 0.77*
After taste	8.3 ± 0.72	8.1 ± 0.83	5.0 ± 1.33	8.0 ± 1.16*	7.5 ± 0.91
Overall acceptability	8.2 ± 0.50	8.2 ± 0.59	7.2 ± 1.08	8.1 ± 1.12	7.7 ± 0.79*

Pl= poppy seed laddoo, *denotes mean difference is significant at 95% confidence level ($p \leq 0.05$)

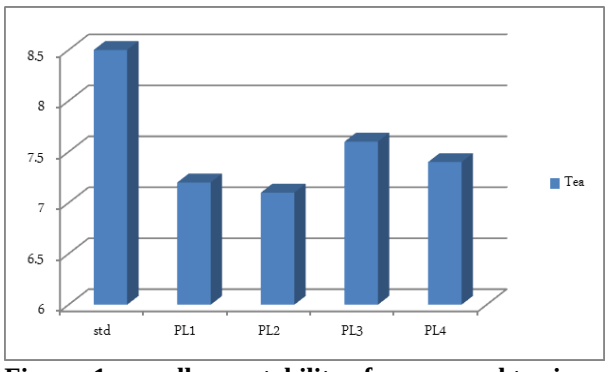

Figure. 1 overall acceptability of poppy seed tea in different ratios

Figure. 2 poppy seed tea

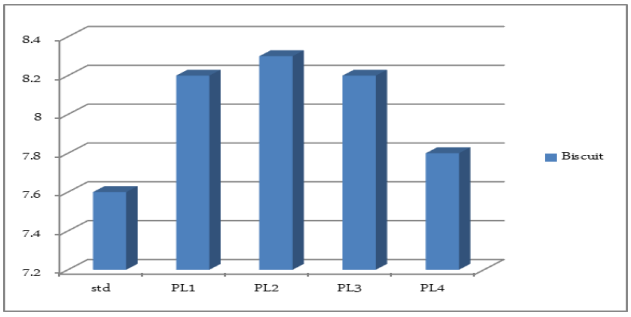

Figure. 3 overall acceptability of poppy seed biscuits in different ratios

Figure. 4 poppy seed biscuits

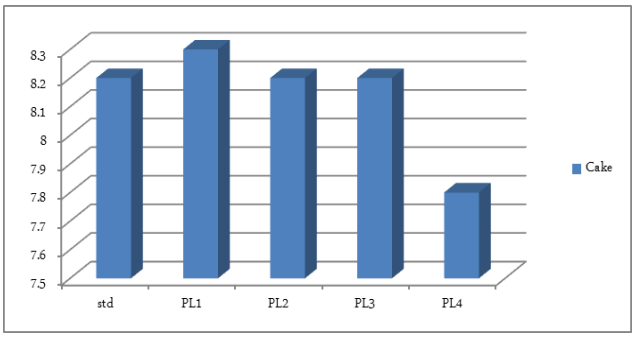

Figure.5 overall acceptability of poppy seed cake in different ratios

Figure. 6 poppy seed cake

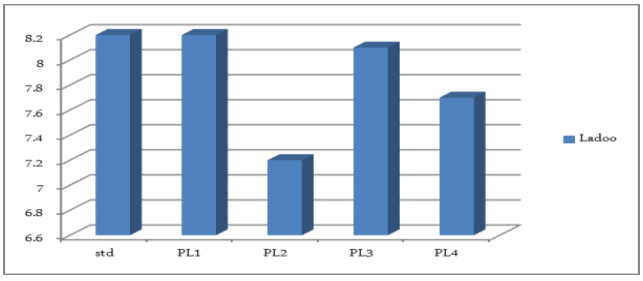

Figure. 7 overall acceptability of poppy seed ladoo in different ratios

Figure. 7 poppy seed ladoo

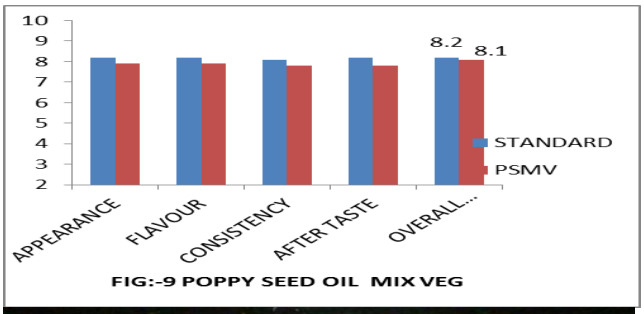

FIG:-9 POPPY SEED OIL MIX VEG

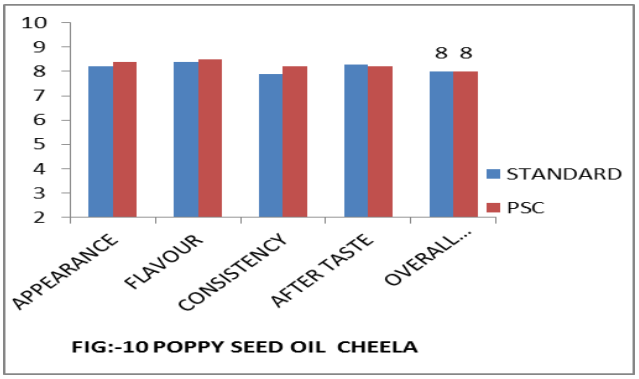

FIG:-10 POPPY SEED OIL CHEELA

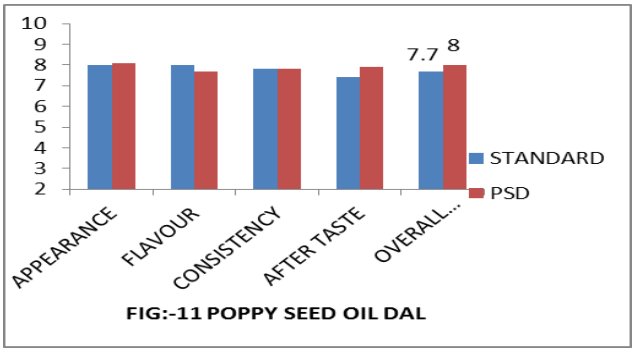

FIG:-11 POPPY SEED OIL DAL

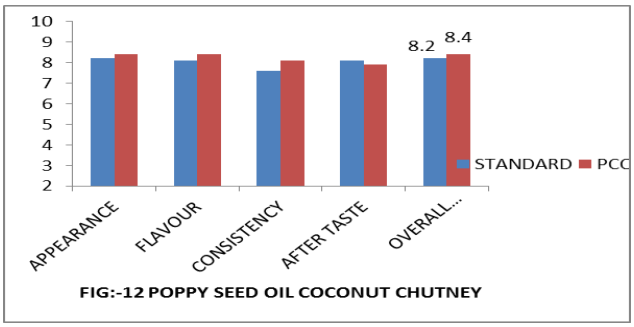

FIG:-12 POPPY SEED OIL COCONUT CHUTNEY

VALUE ADDED PRODUCTS

All recipes were equally 100% incorporated from poppy seed oil. There was found to be no significant difference ($p \geq 0.05$) hence sample was equally accepted with standard.

CONCLUSION

The alkaloid content of poppy seed samples and poppy seed containing food can be reduced by several methods of pre-treatment and processing. Food processing may decrease the alkaloid content by up to about 90% as well as aroma of the product. The most effective methods include washing, soaking and heat treatments, as well as grinding and combinations of these treatments. By the use of seeds of low morphine content from controlled producers and the possibility to reduce morphine level furthermore during food processing, and the proposed limits can easily be kept. Further after the reduction of alkaloids from seeds their bactericidal and liniment food supplements offered a broad range of potential benefits in preventing diseases.

The results of the sensory evaluation data summarized the incorporated recipes were equally acceptable and the scores ranged from 7.1-8.5g/100g on the basis of overall acceptability and value added products made from poppy seed oil were highly appreciated in terms of appearance, flavour, texture, and the mean scores for overall acceptability for prepared products ranged from 7.7- 8.2g/100g on the basis of overall acceptability. Hopefully the traditional variety and craftsmanship of the high quality poppy seed bakery products and poppy seed oil based food supplements will not suffer from risk assessments and exaggerated fear of the public.

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REFERENCES

- AOAC (1993) Official Methods and Recommended Practices of the American Oil Chemists Society. Champaing.
- AOAC (1995) Official and tentative methods of the AOAC International. Maryland.
- Aruna K, Sivaramakrishnan VM (1992) Anticarcinogenic effects of some Indian plant products. Food Chem. Toxicol. 30:953-956.
- Azcan N, Kalender BO, Kara M (2004) Investigation of Turkish poppy seeds and seed oils. Chemistry of Natural Compounds 40:370-72.
- Baydar H, Turgut l (1999) Extraction of fatty acid composition ascending to some morphological and physiological properties and acological regions in oil seed plants. Turkish Journal of Agriculture and Forestry 23:1303-6173.
- Bernath J, Nemeth E Poppy, oil crops. (2009) Handbook of plant breeding. (4eds) Vollmann J and Rajcan I. Springer Science and Business Media, 449-468.
- Bjerver K, Jonsson J, Nilsson A, Schuberth J (1982) Morphine intake from poppy seed food. Journal of pharmacy and pharmacology 34:798-801.
- Bozan B, Temelli F (2003) Extraction of Poppy Seed Oil Using Supercritical CO_2. Journal of Food Science 28(2):1750-3841.
- Cohen HM, Law-Yone B, Lu S (2002) Poppy to Fufu. J. Chinese Med. 15-20.

- Duke JA (1989) CRC Handbook of Nuts. CRC Press. Boca Ratan 240-243.
- Efsa (European Food Safety Authority) panel on contaminants in the food chain (CONTAM) (2011) Scientific Opium on the risks for public health related to the presence of opium alkaloids in poppy seeds. European food safety authority 9(11):2405.
- Erinc H, Tekin A, Ozcan MM (2009) Determination of fatty acid, tocopherol and phytosterol contents of the oils of various poppy (*Papaver somniferum*) seeds. Grasas Y Aceites 60(4):375-381.
- General J, Unbehend G, Lindhauer MG, Kniel B, Moser M (2007) Untersuchungen zur Reduzierung von Morphin in Mohnsamen and Mohngebacken mit praktikablen technologischen Massnahmen. Getreidetechnologie 61:36-42.
- Kryzmanski J, Jonsson R (1999) Poppy. In: Robbelon G, Downey RK, Ashri A. (eds.), Oil Crops of the World. Their Breeding and Utilization. New York, NY:McGraw-Hill, Inc. 389.
- Lachenmeier DW, Sproll C, Musshoff F (2010) Poppy seed foods and opiate drug testing- where are we today? Therapeutic Drug Monitoring 32:11-18.
- Luthra R, Singh N (1989) Changes in Fatty Acid Composition accompanying the deposition of triacylglycerols in developing seeds of opium poppy (Papaver somniferum l.) Plant Science 60:55-60.
- Nencini P (1997) The rules of drug taking: wine and poppy derivatives in the ancient world. VI. Poppies as source of food and drug. Substance Use & Misuse 32:757-766.
- Nergis C, Otles S (1999) The proximate and some minor constituents of poppy seeds. J. Sci. Food Agric. 66:117-120.
- Ozcan MM, Atalay C (2006) Determination of seed and oil properties of some poppy (Papaver Somniferum L.) varieties. Grasas y aceties 57(2):169-174.
- Qidwai W, Alim SR, Dhanani RH, Jehangir S, Nasrullah A et. al., (2003) Use of folk remedies among patients in Karachi Pakistan. J. Ayub. Med. Coll. Abbottabad, 15:31-33.
- Raghuramulu N, Nair KM, Kalyanasundaram S (2003) A Manual of laboratory techniques. National Institute of Nutrition Press. Hyderabad. 50-59.
- Sharma S (2007) Experiments and techniques in biochemistry. Galgotia Publicatios Pvt. Ltd. New Delhi.
- Singh SP, Khanna KR, Shukla S (1999) Breeding strategies in opium poppy (*P. somniferum* L.) at National Botanical Research Institute, Lucknow, India. Applied Botany Abstracts 19(2):121-139.
- Singh SP, Khanna KR, Shukla S, Dixit BS, Banerjee R (1995a) Prospects of breeding opium poppies (P.somniferum L.) as a high linoleic acid crop. Plant Breeding 114:89-91.
- Singh SP, Khanra KR, Dixit BS, Srivastava SN (1990) Fatty acid composition of opium poppy (Papaver somniferm L.) seed oil. Ind. J. Anric. Sci. 60:358-359.
- Sproll C, Lachrnmeier DW (2006) Morphine in poppy seed food: influence of food processing and guidelines for reduction. J Agric Food Chem. 54:5292-5298.
- Sproll C, Perz RC, Buschmann R, Lachrnmeier DW (2007) Guidelines for reduction of morphine in poppy seed intended for for food purposes. European food research and technology 226:307-310.
- Sproll C, Perz RC, Lachrnmeier DW (2006) Optimized LC/MS/MS analysis of morphine and codeine in poppy seed and evaluation of their fate during food processing. J. Agric. Food Chem. 54:5292-52.
- Yadav HK, Shukla S, Singh SP (2008) Discriminant function analysis for opium and seed yield in opium poppy (Papaver somniferum). Genetika 40:109-120.
- Zadeh SS, Begum K (2011) Nutritional supplements and its effect on quality of life and sleep. American medical journal 2(2):104-110.