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Research Article

REDUCTION OF MORPHINE IN POPPY SEEDS THROUGH FOOD PROCESSING FOR ENHANCING NUTRITIONAL QUALITY OF FOOD PRODUCTS

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ABSTRACT
Poppy seed is an oilseed obtained from the opium plant (*Papaver somniferum*). It is one of the oldest known painkillers consisting of many alkaloids (morphine, codeine etc.) and is rich in protein and calcium. The morphine content of poppy seed intended for use in food recently have raised major concern due to intoxication in maximum limits. The federal institute for risk assessment (BFR) derived a provisional guidance value of 4mg morphine/kg body weight poppy seed based on a maximum daily intake of 0.38mg morphine/person. This 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 systematic research was done to determine the influence of food processing and the significant reduction of morphine (by steps like grinding and boiling) was observed. Randomly selected poppy seeds sample was taken for its degradation directly for which the optimal treatment consisted of washing, drying and grinding. The alkaloids were analysed by HPLC method. The results revealed that the percentage of the 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. Bakery products showed significant reduction ($p<0.5$) in alkaloids due to grinding and heat treatment done at 200ºC. In the current study, the kinetics of the morphine degradation during grinding as well as the efficacy of washing steps was evaluated which was reached to 90%. The results were used to establish guidelines for consumers and bakeries with possibilities to minimize the morphine content of poppy seed.

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

INTRODUCTION
Poppy seed is an oilseed obtained from the opium plant (*papaver somniferum*. It is an erect annual herb which has been cultivated from prehistoric times (Bernath *et al.,* 2009). The production of poppy principally occurs in northern India in the region of Madhya Pradesh and Rajasthan (Schiff, 2002; Srinivas and Rao, 1981).

It is one of the oldest known painkillers and is the source of many alkaloids which includes narcotics and analgesic morphine and the codeine, thebaine, nonscapine and many more (Shukla *et al.,* 1995; Shukla *et al.,* 2006; yadav *et al.,* 2006).

The morphine content of poppy seed which is widely used in dishes and pastries has recently raised major concerns (Sproll *et al.,* 2006) and cases of intoxication after consumption of strongly contaminated poppy seed even led to a discussion about maximum limits. The federal institute for risk assessment (BFR) derived a provisional guidance value of 4mg morphine/kg poppy seed based on a maximum daily intake of 0.38mg morphine/person. The maximum value of 4mg morphine/kg 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 systematically the research was done to determine the influence of food processing and the significant reduction of morphine (during steps like grinding and boiling) was observed (Sproll *et al.,* 2006).

In the current study, the kinetics of the morphine degradation during grinding as well as the efficacy of washing steps was evaluated. The results were used to establish guidelines for consumers and bakeries with possibilities to minimize the morphine content of poppy seed.

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, 2007). The protein level was determined by the Khjeldal method, according to official analytical chemists (AOAC) 984.13 (AOAC, 1995). The ash was determined by (NIN, 2003). Crude fibre were estimated by acid alkali method (AOAC, 1985), lipid were extracted in soxhlet apparatus with petroleum ether at 40-60 according to the method 3 75 Ai AOAC (AOAC, 1993) and carbohydrate by difference. Estimation of iron content by Wong's method (NIN, 2003). Estimation of calcium by titrametric method (Sharma, 2007).

Table 1: Proximate composition of poppy seeds

Nutrients (g/100g)	Std	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*

Table 2: Fatty acid composition of poppy seeds oil

Fatty acid component (%)	Std	Test oil
Palmitic acid	12.85	14.3±0.25*
Stearic acid	2.40	10.9±0.11
Oleic acid	13.11	5.5±0.15*
Linoleic acid	71.50	62.6±0.05
Linolenic acid	0.30	4.3±0.15

** Denotes mean difference is significant at 95% confidence interval (p≤0.05)*

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 figure 1, about the correct treatment of poppy seed. The poppy seed should be washed with water as described above; 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.

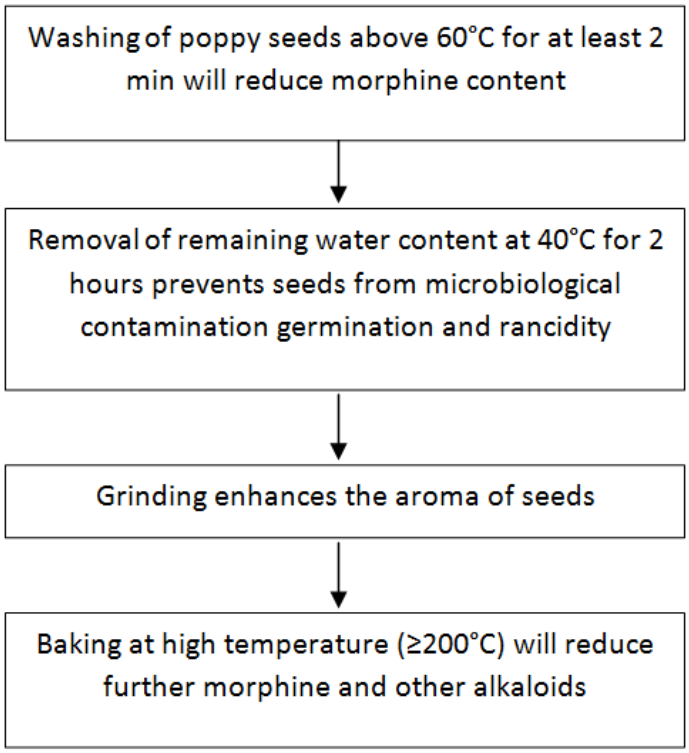

Figure 1: Flowchart shows reduction method of morphine and codeine (Sproll and Lachrnmeier, 2006)

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.

RESULTS AND DISCUSSION

The analytical study for the reduction of alkaloids in poppy seed showed that there was significant reduction in morphine, codeine, thebaine and narcotine after processing. As shown in fig.2, before processing the morphine content in the raw sample was 0.178% which was significantly reduced to 0.046% after processing, reduction of around 59% was observed. The result was in accordance with the previous studies, which showed that about 60 % of morphine was reduced by washing the poppy seed with cold water. At temperatures above 60ºC, significantly higher concentration around 90% can be

reduced. In the present study the raw seed showed 0.038% codeine and 0.041% thebaine content which was reduced to 0.009% and 0.002% (i.e. 81% and 91% reduction) respectively. All the three alkaloids showed a significant difference except narcotine which showed no significant difference and the reduction was only 0.001%.

Table 3: Analysis of reduction of alkaloid content in poppy seeds by processing

Alkaloids	Before processing (%)	After processing (%)
Morphine	0.178*	0.046*
Codeine	0.038*	0.009*
Thebaine	0.041*	0.002*
Narcotine	0.002	0.001

**Denotes mean difference is significant at 95% confidence interval (p≤0.05)*

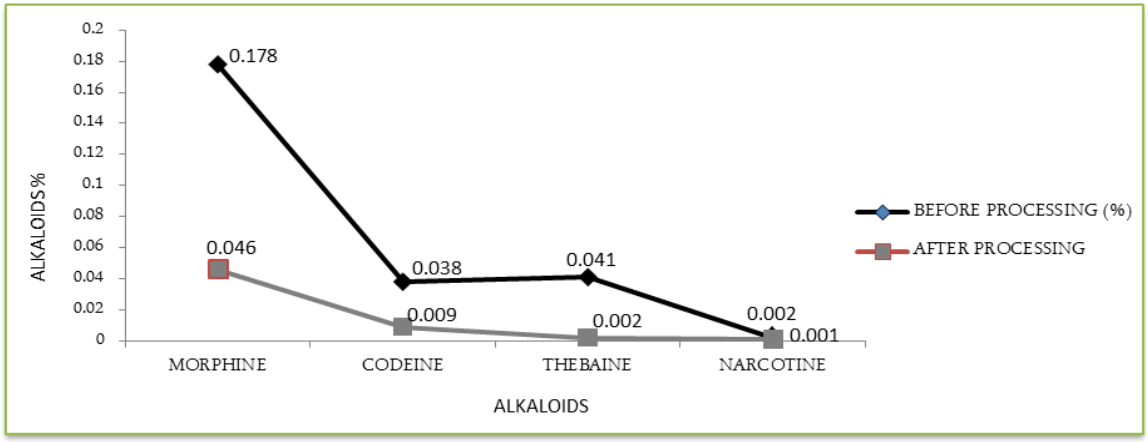

Figure 2: Alkaloid content of raw poppy seed and processed sample

Previous studies showed that the morphine content of poppy seed is significantly reduced during food processing. Mechanical pre-treatment such as grinding was found to have an important contribution on morphine reduction (Sproll, 2007). In the toxicological study by the Bavarian State Office for health and food safety and Federal Institute for Risk Assessment (Bfr) derived a provisional guidance value of 4mg/kg. The influences of food processing found a significant reduction of morphine during steps like grinding and baking. Poppy seed for decorative purposes (e.g. topping and buns) could contain upto 100mg/kg of morphine which was far off from the tolerable daily intake (Sproll, 2006).

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, the 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. 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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