POTENTIAL FOR IMPROVED UTILIZATION OF CANOLA MEAL BY MONOGASTRIC ANIMALS

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Joseph Simbaya

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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DEDICATION

To my mother Melise Ngao Nambela, who will never understand why it had to take so long.

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FOREWORD

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MANUSCRIPT I. Simbaya, J., Slominski, B.A., Rakow, G., Campbell, L.D., Downey, R.K. and Bell, J.M. 1995. Quality characteristics of yellow-seeded *Brassica* seed meals: Protein, carbohydrates and dietary fibre components. J. Agric. Food Chem. 43: 2062-2066.

MANUSCRIPT II. Simbaya, J., Slominski, B.A., Rakow, G. and Campbell, L.D. 1995. Nutritive value of yellow-seeded canola. Part I. Digestible protein, dietary fibre and the effect of moist heat treatment on meal quality. J. Sci. Food Agric. (to be submitted).

MANUSCRIPT III. Simbaya, J., Slominski, B.A., Campbell, L.D., Rakow, G. and Guenter, W. 1995. Nutritive value of yellow-seeded canola. Part II. Chemical composition and feeding quality of meals derived from newly selected varieties. J. Sci. Food Agric. Sci. (to be submitted).

MANUSCRIPT IV. Simbaya, J., Slominski, B.A., Guenter, W., Morgan, A. and Campbell, L.D. 1995. The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: *In vitro* and *in vivo* studies. Anim. Feed Sci. Technol. (accepted).

ABSTRACT

The purpose of this research was to explore the potential for improved utilization of canola meal by monogastric animals. In study one, 26 yellow-seeded and 7 brown-seeded *Brassica* genotypes were evaluated for differences in chemical composition and dietary fibre profiles. On average, in comparison to brown-seeded, yellow-seeded genotypes contained more sucrose (8.7% vs 7.5%) and protein (44.5% vs 42.7%) and less dietary fibre (28% vs 33%). Dietary fibre was negatively correlated (r=-0.71) with protein content and its reduction in yellow-seeded samples was attributed to lower contents of lignin and polyphenols, cell wall protein and minerals associated with the fibre fraction. In a second study, selected *Brassica* genotypes were analyzed for digestible protein, soluble fibre, content of soluble phenolics and extract viscosity. Despite only minor differences in soluble fibre, soluble phenolics and extract viscosity, *B. rapa* and *B. napus* species had relatively high digestible protein content in comparison to *B. juncea* and *B. carinata* species. The measurements of digestible protein and dietary fibre as well as soluble phenolics and extract viscosity were poorly correlated. Based on chemical composition and digestible protein content, four *Brassica* cultivars were selected for use in a third study and the seeds were processed under optimal moist heat treatment conditions (108 +1°C for 20 min). The samples included yellow-seeded *B. rapa* (cv. Parkland), *B. napus* (cv. Y1016) and *B. juncea* (J4316) and brown-seeded *B. napus* (cv. Excel). With the exception of *B. rapa*, all samples had higher than commercial meals protein content with the yellow-seeded *B. napus* canola showing the highest true metabolizable energy value. The overall performance of broiler chickens fed the *Brassica* seed meals was similar to that of the commercial meal from yellow-seeded canola (control) except for *B. juncea* which had a relatively high content of undesirable aliphatic glucosinolates. Of the diets with comparable growth performance, birds fed the yellow-seeded *B. napus* canola showed the highest feed efficiency value. In a forth study an attempt was made to improve the utilization of canola meal by supplementation of broiler chicken diets with exogenous enzymes. A positive and synergistic effect was noted when a combination of protease, carbohydrase and phytase enzymes were supplemented to canola meal-based diets deficient in available phosphorus.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists.
ANOVA	Analysis of Variance.
AMEn	Nitrogen corrected apparent metabolizable energy.
CM	Canola meal.
CP	Crude protein.
DM	Dry matter.
IU	International unit.
LMWC	Low molecular weight carbohydrates.
MWCO	Molecular weight cut off.
NDF	Neutral detergent fibre.
NRC	National Research Council.
NSP	Non-starch polysaccharides.
SAS	Statistical analysis system.
SBM	Soybean meal.
SCFA	Short chain fatty acids.
SCN	Isothiocyanate ion.
SCWL	Single comb white leghorn.
SD	Standard deviation.
SDS	Sodium dedocyl sulphate.
SEM	Standard error of the mean.
TDF	Total dietary fibre.
TMA	Trimethylamine.
TMEn	Nitrogen corrected true metabolizable energy.

1. INTRODUCTION

Seeds of *Brassica* species, canola/rapeseed and mustard rape, have become important oilseeds in many temperate and high altitude climate zones where other oil crops can not thrive and grow (Bell, 1984). *Brassica* crops currently account for 13.2% of the world's edible oil output, which makes them the third most important source of edible oil after soybean and palm (Shahidi, 1990a). In comparison to soybeans, *Brassica* seeds are considerably smaller and contain a higher oil content of more than 40% (dry matter basis), with a profile of fatty acids well suited to modern human consumption (Ackman, 1990). The superiority of canola varieties to the original rapeseed cultivars is well known. Rapeseed cultivars had disadvantages in that they yielded oil which contained 25 to 45% erucic acid and the oil-extracted meal had 110 to 150 μmoles of aliphatic glucosinolates per gram (Bell, 1993a). As a result of genetic selections, current cultivars yield oil with less than 2% erucic acid and less than 30 μmoles of glucosinolates per gram of the meal.

Most of the oil derived from *Brassica* seeds is used in human food products such as margarine, salad and cooking oils (Downey, 1983). The meal which remains after oil extraction contains 38-44% (dry matter basis) of high quality protein with an amino acid profile comparable to that of soybean meal (Fenwick, 1982; Downey and Röbbelen, 1989). While in some Asian countries the high glucosinolate meal is still being used as an organic fertilizer, in Europe and Canada the improved quality meal is exclusively used as a protein supplement in livestock and poultry diets (Downey, 1983; Bell, 1984).

Despite the high protein content, the use of canola meal (CM) as a high quality protein supplement for poultry and other monogastric animals is still limited by high content of dietary fibre (30%)in the meal (Bell and Shires, 1982). Dietary fibre tends to dilute the nutrient content and has also been associated with reduced energy (Sarwar *et al.*, 1981) and protein digestibilities in CM-based diets (Bell, 1993a). The major fibre components in CM include cellulose (4-6%), non-cellulosic polysaccharides (13-16%), lignin and polyphenols (5-8%), and protein and minerals associated with the fibre fraction (Slominski and Campbell, 1990). The nutritive quality of CM may be improved by reducing fibre content through genetic selections, innovations in processing plants or by dietary means. Currently, plant breeding programs are directed towards selection for yellow-seeded varieties of low fibre content (Stringam *et al.*, 1974). Efforts in commercial processing are aimed at reducing fibre content by dehulling of the seed prior to oil extraction and animal nutritionists are examining the possibility of increasing nutrient digestibility through the use of cell wall degrading enzymes (Slominski and Campbell, 1990; Slominski *et al.*, 1993).

Recent reports on chemical composition of yellow-seeded canola indicated only minimal reduction in fibre content which, as opposed to brown-seeded type, was found to contain more of non-starch polysaccharides (NSP) at the expense of lignin and associated polyphenols (Slominski and Campbell, 1991a; Slominski *et al.*, 1994a). Despite the increased nutrient content in dehulled CM, the processing industry is yet to establish the appropriate conditions to improve percolation of hexane through the hull-free and thus very fine meal (Bell, 1993b). While the application of supplementary cell

wall degrading enzymes tend to increase NSP digestibility in laying hens (Slominski and Campbell, 1990), their effect on the performance of growing chickens still remains inconclusive (Slominski *et al.*, 1993).

Lack of visible improvements in energy and protein utilization from yellow-seeded canola, dehulled CM and enzyme supplemented conventional CM suggests that further research is needed to characterize the factors that influence meal quality. The major objective of this study was to provide detailed knowledge on chemical composition of canola with emphasis on the potential relationship between total and digestible protein and soluble and insoluble dietary fibre contents. The effect of various conditions of moist heat treatment on *in vitro* protein digestibility was studied in order to establish optimal parameters required for processing of canola seed. Such parameters were further employed for processing of the seed used in subsequent *in vivo* evaluation of selected samples of canola. Since a wide range of yellow- and brown-seeded strains/varieties were included in the study, it is believed that this research will provide valuable information for future canola selection programs. Knowledge of digestible protein and available amino acid contents and the profile of dietary fibre will assist in identifying the industrial enzyme preparations suitable for canola meal treatment. Therefore, the second objective of the study was to explore the potential for improved utilization of canola meal by supplementation of canola meal containing poultry diets with exogenous protease and carbohydrase enzymes.

2. LITERATURE REVIEW

2.1. CANOLA MEAL: ITS IMPORTANCE AND LIMITATIONS

2.1.1. Historical Perspectives

The real beginning of rapeseed cultivation is not certain as the domestication of the crop occurred at different times in different parts of the world. Domestication of rapeseed occurred whenever the economical value of *Brassica* weed seeds in cereal grain crops was appreciated by local populations (Boulter, 1983; Åppleqvist, 1972). Early Asian Sanskrit writings indicate rapeseed to have been an important oil crop as early as 2000-1500 B.C. Ancient civilizations used rapeseed oil for illumination, cooking, medicinal, paint and soap making (Åppleqvist and Olhson, 1972). Rapeseed cultivation was introduced to Japan from China directly or via the Korean Peninsula in 35 B.C. (Bell, 1982).

In Europe, rapeseed cultivation dates back to the 13th century, when it was mainly confined to the Netherlands where it was grown for land reclamations from the sea (Åppleqvist and Olhson, 1972). The crop was introduced to England in the 16th century for the same purpose probably by Dutch ship builders. In England rapeseed cultivation increased in 1752 due to a Parliamentary bill which emphasized making oil out of locally grown seeds equal to foreign oils (Bunting, 1986). Rapeseed cultivation spread to other European countries in the 18th century, especially those where olive oil and poppy seed trees were not grown. It was from this period that rapeseed oil became

the most important lamp oil in Europe until the end of the 19th century when cultivation declined due to the discovery of mineral oils. However, this was also the time when rapeseed production was spreading eastwards into Switzerland, Poland and Western Russia and northwards into the Scandinavian countries of Denmark and Sweden, where production continued well up to the end of the 19th century. In Western Europe, production started again during the first world war due to blockades on industrial oil imports and the increased demand for edible oils during the hostilities. This initiative did not last long as there was a decrease in rapeseed production after the war and most of the oil demand was being met by cheap imports from the colonies in Africa and Asia.

Production increased again in the early 1940s when imports could not reach Europe with the start of the second world war. After the world war II, Sweden was the first nation to establish a guaranteed price system on locally grown rapeseed oil to boost domestic production and avoid future shortages as was experienced during the war. Other nations adopted the guaranteed price scheme, though in the early 1960s, the European rapeseed industry could not compete with cheap Canadian imports. In 1960 the formation of the EEC and its support policy of promoting local agricultural crops saved the oil industry from collapsing. Since then, the European rapeseed industry has been on the increase up to the present.

In Canada, rapeseed cultivation started in 1936 when a Polish farmer at Shellbrook, Saskatchewan received seeds of *Brassica rapa* from a contact in Poland (Bell, 1982). These seeds became the resource material for yield and establishment trials by the Canadian Department of Agriculture. In 1942, seeds of *Brassica napus* from

Argentina were introduced through the USA to Saskatchewan farmers who grew the crop on contract (White, 1979). For a long time, the two types of rapeseed were grown and came to be known as *Polish* and *Argentine* rape, respectively (Bell, 1982). The original interest in rapeseed production was centred on its high oil content and the unique property of sticking to steam and marine engines (Bell, 1982).

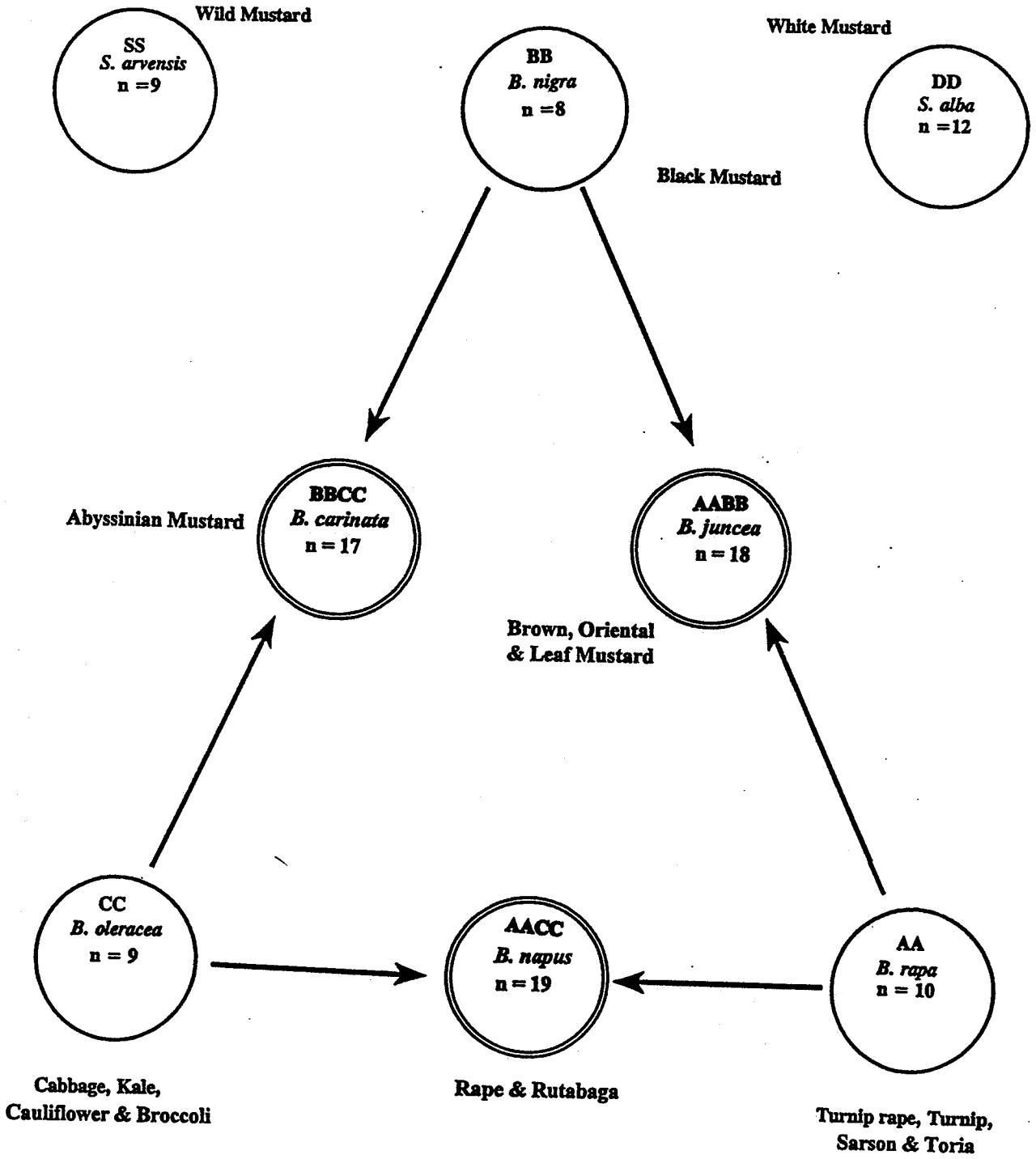
The increase in demand for industrial and human consumption created by war blockades led to increased local supply of rapeseed oil. But after the war, there was a decrease in the demand of industrial oil and there were concerns regarding the use of rapeseed oil for human consumption due to high erucic acid content which was considered a health hazard by the Food and Drug Directorate of the Department of National Health. While most of the oil was being exported to European markets, the limitations on erucic acid provided a motivation for the search of rapeseed strains with lower erucic acid content in their oil. Many cultivars were developed through plant breeding, and in 1970 a decision was made to change from growing high erucic acid varieties to low erucic acid varieties.

In parallel with expanded use of rapeseed oil on the domestic market, there was an urgent need to increase the use of rapeseed meal for animal feeding to make the crop more competitive (Bell and Wetter, 1979). But feeding of rapeseed meal to livestock and poultry resulted in thyroid disorders and was also associated with reduced feed intake and poor animal performance. This lack of response from rapeseed feeding set a stage for future research on meal improvement techniques. But progress on meal utilization was limited for many years by lack of analytical and appropriate processing techniques (Bell

and Wetter, 1979). With the analytical procedure developed by the Prairie Regional Laboratory, it was learnt that glucosinolates, which yield toxic and goitrogenic products upon hydrolysis, were the main factor limiting rapeseed meal utilization in swine and poultry. The break through came in the late 1960s when a variety "Bronowski" from Poland was found to contain only one seventh of glucosinolates normally present in rapeseed cultivars. This led to the development of low erucic acid and low glucosinolate varieties. By 1980, most of the rapeseed varieties grown in Canada were low erucic acid and low glucosinolate (double zero) cultivars. These were patented in 1981 under the name "canola" to differentiate them from the original rapeseed varieties.

2.1.2. Adaptation and Distribution

All current canola oil and meal producing varieties belong to the genus *Brassica* in the family of *Cruciferae*. Other members of the family and their cytogenetic relationships are presented in Fig. 1. The relationships have been verified by both chemotaxonomy and artificial hybridization of amphidiploids (Dass and Nybom, 1967). *Brassica* species appear to have evolved from a now extinct common ancestor in the Himalayan region (Hedge, 1976), though nearly all species seem to have secondary centres of origin and appear to have developed at different times wherever parental species coexisted. Due to natural selection and/or perhaps crossbreeding over several hundreds of years, the *Brassica* species have subdivided into different subspecies, forms and varieties or cultivars (Downey, 1983). Turnip rape (*Brassica rapa =campestris)* is the most variable and widely distributed of all the *Brassica* species (Thompson and


Wild Mustard
SS
S. arvensis
n =9
BB
B. nigra
n =8
White Mustard
DD
S. alba
n =12
Black Mustard
Abyssinian Mustard
BBCC
B. carinata
n = 17
AABB
B. juncea
n = 18
Brown, Oriental
& Leaf Mustard
CC
B. oleracea
n = 9
AACC
B. napus
n = 19
AA
B. rapa
n = 10
Cabbage, Kale,
Cauliflower & Broccoli
Rape & Rutabaga
Turnip rape, Turnip,
Sarson & Toria

Hughes, 1986). Its secondary centres of diversity range from the Atlantic Islands in the West to the shores of China and Korea in the East and from Norway in Northern Europe to Northern India in the South. The origin of *Brassica napus* is somehow obscure and it appears that the species have evolved at a much latter date; most probably in the Mediterranean region where the parental species of *B. rapa* and *B. oleracae* occurred together (Thompson and Hughes, 1986). The origins of *B. juncea* has also not been established with certainty, though most studies point to the Middle East as its secondary centre of diversity where *B. rapa* and *B. nigra* existed in proximity.

Because of their ability to survive and grow under relatively cold conditions, canola/rapeseed crops are restricted to the temperate regions of Canada, Northern Europe and the high altitude subtropical regions of China and the Indian subcontinent. As newly adapted varieties get released each year, there is a potential for Australia, the United States and perhaps South America to join the major rapeseed/canola producing regions of the world (Downey and Röbbelen, 1989). In Canadian and European temperate regions, *B. napus* and *B. rapa* are the predominant species grown whereas in China and the Indian subcontinent *B. rapa* (brown and yellow Sarson) and *B. juncea* (Toria) are the main species (Bunting, 1986). The Asian and European forms of *B. rapa* must have separated early in their development as the two forms differ significantly in their morphology and chemical compositions. Both European and Canadian *B. napus* and *B. rapa* varieties yield oil and meal of similar characteristics with each having spring and winter grown forms. In both cases, winter cultivars out yield spring ones by approximately 20%. There are also variations in seed yield between *B. rapa* and *B.*

napus varieties with the latter out yielding the former by approximately 20% and its seeds having more oil and protein.

Brassica carinata or Ethiopian mustard is yet to be incorporated into canola quality varieties and its production is still confined to its region of origin in North Eastern Africa in the Abyssinian highlands (Bunting, 1986). It should be pointed out, however, that its superior agronomic characteristics and resistance to pests and diseases has attracted attention in many current canola breeding programs. *Brassica juncea* forms are mostly grown in China and the Indian subcontinent though the recent increase in rapeseed production in Asia is mostly attributed to expanded use of *B. napus* varieties. In Canada and the United Kingdom, there has been an ongoing small scale cultivation of *B. juncea* varieties for commercial production of condiment mustard (Downey and Röbbelen, 1989). In recent years, *B. juncea* varieties have been undergoing trials for canola oilseed production in Canada, Australia and the United States (Shahidi, 1990b).

2.2. COMMERCIAL CRUSHING OF CANOLA SEED

In Canada, the seed that is destined for processing is either obtained directly from the farm or indirectly from primary grain elevators via the Canadian Grain Commission. Seeds of *B. napus* and *B. rapa*, the most common canola species grown, and since the two species yield oil and meal of similar quality characteristics (Beach, 1983; Downey, 1983), the seed is usually mixed on delivery and is processed as such.

2.2.1. Seed Cleaning

The first step in canola processing is cleaning of the seed from any docking materials such as sticks, leaves, stones, chaff and cereal grains (Beach, 1983; Unger, 1990). Metallic fragments in the seed are removed by passing the seed though a series of magnetic steel bars (Carr, 1989). The seed is then passed through a series of screens to remove undersized and oversized seeds with air aspiration being employed through out to remove dust particles (Brogan, 1986).

2.2.2. Seed Flaking

The cleaned seed, which usually contains less than 2.5% dockage, is flaked by passing through a series of roller mills adjusted to a narrow clearance of 0.2-0.3 mm to physically rapture the seed coat and some of the oil containing cells (Pickard, 1993). In Canada, where the canola seed is usually stored under freezing conditions, the seed must first be pre-conditioned to flaking by heating at 30-40°C indirectly or by direct hot air applications (Unger, 1990). Pre-conditioning of the seed is a pre-requisite for proper flake formation, screw pressing, cake formation, oil extraction and subsequent solvent removal from the oil extracted meal.

2.2.3. Cooking

The flaked seed is passed on into a series of closed cylindrical kettles stacked one on top of the other with each having a sweep type handle device for continuous mixing